

Arabidopsis thaliana phytaspase: identification and peculiar properties

ABSTRACT

Phytaspases are plant cell death-related proteases of the subtilisin-like protease family that possess an unusual aspartate cleavage specificity. Although phytaspase activity is widespread in plants, phytaspase of *Arabidopsis thaliana* (L.) Heynh. has escaped detection and identification thus far. Here, we show that a single gene (At4 g10540) out of 56 *A. thaliana* subtilisin-like protease genes encodes a phytaspase. The recombinant phytaspase was overproduced in *Nicotiana benthamiana* Domin leaves, isolated, and its substrate specificity and properties were characterised. At pH 5.5, at physiological mildly acidic reaction conditions, the *Arabidopsis* phytaspase was shown to be strictly Asp-specific. The strongly preferred cleavage motifs of the enzyme out of a panel of synthetic peptide substrates were YVAD and IETD, while the VEID-based substrate preferred by the tobacco and rice phytaspases was almost completely resistant to hydrolysis. At neutral pH, however, the *Arabidopsis* phytaspase could hydrolyse peptide substrates after two additional amino acid residues, His and Phe, in addition to Asp. This observation may indicate that the repertoire of *Arabidopsis* phytaspase targets could possibly be regulated by the conditions of the cellular environment. Similar to tobacco and rice phytaspases, the *Arabidopsis* enzyme was shown to accumulate in the apoplast of epidermal leaf cells. However, in stomatal cells *Arabidopsis* phytaspase was observed inside the cells, possibly co-localising with vacuole. Our study thus demonstrates that the *Arabidopsis* phytaspase possesses both important similarities with and distinctions from the already known phytaspases, and is likely to be the most divergent member of the phytaspase family.

Keyword: Apoplast; Aspartate specificity; Proteolysis; Subtilisin-like protease