根温20℃で生育したキュウリとクロダネカボチャの分離根の呼吸の低温感受性の比較

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Respiratory Response of Detached Roots to Lower Temperatures in Cucumber and Figleaf Gourd Grown at 20°C Root Temperature

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Summary

Respiratory response of detached roots to lower temperatures was compared between a chilling-sensitive species, cucumber (*Cucumis sativus* L. cv. Suyô) and a less sensitive species, figleaf gourd (*Cucurbita ficifolia* Bouchè), which were grown at a root temperature of 20°C. Respiration of figleaf gourd roots was less susceptible to low temperature than that of cucumber roots. It was mainly the cytochrome respiration that was responsible for the differential susceptibility of root respiration. Experiments with 2,4-dinitrophenol(DNP) indicated that the oxidative phosphorylation rate was higher in figleaf gourd than in cucumber roots at low temperature. These results strongly suggest that the response to low temperature of the cytochrome respiration of roots, coupled with oxidative phosphorylation, is an important aspect of differential root-chilling tolerance between cucumber and figleaf gourd.

Introduction

Root growth and many root functions are dependent on respiratory energies of the root(3). Among many factors that affect respiratory activity of roots, temperature is undoubtedly a decisive one. Root respiration is suppressed by low temperature differently with plant species(2, 6). There is a positive correlation between the chilling tolerance of plants and the respiratory activity of roots at lowered root temperatures(4, 13). Masuda and and Gomi(10) observed that figleaf gourd roots showed higher respiration rates at lower temperatures than cucumber roots. They indicated this to be one of the physiological bases of higher root-chilling tolerance of figleaf gourd. In their results, however, respiration rates of detached figleaf gourd roots at 12° and 16°C were only 23 and 32%, respectively of the rate at 24°C. On the other hand, Tachibana(14, 15) showed that growth of intact figleaf gourd roots was more active at 12～14 °C than at higher temperatures, and that their nutrient absorption rate was little effected by low root temperature. In the present invesigation, therefore, figleaf gourd was compared with cucumber for response to low temperature of the root respiration, via either the cytochrome pathway or the cyanide insensitive pathway not accompanied by oxidative phosphorylation(8).

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Meterials and Methods

Plant materials

Germinated seeds of a summer type cucumber cultivar 'Suyô' and of figleaf gourd were grown in vermiculite in a glasshouse. Seedlings with fully expanded cotyledons were pricked out to clay pots filled with small-sized gravel, which was dipped in one-half strength Hoagland No. 1 solution. When the first leaf was fully expanded, the plants were transplanted to a culture vessel in a growth room. The vessel contained 110 *l* of one-third strength Hoagland No. 1 solution. Day/night air temperatures in the room were 26/20°C. Light was provided by plant growth fluorescent lamps (Toshiba FL 40 S-BRN) for 12 h per day at an irradiance of ca 300 $\mu mol \cdot s^{-1} m^{-2}$ at plant level. Unless noted otherwise, solution temperature was maintained at 20°C.

Respiration rate measurement

Root segments of ca 10 cm from tips were detached from the plants with 7～9 leaves during the light regime, and five 1 g samples were prepared for respiration measurements.

Care was taken to select roots of similar thickness.

The direct manometric method of Warburg was used to measure oxygen consumption rates. Detached roots were immediately immersed in 5 ml of 0.04 M phosphate buffer (pH 6.8) of a desired temperature ranging from 8 to 23°C. After pre-shaking for 15 min, the roots were measured for their respiration rates for 30 min. Care was taken to immerse the roots completely in the buffer solution, since it is known that respiration rates differ significantly between aerial and underwater roots(5, 7). When the effects of cyanide and DNP on respiration rates were evaluated, KCN at 10^{-3} M or DNP at 10^{-4} M was added to buffer solution at the end of measurements without additives, and the rate was measured for another 30 min. Changes in pH of the buffer solution with these additions were less than 0.1. Different temperature regimes were run consecutively with fresh root preparations. About 6 h were required to complete the determination on a single species for a temperature series. However, a preliminary result showed no significant variations in the root respiratory activity during that period.

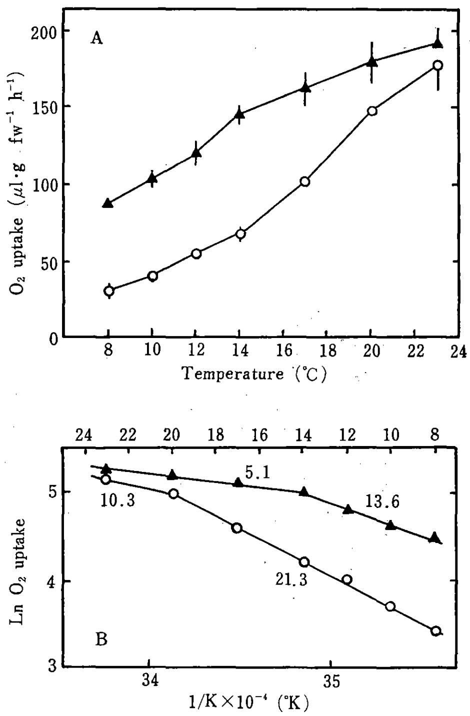

Fig. 1. A; Temperature response of respiration rates, and B; Arrhenius plots of respiration, in detached roots of cucumber (○) and figleaf gourd (▲). Numerals in B indicate activation energies in kcal·mol^{-1}. Standard errors are smaller than the symbols, unless otherwise indicated.

Results

Fig. 1 shows respiration rates of cucumber and figleaf gourd roots at various temperatures together with the Arrhenius plots of respiration. Respiration rates at 23°C did not differ significantly between two species. The rates decreased with decline of temperature in both species. However, the decreasing rate was clearly greater in cucumber than in figleaf gourd roots. As a result, figleaf gourd showed significantly higher rates than cucumber below 20°C. The temperature 'break' in the Arrhenius plots of respiration occurred at 14°C in figleaf gourd, and at 20°C in cucumber. Activation energies of respiration, equal to the slope of the corresponding straight lines, were significantly smaller in the former than in the latter below 20°C.

Respiration rates of roots at 12° and 16°C relative to 23°C were 31 and 50% in cucumber, and 63 and 85% in figleaf gourd, respectively. These values were rather inconsistent with those reported by Masuda and Gomi. They measured the rate with detached roots over 3~5 h for the lower temperature regimes. A preliminary experiment showed that detached roots began to lose their original rate after 1.5 h following root excision. Therefore, it is necessary to complete the measurement within 1 h after root excision.

Temperature response of respiration via the cytochrome and cyanide insensitive pathways was determined. The difference between the respiration rates with and without 10^{-3} M KCN was regarded as the cytochrome respiration rate. As shown in Fig. 2, the cyanide insensitive respiration rate decreased with lowering in temperatures similarly in both species. In contrast, the cytochrome respiration of figleaf gourd roots was clearly less susceptible to low temperature than that of cucumber roots. In cucumber roots cytochrome respiration rates decreased markedly toward lower temperatures, and reached nearly zero

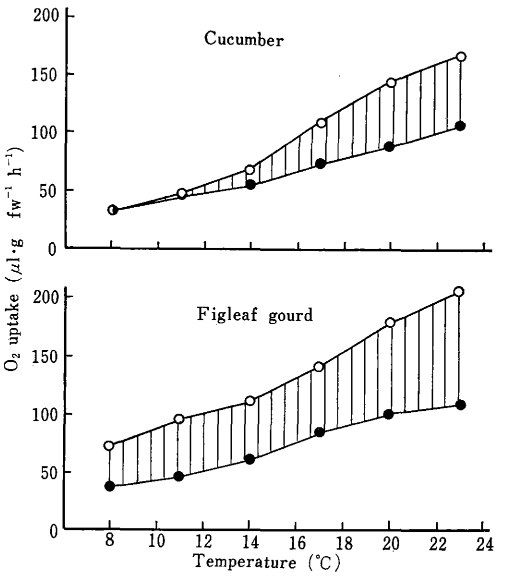

Fig. 2. Total (○), cyanide insensitive (●) and cytochrome (|||) respiration rates in detached roots of cucumber and figleaf gourd at different temperatures.

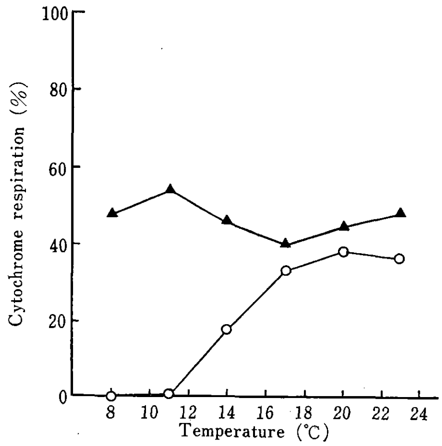

Fig. 3. Cytochrome respiration rates in percent of total respiration rates in detached roots of cucumber (○) and figleaf gourd (▲) at different temperatures.

below 11°C. However, figleaf gourd roots showed a positive rate even at the lowest temperature tested. Fig. 3 indicates that the cytochrome respiration is more susceptible to low temperature than is cyanide insensitive respiration in cucumber roots, but not in figleaf gourd roots.

The increase in respiration rates by 10^{-4} M DNP addition was used to estimate the oxidative phosphorylation rates in the roots(1, 12).

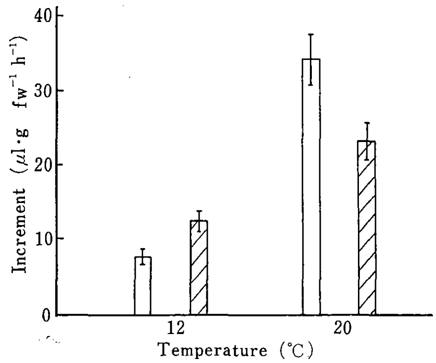

Fig. 4. Magnitude of respiratory stimulation by 10^{-4}M DNP over—DNP control in detached roots of cucumber (□) and figleaf gourd (▨) grown at 20°C root temperature. The rates were measured at 12° and 20°C.

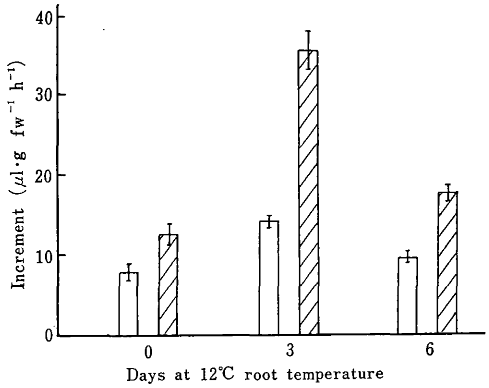

Fig. 5. Magnitude of respiratory stimulation by 10^{-4}M DNP over—DNP control in detached roots of cucumber (□) and figleaf gourd (▨) grown at 12°C root temperature for 0 (just prior to change of root temperature from 20° to 12°C), 3 and 6 days. The rates were measured at 12°C.

Respiration rates were determined at 12° and 20°C and the results were summarized in Fig. 4. Magnitude of respiratory stimulation by DNP was greater in cucumber than figleaf gourd roots when the rate was measured at 20°C, while the reverse was the case at 12°C. In another experiment, temperature of the rooting medium of the plants was changed from 20° to 12°C, and respiration rates with and without DNP were measured with root samples prepared at 0 (just prior to temperature change), 3 and 6 days after the change in the medium temperature. As shown in Fig. 5, a marked increase in DNP stimulation was observed in figleaf gourd roots 3 days after the temperature change. This trend was less marked in cucumber roots.

Discussion

It was clearly demonstrated in the present investigation that the respiration of figleaf gourd roots was far less susceptible to lower temperatures than that of cucumber roots. It was mainly the cytochrome respiration coupled with oxidative phophorylation that was responsible for the differential chilling susceptibility of root respiration. Experiments with DNP seem to offer additional evidence that the oxidative phosphorylation rate is higher in figleaf gourd than in cucumber roots at low temperatures.

These results are consistent with the findings by Leopold and Musgrave(9). They found that chilling temperature predominantly limited respiration via the cytochrome pathway in soybean. Also Yoshida and Tagawa(17) showed the cytochrome respiration of calli from chill-sensitive *Cornus stronifera* to be more susceptible to low temperature than that from chill-resistant *Sambucus Sieboldiana*.

Occurrence of temperature 'break' in the Arrhenius plots of biological phenomena is believed to be indicative of the phase change in the membrane lipids from liquid-crystalline to gel state(11). A previous study showed that the degree of fatty acid unsaturation of phospholipids was higher in figleaf gourd than in cucumber roots when they were subjected to low temperature(16). Whether the lower 'break' temperature for root respiration in figleaf gourd, than in cucumber, is due to a higher degree of fatty acid unsaturation of mitochondrial membrane lipids needs further investigation.

A most likely suggestion given by the present data is that a physiological basis for the different root-chilling tolerance between cucumber and figleaf gourd is based on the differential susceptibility of roots to low temperature in the cytochrome respiration, coupled with oxidative phosphorylation. Further study with plants grown at low root temperature is necessary to comfirm the above suggestion.

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根温 20°C で生育したキュウリとクロダネカボチャの分離根の呼吸の低温感受性の比較

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摘　要

低根温耐性の異なるキュウリ（品種；四葉）とクロダネカボチャの根の呼吸系の低温感受性を比較するために，根温20℃で生育した植物体から分離根を調製し，それらの呼吸の温度反応をワールブルグ検圧法によって調べた．クロダネカボチャの根はキュウリの根に比べて低温による呼吸抑制の程度が小さく，この違いは主としてチトクロム系呼吸鎖の低温感受性の違いによることが明らかとなった．また，根の酸化的リン酸化反応は低温下ではクロダネカボチャがキュウリより旺盛であることが示唆された．これらの結果から，キュウリとクロダネカボチャの低根温耐性の違いには，酸化的リン酸化反応と共役したチトクロム系呼吸鎖の低温感受性の違いが関与していると推察される．