Effect of Low Root Temperature on Ribonucleic Acid Concentrations in Figleaf Gourd and Cucumber Roots Differing in Tolerance to Chilling Temperature

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Summary

To clarify the involvement of RNA metabolism in chill-tolerant figleaf gourd (*Cucurbita ficifolia* Bouché) and chill-intolerant cucumber (*Cucumis sativus* L), the effects of root temperatures ranging from 12 ° to 26 °C on root RNA concentrations and root growth were studied. RNA concentrations in figleaf gourd roots were affected by root temperature, being lowest at 23 ° to 26 °C, increasing with lower temperatures and peaking at 14 °C; concentrations then decreased at 12 °C. The concentration at 14 °C was 2.5 times higher than it was at 23 ° to 26 °C. A similar trend was observed in cucumber roots, but much less dramatic. The marked increase of RNA concentration in figleaf gourd roots was due to increases of such RNA species as rRNA, mRNA, tRNA, and RNA precursors. The extent of increases was most prominent in tRNA. Six days after exposure to 14 °C, the roots were transferred to 23 °C. The move caused a rapid drop of RNA concentration within 2 days to a pre-chill level in figleaf gourd roots but no change in cucumber roots. The marked increase of RNA concentration in figleaf gourd roots exposed to 14 °C may be caused by the low temperature-induced net accumulation of RNAs. Root growth of figleaf gourd paralleled that of RNA concentration/temperature curve; that of cucumber was nearly linear from 12 °C to 26 °C. The marked increase in RNA concentration in figleaf gourd roots exposed to 14 °C is discussed in relation to chilling tolerance.

Introduction

After the steep oil price increases in the 1970's, strenuous efforts were made to reduce oil consumption in the cultivation of horticultural crops. Improving the chill tolerance of crops is considered cardinal; some trials have been successful.

To date breeding of chill-tolerant cultivars has been achieved mainly by traditional crossbreeding, using chill-tolerant wild species and varieties. However, recent advances in plant biotechnology shed light on the possibility of using a recombinant DNA technique. For example, Houde et al. (1992) succeeded in cloning genes specifically expressed under chilling temperature regimes in wheat. Murata et al. (1992) were able to improve

chill-tolerance of tobacco plants by introducing *Arabidopsis* genes coded for glycerol-3-phosphate-acyl-transferases.

Figleaf gourd is unique among Cucurbitaceous species in that its root growth is favored at a temperature of about 14 °C; root growth of other species is greatly suppressed at low temperatures, compared to higher temperatures of about 25 °C (Hori et al., 1970; Tachibana, 1982). Shoot growth is better achieved at higher root temperatures in all species including figleaf gourd. Since figleaf gourd is an overwintering plant (Whitaker and Davis, 1962), this characteristic feature of the roots may have been acquired during its evolution. According to Tachibana (1982, 1987b, 1988, 1989), figleaf gourd roots exhibit the following characteristic changes in morphology and physiology when exposed to chilling temperatures; great increases in thickness, fatty acid unsaturation of

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phospholipids, cytokinin content, and respiration via cytochrome pathway. Some of these changes have been implicated to be characteristic of chill-tolerant plants (Graham and Patterson, 1982). Thus, we deduce that figleaf gourd roots have the ability to adapt themselves to chilling temperatures.

Undoubtedly, these traits of figleaf gourd roots are genetically controlled, and it is expected that when the root is exposed to chilling temperatures, genes are expressed in the root which are responsible for the morphological and physiological changes in the root as described above. Mounting evidence demonstrate that cold acclimation processes involve accumulation of soluble protein, changes in electrophoretic patterns of protein, synthesis of new enzymes, accumulation of rRNA and soluble RNAs, and altered mRNA content (Guy, 1990). However, most of these evidence have been obtained with plants as they cold-acclimate to freezing temperatures; to our knowledge, little information is available as to whether similar events occur in chill-tolerant or chill-favourite roots, when only the root is chilled. The objective of the present investigation was, therefore, to elucidate what happens in RNA metabolism in chilled roots of figleaf gourd, using a chill-sensitive cucumber as a control plant (Tachibana; 1982).

Materials and Methods

1. Effect of root temperature on RNA concentrations in roots (Experiment 1)

Seedlings of figleaf gourd and cucumber cv. Suvo, raised by gravel culture in a greenhouse, were planted at the 2-leaf stage into hydroponic culture vessels containing 250 liter of 1/2 strength Hoagland solution. Solution temperature was held at 23 °C at planting time and changed to 12 °, 14 °, 18 °, 23 ° and 26 °C at the 3-leaf stage. After 7 days of treatment, three 2-g portions of roots were taken and frozen immediately in liquid nitrogen and stored at -80 °C until analyzed for RNA. Air temperatures during treatment fluctuated between 30 °C day and 22 °C night with an average daily solar radiation (400-700 nm) of about 5.3 ly · day⁻¹.

2. Time course of changes in RNA concentrations in roots following exposure to 14 °C root temperature (Experiment 2)

Figleaf gourd and cucumber roots were exposed to 14 ° and 23 °C for 7 days as in Experiment 1. Roots grown at 14 °C were sampled as above just before treatment (day 0; the roots were growing at 23 °C) and 2, 4 and 6 days later. Some plants were transferred to 23 °C after 6 days, and the roots were re-sampled 2 days later. Roots grown at 23 °C were sampled on day 0 and day 6. Air temperatures during the treatment fluctuated between 30 °C day and 22 °C night, and the average daily solar radiation was about 4.1 ly · day⁻¹.

3. Determination of total RNA and mRNA

Total RNA (RNA species plus RNA precursors) in the roots was extracted and purified according to guanidine HCl/phenol method (Watanabe, 1989), except that guanidine thiocyanate was substituted for guanidine HCl and crude RNA extracts were treated with proteinase K prior to purification by SDS/phenol method (Izumi et al., 1986). Purified RNA was dissolved in 0.5 ml water and its absorbance read at 260 nm. RNA concentrations in the solution were determined using the following equation; $\text{RNA } (\mu\text{g} \cdot \text{ml}^{-1}) = 45 \times \text{OD}_{260}$ (Watanabe, 1989). Messenger RNA was isolated from total RNA solutions by oligo (dT)-cellulose column chromatography according to Watanabe (1989), and quantified with the same method as total RNA.

4. Electrophoresis of RNA

To estimate rRNA species concentrations, an aliquot of total RNA solutions (equivalent to 30 mg root fresh weight) was loaded on 2.4% polyacrylamide gels and electrophoresed at 6 mA/gel following the procedure of Loening (1967). To estimate tRNA concentrations total RNA solutions were salted out with LiCl before loading an aliquot (equivalent to 50 mg root fresh weight) on 5% gels. After electrophoresis, the gels were scanned at 260 nm using a scanning spectrophotometer (Shimadzu, UV-400). Amounts of RNA species in the gels were estimated by calibrating the peak areas with those of known amounts of authentic tRNA.

Results

1. Effect of root temperature on RNA concentrations in roots (Experiment 1)

The optimum root temperature for root growth was different between the two species; in figleaf gourd root growth increased as temperature was lowered from 26 °C; it peaked at 14 °C and declined at 12 °C. In the cucumber, growth was maximal at 26 °C and declined almost linearly to 12 °C. Maximal shoot growth occurred at 23 ° to 26 °C in both species. However, reduction of shoot growth at lower root temperatures was much smaller in figleaf gourd than in cucumber (data not indicated).

Total RNA concentration in figleaf gourd roots was lowest at 23 ° to 26 °C. It increased significantly at 18 °C, peaked at 14 °C and then dropped at 12 °C to the same level as that at 18 °C (Fig. 1). A similar trend was observed in cucumber roots, but the increase at 14 °C was much less dramatic. RNA concentration at 14 °C was 2.5 times the level it was at 23 °C in figleaf gourd, and only 1.5 times that in the cucumber. Another important difference was that RNA concentration in roots was highest at the optimal temperature for root growth in figleaf gourd roots; no such relationship was noted in cucumber roots.

Fig. 1. Relationship between total RNA concentration in figleaf gourd (▲) and cucumber (○) roots and root temperature (Experiment 1). Plants were grown at different root temperatures for 7 days. Vertical bars indicate $\pm$ S. E..

The electrophoretic analysis of total RNA in figleaf gourd roots revealed that a marked increase in total RNA at 14 °C was accompanied by an increase in all species RNA rather than an increase in any one RNA species (Fig. 2). Peaks on the electrophoregram were identified based on S values, disappearance of peaks after salting-out the RNA extracts with LiCl and the color of bands on the gels stained with Stains-all reagent (Dahlberg et al., 1969). An extract equivalent to a given root fresh weight was loaded onto the gels; thus, the peak areas are proportionate to the concentration of RNA species in the roots. A broad peak that migrated behind 25S rRNA, which consisted of RNA precursors, was highest at 14 °C. Peak D probably represents DNA contaminants because it disappeared when the extract was treated with LiCl. Furthermore, the color of the band in the gel stained with Stains-all was characteristic of DNA. Concentrations of mRNA, quantified after isolation from total RNA solutions with oligo (dT)-cellulose, were also highest at 14 °C (data not indicated).

Although the concentrations of all RNA species in figleaf gourd roots were highest at 14 °C, the rate of increases was much greater in tRNA than in rRNA and mRNA (Fig. 3). Consequently, the ratio of tRNA to total RNA rose from 4% at 23 °C to 10% at 14 °C. Concentrations of mRNA changed with root temperatures, paralleling those of rRNA; mRNA consisted of about 2% of total RNA, regardless of root temperatures.

2. Time course of changes in RNA concentrations in roots following exposure to 14 °C root temperature (Experiment 2)

RNA concentrations in figleaf gourd roots grown at 14 °C increased slightly during the first 2 days and very markedly during the next 2 days (Fig. 4). It continued to increase into the last 2 day-period with a little reduced rate. In cucumber roots, the increase in RNA concentration during the first 2 days at 14 °C was comparable to that of figleaf gourd roots. During the next 2 days, however, the increase was reduced to a half that in figleaf gourd roots; no further increase occurred during the last 2 days. No change in RNA concentration in the roots grown at 23 °C was noted during the 6-day treatment in both species. Thus, the increase of RNA concentration observed in the roots exposed to 14 °C was age-independent.

Fig. 2. Electrophoretic patterns of total RNAs in figleaf gourd roots grown at 12°, 14° and 23 °C for 7 days (Experiment 1). Upper and lower figures show the pattern on 2.4 and 5% gels, respectively. RNA extracts equivalent to a given root fresh weight was loaded on the gels. Peaks : 1; 25S rRNA, 2; 18S rRNA, 3; 5S rRNA, 4; tRNA, D; DNA.

When roots of plants were exposed to 23 °C after 6 days at 14 °C, the root RNA concentrations in figleaf gourd dropped dramatically to the pre-chill level within 2 days (Fig. 4). In contrast, no such a drop occurred in the cucumber roots. The increase in RNA concentration in figleaf gourd roots at 14 °C was accompanied by an increase in all kinds of RNA species (Figs. 5, 6). The significant increase in tRNA was linear with time, whereas, rRNA and mRNA increased with time rather parabolically. Two days after transfer of figleaf gourd roots from 14 ° to 23 °C, rRNA and mRNA concentrations dropped to the pre-chill levels but the concentration of tRNA remained higher than the pre-chill level.

Discussion

Generally, cool season plants have a lower optimal root temperature than warm season plants. In both cold- and warm-season plants, the optimum root temperature for root growth is several degrees lower than that for shoot growth, which leads to an increase in root/shoot dry weight ratios with lower root temperatures (Davidson, 1969; Cooper, 1973). In figleaf gourd, root growth is usually most rapid at 14 °C as found in the present investigation, although it is sometimes greater at 12 °C and in rare cases at higher temperatures than 14 °C (Tachibana, 1982; Bulder et al., 1991). The increased root growth at low temperature could compensate for the reduced root functions

Fig. 3. Concentrations of RNA species in figleaf gourd roots as affected by root temperature (Experiment 1). Concentrations are expressed in percent of those found at 23 °C (rRNA; 411, mRNA; 10, tRNA; 24 $\mu\text{g} \cdot \text{g}^{-1}$ fw). Ribo RNA is the sum of 25S, 18S and 5S rRNA. All standard errors are within the symbols.

such as water and nutrient uptake and cytokinin supply to shoots on the unit weight basis.

We speculate that prerequisites for the increase in root growth at low root temperatures are : the stimulation of photosynthate translocation to roots, sustained or elevated rates of photosynthate metabolism such as respiration, synthesis of protein and deposition of cell wall materials. In a number of plants, increases of protein synthesis during cold acclimation is accompanied with enhanced RNA synthesis (Tseng and Li, 1987).

In figleaf gourd roots RNA concentration increased markedly at lower temperatures with a peak at 14 °C. That the concentration dropped rapidly to the pre-chill level during readaptation to 23 °C, simultaneously with increased root RNA concentrations and root growth rates in response to root temperatures, strongly suggests that the increase in RNA concentration in figleaf gourd roots was caused by net accumulation of RNAs in re-

Fig. 4. Time course of changes in total RNA concentrations in figleaf gourd (Δ , $\blacktriangle$) and cucumber ($\circ$, $\bullet$) roots after transferring the roots from 23°C to 14°C (Experiment 2). Some plants were transferred back to 23°C after 6 days as shown by arrows. Open and closed symbols indicate that the roots were growing at 23°C and 14°C, respectively, at sampling time. Vertical bars indicate $\pm$ S. E..

sponse to chilling temperatures. Hence, RNA fluctuation is involved in the cold adaptation processes in figleaf gourd roots, similarly to those in freeze-tolerant species (Jung et al., 1967; Devay and Paldi, 1977; Sarhan and Perras, 1987). Increases in RNA precursors, as observed in figleaf gourd roots exposed to lower root temperatures, have also been observed in chilled plants of a freeze-tolerant wheat cultivar (Paldi and Devay, 1977), while Gusta and Weiser (1972) found no increases in soluble RNAs (4S and 5S RNA) in cold acclimating Korean boxwood leaves.

On the other hand, increased RNA concentration in cucumber roots exposed to 14 °C seems to be caused by a "concentration effect" rather than net accumulation, because root growth was severely inhibited at this temperature, and RNA concentration did not change 2 days after transfer of the roots from 14 °C to 23 °C. A rapid deletion of cold acclimation processes including accumulation of RNAs during readaptation to normal temperatures in cold tolerant plants has been well documented (Tseng and Li, 1987).

The mechanisms by which RNA concentration increases during adaptation to low temperature

Fig. 5. Electrophoretic patterns of total RNA in figleaf gourd roots before chilling treatment (23 °C), chilled at 14 °C for 2 and 6 days, and 2 days following transfer back to 23 °C after 6 days at 14 °C (Experiment 2). Upper and lower figures show the pattern on 2.4 and 5% gels, respectively. RNA extracts equivalent to a given root fresh weight was loaded on the gels. Peaks : 1; 25S rRNA, 2; 18S rRNA, 3; 5S rRNA, 4; tRNA, D; DNA.

stress have been investigated mainly in terms of enzyme activity involved in RNA synthesis and degradation (Gusta and Weiser, 1972; Brown and Bixby, 1973; Sarhan and Chevrier, 1985; Kritenko et al., 1985). According to Sarhan and Chevrier (1985) the increase of RNAs in cold acclimating wheat plants was mainly a consequence of enhanced RNA polymerase activity. A greater increase of rRNA synthesis at low temperature in frost-hardy than less hardy wheat cultivars has been related to higher rRNA cistron number in the hardy cultivar (Paldi and Devay, 1983). Plant hormones are also known to regulate rRNA synthesis (Chen et al., 1983; Kukina et al., 1985). We are presently attempting to elucidate the physiological basis for the increase in RNA in chilled figleaf gourd roots. In this context, it should be noted that inorganic P concentration in figleaf gourd roots remains nearly constant at low root temperature, in contrast to a large reduction in

cucumber roots (Tachibana, 1987a).

It seems quite reasonable to speculate that low temperature-induced increases of RNA concentration in figleaf gourd roots play an important role in the high chilling tolerance of the roots, probably through stimulation of protein synthesis. Increases of rRNA together with mRNA may cause increased polysome populations as well as enhanced polymerization of polysomes (Laroche and Hopkins, 1987). Increases in tRNA may contribute to increased amino acid transport to ribosomes. Besides these protein synthesizing processes, the increase of tRNA may be responsible for a marked rise in cytokinin concentration in chilled figleaf gourd roots (Tachibana, 1988), because cytokinins are the components of tRNA molecules (Skoog and Armstrong, 1970). To test whether or not the increase in mRNA involves expression of genes responsible for chill-tolerance of figleaf gourd roots poses a very interesting challenge.

Fig. 6. Time course study of changes in RNA species concentrations in figleaf gourd roots following exposure to 14 °C (Experiment 2). Concentrations are expressed in percent of those before exposure to 14 °C (day 0; rRNA; 361, mRNA; 9, tRNA; 17 $\mu\text{g} \cdot \text{g}^{-1}$ fw). Plants were transferred from 23 °C to 14 °C root temperature at day 0, and some plants were transferred back to 23 °C at day 6 as shown by arrows. Open and closed symbols indicate that the roots were growing at 23 °C and 14 °C, respectively at sampling time. Ribo RNA is the sum of 25S, 18S and 5S rRNA. All standard errors are within the symbols.

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低温耐性の異なるクロダネカボチャとキュウリの根の RNA 濃度に及ぼす低根温の影響

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摘 要

クロダネカボチャの根の高い低温耐性に RNA 代謝が関与しているかどうかを明らかにするために、12 °C ~ 26 °C の根温で 6 ~ 7 日間生育したクロダネカボチャとキュウリの根の RNA 濃度を調べた。クロダネカボチャでは、根の全 RNA 濃度は 23 °C ~ 26 °C で最も低く、根温の低下につれて増大して、根の生長が最も優れた 14 °C で最大になり、12 °C では根の生長抑制を伴って低下した。rRNA, mRNA, tRNA のいずれの濃度も全 RNA 濃度と同様に変化した。14 °C での増大程度は tRNA が最も高かった。また、14 °C 遭遇 6 日後に根

を 23 °C に移すと、根の全 RNA の濃度は 2 日以内に 23 °C でのレベルに低下した。一方、キュウリの根においても全 RNA 濃度は 14 °C で最大になったが、増大の程度はクロダネカボチャに比べて非常に小さく、根を 14 °C から 23 °C に移しても全 RNA 濃度はまったく変化しなかった。また、根の生長は低根温ほど抑制された。

以上の結果から、根温 14 °C に遭遇したクロダネカボチャの根における RNA 濃度の著しい増大は、根域の低温によって誘導された RNA 蓄積の結果であり、根の高い低温耐性に密接に関係していると考えられる。

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