CLONING AND CHARACTERISATION OF GENE CODING FOR CHITINASE IN DEVELOPING WINGED BEAN SEED

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Introduction

Improvement of crop plants is an ongoing effort of human kind beginning from the domestication of wild plant species for food and fodder until today. Ever since the early days, convention plant breeding has been the method of choice for crop improvement. Recent advances in genetic engineering offers an alternative method of crop improvement that is faster, more precise and most importantly allow exchange of genetic materials between distant species. In an effort to engineer crop plants with increased resistance to disease, a study was undertaken to isolate novel disease resistance gene from winged bean seed, which is known for its high resistance to many diseases. The objective of the study was to clone and characterise novel gene from winged bean. The isolated gene will in turn be transferred to other crop plants in an attempt to increase its ability to resist pathogen infection.

Materials and Methods

cDNA library was constructed from mRNA isolated from 4-week-old developing winged bean seeds and the quality of the constructed library was assessed. Sets of PCR primers were designed using WebANGIS Bioinformatics Tools based on the available chitinase gene sequences from other plants. Total RNA isolated from developing seeds was used as RT-PCR template and the resulting amplified product was used as probe for the screening of the library. Putative clones were identified and subsequently characterised. Southern and Nothern blot analyses were carried out on the DNA and RNA samples respectively to determine copy number, gene family, and temporal as well as spatial expression patterns of the isolated gene. The best candidate clone carrying chitinase gene was then subjected to DNA sequencing and subsequently compared to the available databases for similarity.

Results and Discussion

Assessment of the quality of the constructed cDNA library revealed that the estimated number of independent clones were greater than 10^6 pfu/ug indicating a representative library of the developing seeds. After analysing a number of clones randomly selected from the library, it was determined that the percentage of recombinant was 98% with the average size more than 1.0 kb. Reverse transcriptase polymerase chain reaction was done in order to get a chitinase fragment from seed of winged bean and this fragment was used as a probe to screen a cDNA library. A chitinase gene was obtained after screening 250,000 plaques by using a plaque lifts method. Southern blot analysis was carried out by using the isolated fragment from chitinase containing clone as a probe. After low and high stringency wash, only a single single band was detected indicating the gene is a single copy gene family in a genome of winged bean. Northern blot analysis was also undertaken by using this probe to determine the expression level of this gene in various tissues of winged bean. Compared to the other parts of winged bean leaves give higher expression with the transcript size of 1.0 kb. The expression level was non detectable at 1, 2 and 3 week-old seed. The expression level of this gene was higher in 6 weeks seeds compared to 4 and 5 weeks seeds with the same transcripts size in leaves. No expression level was detected in root and stem. DNA sequencing was carried out on the positive clone and the complete sequence of the chitinase gene was determined to be 1350 bp in length. Upon homology search analysis which compared the sequence obtained with published sequences in GenBank databases, it was found that the isolated chitinase gene was 86% homologous to *Oryza sativa* chitinase, 78% homologous to *Hordeum vulgaris* chitinase and 76% homologous to *Phaseolus vulgaris* chitinase.

Conclusions

Chitinase gene from developing winged bean seed was successfully cloned and characterised and was shown to be highly homologous to chitinase genes from *Oryza sativa*, *Hordeum vulgaris* and *Phaseolus vulgaris*. The gene is a single copy gene with 1350bp in length and expressed in seed, leaf and tuber tissues but not in stem and root tissues.

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