MPRINT Research Thrusts

Potential Use of Cysteine Proteinase Inhibitors in Creating Insect Resistance in Plants

While studying the nutritive value of legume proteins, Suzanne Nielsen questioned how gut proteases of the bean weevil are able to degrade and utilize undenatured bean proteins. These proteins are resistant to mammalian digestive protease and so are nutritionally unavailable to humans. From MPRINT colleague Larry Murdock, she learned that some insects utilize a digestive cysteine proteinase (unlike the serine proteinases used by humans).

Nielsen's research group isolated and characterized a cysteine proteinase (CP) from the gut of the bean weevil and compared its ability to degrade bean protein to that of human digestive proteases. In collaboration with Larry Murdock and MPRINT's Dick Shade, Nielsen's group showed that inhibiting the gut CP prolonged developmental time and increased mortality of the bean weevils feeding on seeds. This led to studying digestive CPs of certain insects as a vulnerable site that could be exploited as a means to control such insects. Nielsen's research group screened legume seeds for cysteine proteinase inhibitor (CPI) activity, and a CPI from soybean seeds was isolated and characterized.

She then worked with MPRINT colleagues Ray Bressan and Mike Hasegawa, who cloned the gene from soybean CPI. The bacterial recombinant proteins obtained from the expression of two soybean CPI cDNAs substantially inhibit papain and the gut proteases of three insects that utilize digestive CPs: Western corn rootworm, Colorado potato beetle, and cowpea weevil. In vivo studies done with the help of Dick Shade have shown that these recombinant soybean CPIs effectively inhibit growth and development of these three insects. These studies led by Nielsen have provided information on the biochemical interactions of CPs and CPIs, and the potential of using CPIs as sources of insect resistance.

Osuala, C. I., R. L. Donner, and S. S. Nielsen. (1994) Partial purification and characterization of an aminopeptidase from the bean weevil larvae *Acanthoscelides obtectus* Say (Coleoptera, Bruchidae). Comp. Biochem. Physiol. 107B:241-248.

Hines, M. E., C. I. Osuala, and S. S. Nielsen. (1992) Screening for cysteine proteinase inhibitor activity in legume seeds. J. Sci. Food. Agric. 59:555-557.

Hines, M. E., C. I. Osuala, and S. S. Nielsen. (1991) Isolation and partial characterization of a soybean cystatin cysteine proteinase inhibitor of Coleopteran digestive proteolytic activity. J. Agric. Food Chem. 39:1515-1520.

Hines, M. E., S. S. Nieslen, R. E. Shade, and M. A. Pomeroy. (1990) The effect of two proteinase inhibitors, E-64 and Bowman-Birk trypsin inhibitor, on the developmental time and mortality of *Acanthoscelides obtectus* (Say). Entomol. Exp. Appl. 57:201-207.

Wieman, K. F. and S. S. Nielsen. (1988) Isolation and partial characterization of a major gut proteinase from larval *Acanthoscelides obtectus* Say (Coleoptera: Bruchidea). Comp. Biochem. Physiol. 89B:419-426.