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The Function of the ABA-induced Thaumatin-like Protein in Winter Wheat Cells

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WAS-3a cDNA was isolated from ABA-treated cells of winter wheat (*Triticum aestivum* L. cv. Chihokukomugi). *WAS-3a* was homologous to thaumatin-like proteins (TLPs) belonging to a class of pathogenesis related proteins which were known to inhibit hyphal growth of various fungi. *WAS-3a* transcript was induced by the treatment with ABA and snow mold-derived elicitor in the cultured cells and induced by cold-hardening in seedlings. In this study, to characterize the function of *WAS-3a*, we introduced *WAS-3a* cDNA into suspension-cultured HY-1 cells derived from spring wheat (*T. aestivum* L. cv. Haruyutaka). The overexpressed *WAS-3a* protein from HY-1 transformants was purified by cation-exchange chromatography. The purified *WAS-3a* showed an antifungal activity against phytopathogenic fungi. These results suggest that ABA-induced *WAS-3a* is involved in the defense response against pathogens in winter season.

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p- COUMARIC ACID FROM WHEAT INACTIVATES ICE-NUCLEATING ACTIVITY BY ICE-NUCLEATING BACTERIUM

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Recently apoplast is noted as an intermediate region between outer environments and plant cell. We research antifreeze substances including antifreeze proteins (AFPs) from wheat apoplast fraction.

We found that phenolic compounds, especially *p*- coumaric acid, in apoplast fraction increased during cold treatment. Freezing temperature (*T_f*) of *p*- coumaric acid solution by measurement including supercooling was -3.5°C lower than control buffer. However the *T_f* by measurement without supercooling was equal to control buffer's one. *p*- Coumaric acid clearly inactivated ice-nucleating activity by ice-nucleating bacterium. And *p*- coumaric acid did not inactivate ice-nucleating activity by Agl. Ice crystals of *p*- coumaric acid solution was not bi-pyramidal shape such as AFPs solution. Thus, it is possible that *p*- coumaric acid regulates freezing of water in a different mechanism from AFPs' and specifically inactivates ice-nucleating activity by ice-nucleating bacteria.

p- Coumaric acid is an important intermediate on plant secondary metabolism. We studied whether metabolic derivatives inactivate ice-nucleating activity. Ferulic acid and caffeic acid inactivated ice-nucleating activity, while tyrosine did not. Interestingly inactivation of ice-nucleating activity of *o*- coumaric acid, that is an isomer of *p*- coumaric acid, was half than other phenolic derivatives'.

As our results, it is possible that phenolic compounds acclimated apoplast regulate freezing of apoplast by ice-nucleating bacteria etc. We expect that a role of phenolic compounds gives us a new viewpoint for cold adaptation of plants.