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Differential impact of low temperature on fatty acid unsaturation and lipoxygenase activity in figleaf gourd and cucumber roots ☆

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Abstract

Previous studies show that low temperature strongly induces suberin layers in the roots of chilling-sensitive cucumber plants, while in contrast, low temperature produces a much weaker induction of suberin layers in the roots of the chilling-tolerant figleaf gourd [S.H. Lee, G.C. Chung, S. Steudle, Gating of aquaporins by low temperature in roots of chilling-sensitive cucumber and -tolerant figleaf gourd, J. Exp. Bot. 56 (2005) 985–995; S.H. Lee, G.C. Chung, E. Steudle, Low temperature and mechanical stresses differently gate aquaporins of root cortical cells of chilling-sensitive cucumber and figleaf gourd, Plant Cell Environ. (2005) in press; S.J. Ahn, Y.J. Im, G.C. Chung, B.H. Cho, S.R. Suh, Physiological responses of grafted-cucumber leaves and rootstock roots affected by low root temperature, Scientia Hort. 81 (1999) 397–408]. Here, the effect of low temperature on fatty acid unsaturation and lipoxygenase activity was examined in cucumber and figleaf gourd. The double bond index demonstrated that membrane lipid unsaturation shows hyperbolic saturation curve in figleaf gourd roots while a biphasic response in cucumber roots to low temperature. In figleaf gourd, the hyperbolic response in the double bond index was primarily due to accumulation of linolenic acid. Chilling stress also significantly induced lipoxygenase activity in figleaf gourd roots. These results suggest that the degree of unsaturation of root plasma membrane lipids correlates positively with chilling-tolerance. Therefore, studies that compare the effects of chilling on cucumber and figleaf gourd may provide broad insight into stress response mechanisms in chilling-sensitive and chilling-tolerant plants. Furthermore, these studies may provide important information regarding the relationship between lipid unsaturation and lipoxygenase function/activity, and between lipoxygenase activity and water channeling during the response to chilling stress. The possible roles of these processes in chilling tolerance are discussed.

Keywords: Chilling stress; Cucumber; Double bond index; Figleaf gourd; Lipoxygenase

Low temperature is the major factor limiting the growth of warm-season plants. One of the adverse effects of low temperature on plants is altered functional properties of cellular membranes in plant leaves and/or roots. At the cellular and molecular level, low temperature can have a severe impact on membrane fluidity, which also affects metabolic rate and protein turnover [1–3]. Previous studies showed that low temperature has differential effects on the lipid composition of membranes in chilling-sensitive and chilling-tolerant plants. For example, the proportion of unsaturated fatty acids tends to increase in the thylakoid membrane, mitochondrial membrane, and plasma membrane during low temperature stress [3–5]. Chilling stress often causes peroxidation of membrane lipids, a type of membrane damage that is also caused by oxidative stress.

☆ *Abbreviations:* DBI, double bond index; EL, electrolyte leakage; LOX, lipoxygenase; GC, gas chromatography; SDS, sodium dodecylsulfate.

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Peroxidation of unsaturated lipids has been mentioned as a possible cause of increased membrane rigidity in tropical and sub-tropical plants exposed to low temperature stress [6,7]. Lipoxygenase (LOX, EC 1.13.11.12) is thought to play a primary role in generating peroxidative damage in membrane lipids in common bean [6,8], and reducing sugar that accumulates in potato tubers stored at low temperature may also alter membrane permeability [9].

Another factor in low temperature stress could be the development of hydraulic resistance. For example, cold-induced hydraulic resistance could lead to water imbalance in new shoots in chilling-sensitive plants [7]. Major parameters that affect root hydraulics are anatomy, water flow across the root cylinder, and the structure and activity of water channels in root cells. The activity of water channels in the plasma membrane of root cells affects cell-to-cell water flow, and it has been suggested that gating of water channel activity plays an important role in determining root hydraulic resistance [10,11]. Since lipid peroxidation, water channel activity, and membrane fluidity are very sensitive to changes in temperature and the composition of the plasma membrane, the level of saturation of plasma membrane fatty acids is a crucial determinant of low temperature stress. Several reports indicate that increased unsaturation of membrane fatty acids correlates with chilling-tolerance [4,6,9]. However, these results are controversial and additional data on the mechanisms by which low temperature could alter membrane lipid composition are needed.

Cucurbita is a species that grows particularly well at relatively low temperatures. Thus, *Cucurbita* have been widely used as rootstocks for *Cucumis*, allowing for successful production of cucumbers during the cold season [12]. Earlier studies suggest that a comparative study of the chilling-induced physiological response of cucumber (*Cucumis sativus* L.) and figleaf gourd (*Cucurbita ficifolia* Bouche) could provide significant insight into mechanisms of chilling-resistance [7,12,13]. Because cucumber and figleaf gourd may represent typical chilling-sensitive and chilling-tolerant species, respectively, this could provide an ideal model system for studying the chilling-sensitivity and chilling-tolerance. The present study analyzes chilling-induced changes in fatty acid composition and lipoxygenase activity in the roots of chilling-sensitive (cucumber) and chilling-tolerant (figleaf gourd) species. Membrane fluidity was measured using the double bond index (DBI). The results are interpreted with respect to the possible involvement of lipoxygenase in mechanisms of chilling-tolerance.

Materials and methods

Plant materials and growth conditions. Seeds of cucumber (*C. sativus* L. cv. Chung-Jang, Heung-Nong Seed, Korea) and figleaf gourd (*Cu. ficifolia* Bouche, Sakada Seed, Japan) were germinated for 2–3 days at 23 °C in the dark on filter paper soaked with tap water. After germination, seedlings were transferred to containers (5 L with 8 seedlings per container) with aerated 1/5 strength Cooper solution [14] in a climate chamber (day/night cycle: 12/12 h; temperature: 23/21 °C; and light intensity: 300 μmol m^{-2} s^{-1} PAR). The nutrient solution was replaced frequently to avoid excessive depletion of nutrients. Cucumber and figleaf gourd seedlings used in the experiment were 14–20 and 7–14 days, respectively, including the time for germination. At these ages, root systems of cucumber and figleaf gourd were comparable in size (length of root systems: cucumber, 450–500 mm; figleaf gourd, 400–500 mm). A cooler was used for low root temperature conditions of 6 °C for 7 days. A climate chamber was used when necessary.

Fatty acid analysis. Total lipids were extracted from plasma membranes of root systems according to Ryyppö et al. [15]. Roots were ground into a powder in liquid nitrogen and the powder was mixed with isopropanol. Then methanol, chloroform, and butylated hydroxytoluene (5 mg mL^{-1} chloroform) were added and the suspension was mixed for 90 min at 4 °C. After removal of water-soluble contaminants by Folch wash, total lipids were fractionated into neutral lipids and phospholipids by silica gel chromatography. The phospholipids were converted to methyl esters by adding 0.6 M methanolic sodium hydroxide and neutralized with 0.6 M hydrochloric acid. The methyl esters of fatty acids were extracted twice with hexane and separated on a gas chromatograph (GC-17B, Shimadzu) equipped with a Rtx-1 column (Resteck) and a flame ionization detector (Shimadzu). Initially, column temperature was maintained at 145 °C for 2 min and then subjected to a step gradient of 145–160 °C 5 °C min^{-1}, 160 °C for 1 min, 160–170 °C 2 °C min^{-1}, and 170 °C for 90 min. The injector and detector temperatures were 225 and 275 °C, respectively. DBI was calculated according to the formula of Wismer et al. [16]: $DBI = [(2 \times \%C18{:}2) + (3 \times \%C18{:}3)]/[(\%C16{:}0) + (\%C18{:}0) + (\%C18{:}1)]$.

Northern blot analysis. Total cellular RNA was extracted and Northern blot analysis was conducted according to standard procedures. Northern blot hybridization was performed for 20 h at 68 °C in prehybridization solution supplemented with 10% dextran sulfate. The LOX cDNA from *Zea mays* (GenBank Accession No. AF271894) was labeled with [γ^{32}P]dCTP (Amersham pB10205) by nick translation (BRL, 9160SB), and *Bam*HI and *Kpn*I fragments of the LOX cDNA were used as a probe [17]. The membrane was washed twice at room temperature in 0.1× SSC, 0.1%(w/v) SDS for 10 min and then once at 42 °C in 0.1× SSC, 0.1%(w/v) SDS for 30 min. The Northern blot was visualized using a BAS 1500 image system (Fuji Photo Film, Tokyo, Japan).

Lipoxygenase assay. Roots of cucumber and figleaf gourd were frozen in liquid nitrogen and pulverized using mortar and a pestle. The powdered sample was suspended in 50 mM potassium phosphate, pH 6.0, centrifuged at 12,000*g* for 10 min, and the supernatant was removed for activity assay. Linolenic acid or linoleic acid were used as substrates. Substrate solution was prepared as described previously [18]. The reaction was started by mixing the substrate solution with enzyme, stopped by adding ethyl alcohol to aliquots of the reaction mixture at 1, 4, 5, 6, and 7 min, and the mixture was diluted with 60% ethyl alcohol. The absorbance was measured at 234 nm.

Results

Chilling-induced change in plasma membrane DBI in figleaf gourd and cucumber roots

We previously reported that low temperature strongly induces suberin layers in the endodermis of cucumber roots, but it induces much weaker suberization in figleaf gourd roots [10,11]. Here, the effect of