INTRODUCTION
CHAPTER 1

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Plant diseases are one of the major concerns to agricultural production. It has been estimated that total loss as a consequence of plant diseases reaches 25% of the yield in western countries and almost 50% in developing countries. Of this, one third yield loss is due to fungal infections (Bowyer, 1999). There is a pressing need to control fungal diseases to ensure a steady and constant food supply to feed the ever increasing population of the world. Conventional practice to control fungal diseases has been the use of chemical fungicides which have adverse environmental effects causing health hazards to humans and other non-target organisms, including beneficial life forms. Due to the increasing concern towards the toxicity and biomagnification potential of chemical fungicides in agriculture, current practices based on molecular biology techniques which involve the development of transgenic resistance in crop plants merit attention (Schickler and Chet, 1997). In response to developmental signals and pathogen attack plants evolve a number of defense responses (Bell, 1981) and synthesize an array of proteins and peptides such as chitinase, glucanase, α-defensins, lipid transfer proteins, thionins, hevein- and knottin-type peptides, cyclopeptides, alkaloids and other unique peptide groups as part of their natural defense systems (Broekaert et al., 1997; Yun et al., 1997; Somssich and Halbrock, 1998).

Efforts have been made for isolation and characterization of pathogenesis-related (PR) proteins (Carmona et al., 1993; Terras et al., 1995; Molina and Garcia-Olmedo, 1997; Datta et al., 1999). It is reported that not all the PR-proteins are
antimicrobial and therefore, *in vitro* screening of proteins for their antimicrobial potential is important to use them for developing transgenic resistance in crop plants.

Selectable markers such as phosphinothricin acetyl transferase (*bar*), dihydrofolate reductase (*dhfr*), hygromycin phosphotransferase (*hph*), neomycin phosphotransferase (*nptII*), glyphosate-tolerant CP4 and GOX genes have been used in plant transformation work for screening of transgenic plants. Each of these selectable markers has its particular advantages and disadvantages. The use of herbicide resistance genes including *bar*, CP4 and GOX concerns the outcrossing through sexual transmission to wild relatives producing herbicide-resistant weeds (Vasil, 1994; Arriola and Ellstrand, 1996). In comparison to other selectable markers, cyanamide hydratase (*cah*) gene is being considered as a unique marker because of its ability to convert calcium cyanamide into urea, a useful nitrogen compound, which can be considered as a fertilizer source to plants (Maier-Greiner et al., 1991a; Sahrawat et al., 2003).

Recombinant DNA technology has paved the way for obtaining and combining genes from a variety of sources and the possibility of expressing genes in different host cells. *Escherichia coli* has been successfully used for the production of relatively complex proteins in large quantities (Rudolph, 1996). Considering the number of proteins produced during biological stress in plants, only few of them have been characterized and utilized for developing transgenic resistance against fungal pathogens.
Present investigation was aimed for construction of plant transformation cassettes and heterologous expression of cyanamide hydratase \((cah)\) marker gene and antifungal genes encoding chitinase and lipid transfer protein (LTP).

Objectives of the present study have been formulated as follows:

1. Cloning and expression of chitinase and LTP genes in \(E.\ coli\) and evaluation of their antimicrobial potential towards phytopathogens.

2. Site-directed mutagenesis, cloning and expression of \(cah\) gene in \(E.\ coli\).

3. Construction of gene cassettes with \(cah\) marker and antifungal genes encoding chitinase and LTP.

4. Transgenic expression of \(cah\) marker and antifungal genes in tobacco.