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# Plasma membrane $H^+$ efflux and aquaporin in cucumber and figleaf gourd root differing in tolerance to low temperature

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## Objectives

The susceptibility or tolerance of plant to low temperature may well be related to the $H^+$-ATPase activty ($H^+$ efflux) and capability of the root system to transport water at low temperature since low temperature-sensitive species often exhibit water-stress symptoms described as low temperature-induced water stress. Therefore, we compare that the $H^+$ efflux across the PM and induction of plasma membrane (PM) quaporins in cucumber and figleaf gourd roots by low temperature.

## Materials and Methods

Seeds of cucumber and figleaf gourd were germinated and transferred to containers with aerated 1/5 strength HS in a growth chamber. A cold chamber was used for low temperature conditions of 4±2°C for 5 d. To visualize acidification along single intact roots by low temperature, a modified agarose plate technique was used. The pH values were obtained by comparing a standard colored scale in same plate but without plants (range of color pH scale:4.0 to 6.5 with intervals of 0.5 pH unit). Each experiment was carried at least three times with four replicates. The PM aquaporin was detected using standard western blot protocol with an antibody after determination of protein content.

## Results and Discussion

In present work shows that acidification, the $H^+$ efflux across PM, along the root surface was detected by observing color change from purple to yellow in normal condition (22°C). The strong pH decrease (from pH 6.0 to 4.0) was visible along the root surface both of cucumber and figleaf gourd by the time (Fig. 1A,B,C,D). In low temperature-treated roots, acidification was detectable just in figleaf gourd root (Fig. 1E,F,G,H). When both cucumber and figleaf gourd transferred to normal condition, pH decrease was visible along the root surface in both of plants by the time (Fig. 1I,J,K). In addition, we have conducted Western blotting of aquaporins(PIP-type) using broad detection of aquaporins in PM. The level of PM aquaporin proteins slightly decreased in cucumber as the exposure time to low temperature prolonged up to day 6 but increased in figleaf gourd from day 1 (Fig. 2A and B). This results indicates that the response of the $H^+$ efflux across PM and aquaporin expression to low temperature have differences between two species.

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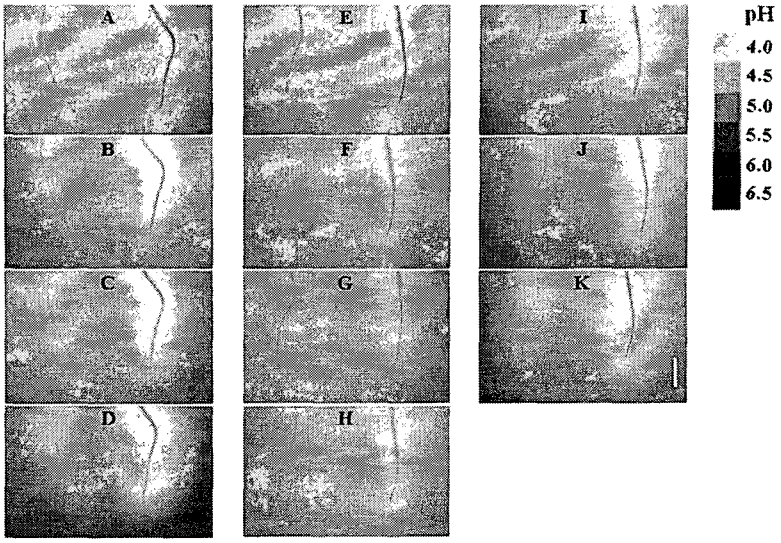


Fig. 1. Time-course effect of low temperature on shift of surface pH in cucumber and figleaf gourd roots. Seedlings were grown up to normal condition for 3h(A), 6h(B), 12h(C) and 24h(D) and to low temperature for 3h(E), 6h(F), 12h(G) and 24h(H) and then transferred to normal condition again for 3h(I), 6h(J), 12h(K). Images from a representative replicate are shown. Bar represents 10 mm.

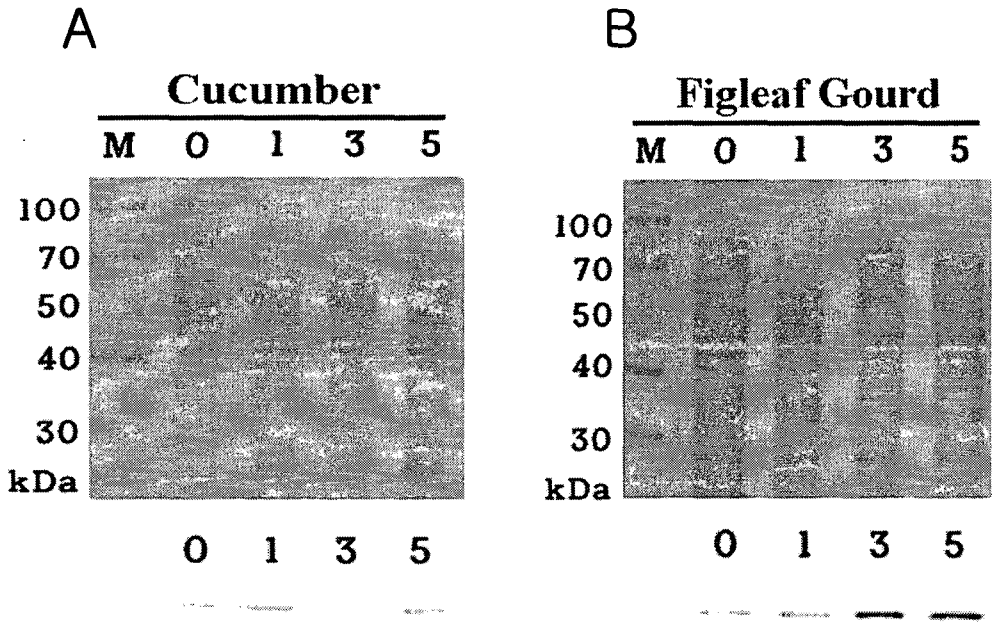


Fig. 2. Coomassie blue-stained SDS-PAGE profile of PM proteins isolated from cucumber and figleaf gourd roots exposed to low temperature for 0, 1, 3, 5 days and western blot analysis of PM aquaporin using an antibody.