

Soluble protein changes in cucumber plants: their characterisation and involvement with induced resistance, following hypersensitive reactions and/or stress responses

R.H.A. COUTTS (Department of Pure and Applied Biology, Imperial College, Prince Consort Road, London SW7 2BB, United Kingdom)

Leaves and cotyledons from several cultivars of cucumber (*Cucumis sativus* L.) have been used to study the alterations occurring in the soluble protein fraction following tobacco necrosis virus (TNV)-elicited necrosis, *Colletotrichum lagenarium* fungus-elicited necrosis, tobacco mosaic virus infection, a period of mannitol-induced osmotic stress, or polyacrylic acid (PA) treatment. Following the various treatments, one apparently novel fraction (γ) was identified among the soluble proteins of all cultivars investigated by discontinuous polyacrylamide gel electrophoresis (disc PAGE) and gel staining. An additional novel fraction of lower mol. wt (γ_A) was identified in the cv. Explorer. Smaller amounts of the γ fraction were also present in apparently healthy tissue adjacent to the necrotic halves of cotyledons (AH tissue) but were absent from any other leaves on these plants. The γ fraction is probably representative of at least three different proteins, two of which are also constituents of control tissue (a glycoprotein and possibly one or two RNase isozymes), whereas the third is a protein of unknown nature, present as the bulk of the fraction and possibly synthesised de novo. The γ fraction does not contain peroxidase or polyphenoloxidase isozymes.

Following the radioactive labelling of AH tissue, it would appear that an increased protein synthesis, which is sensitive to the effects of translational inhibitors, may result in the production of the γ fraction, but this is speculative. The major component of the fraction gave a single band on SDS-PAGE, with a mol. wt of ca. 22 000, and a single band at pI 4.1 on isoelectric focussing. While it is thought that the bulk of the novel portion of the γ fraction is represented in these gel systems, increased amounts of the constitutive glycoprotein component should also be taken into consideration.

Soluble extracts of protoplasts isolated from AH tissue retained only constitutional RNase isozyme(s), and were totally devoid of the rest of the γ fraction. This result suggests that the other component(s) of the fraction is (are) subcellularly located in the plasmodesmata, between the cell wall and plasma membrane or non-covalently attached to either.

Induced resistance to homologous challenges with either TNV or *C. lagenarium* was demonstrated in the upper leaves of cucumber plants, following hypersensitive necrosis on both cotyledons. A similar resistance developed in AH tissue and PA-sprayed cotyledons using TNV as the challenger. In all instances of induced resistance to TNV, the resistance was against symptom expression rather than virus multiplication. Tissue exhibiting resistance contained either small or undetectable (by PAGE) amounts of the γ fraction suggesting that its induction may only be involved with an overall stress response of cucumber, with no obvious role(s) in resistance.