

## **Protease inhibitors defense plant: The analysis of process induction and activation**

### ***Abstract***

Trypsin proteinase inhibitors (TPIs) of *Nicotiana attenuata* are major anti-herbivore defenses that increase dramatically in leaves after attack or methyl jasmonate (MeJA) elicitation. To understand the elicitation process, we characterized the proteolytic fragmentation and release of TPIs from a multidomain precursor by proteases in MeJA-elicited and unelicited plants. A set of ~6 kD TPI peptides were purified from leaves and their posttranslational modifications were characterized. The presence of single-chain and two-chain TPIs suggested a circular TPI precursor. In MeJA-elicited plants, the diversity of TPI structures was greater than that predicted to be encoded by the precursor gene. This elicited structural heterogeneity resulted from differential fragmentation of a linker peptide (LP) that separates the 7-TPI functional domains. With an *in vitro* FRET assay and synthetic substrates derived from the LP sequence, we characterized proteases involved in both the processing of the TPI precursor and its vacuolar targeting sequence.