The isolation and expression analysis of a class I chitinase from developing winged bean seed (Psophocarpus tetragonolubus)

ABSTRACT

Chitinase catalyses the hydrolysis of ϐ-1,4-N-acetyl-D-glucosamine linkages of the fungal cell wall polymer chitin and is involved in the inducible defenses of plants. The aim of this research was to isolate and clone chitinase cDNAs from the seed of winged bean. Chitinase gene fragments were isolated from a winged bean seed cDNA library using two sets of degenerate primers corresponding to the conserved regions of chitinase class I and IV. The poly A+ mRNA was reversed transcribed and further amplified using RT-PCR. A 1.1 Kb fragment was selected, cloned and sequenced. A nucleotide sequence comparison identified the fragment as a Class I basic chitinase cDNA; this fragment was subsequently used as a probe to screen for a full length transcript from the cDNA library. Library screening resulted in the isolation of a 1324 bp clone designated CHRZP; encoding a polypeptide of 289 amino acids containing the diagnostic N-terminal cysteine-rich domain of class 1 chitinases. CHRZP showed 47% similarity to a chitinase sequence from Rice and to another class I winged bean chitinase (ChitiWb1) at the amino acid level. RNA blot hybridization revealed that CHRZP mRNA accumulates to the highest level in leaves followed by tubers and pods. Southern hybridization analysis indicated that this gene is likely to be present as a single copy in the winged bean genome.

Keyword: Winged bean; Chitinase; Pathogen; Pathogenesis response