E2 22

Production of antibody and histochemical analysis of Nepenthes major Acid proteinase

Previously reported results clearly demonstrated the salient properties of *Nepenthes* acid proteinase and suggesting that they are novel members of the aspartic proteinase family. Therefore, it is very important to study exact tissue localization of these two proteinases for messenger RNA isolation, cDNA cloning and structure elucidation.

Preliminary investigations reported last year suggested that acid proteinases are secreted from the tissues of the lower part of the pitcher. In this study antibody of *Nepenthes* major acid proteinase was produced and histochemical staining was performed on transverse sections obtained under freezing conditions.

Immunochemical staining of dot blotted *Nepenthes* major acid proteinase with serum collected before primary inoculation after first and second booster suggests that rabbits produce a specific antibody against the *Nepenthes* proteinase. Results clearly showed that 1:4000 diluted serum can be used to detect 0.05 µg of *Nepenthes* major acid proteinase. Further immunochemical staining of blotted Nepenthes minor acid proteinase and other standard proteins with 1:1000 diluted serum did not produce any protein and Nepenthes minor acid proteinase IgG preparation is 2.5 mg/ mL and immunochemical staining of dot blotted Nepenthes major acid proteinase suggest that 1:8000 diluted purified antibody preparation can be used to detect 0.01 µg of protein.

Nepenthes pitchers were divided into 4 parts [1 and 2 in bottom 1/3 glandular region and 3 and 4 in upper 2/3 non glandular region]. Transverse tissue sections of each part were subjected to immunochemical staining with 1:8000 diluted antibody preparation. A blue coloration with alkaline phosphotase or brown colour with peroxidase substrate was observed only in the transverse sections of the lower 1/3 part of the pitcher. Microscopic examinations of stained slides suggest that most of the parenchyma cells which are present as clusters and located near the inner surface of the pitcher had been stained with antibody.

This result suggests that the major Nepenthes acid proteinase is synthesized in parenchyma cells located in the lower 1/3 part of the pitcher.