Chapter 1

INTRODUCTION
Plants are the source of food and shelter for a wide range of organisms including other plants (parasitic plants). Plants are constantly under heavy stress from various biotic and abiotic sources, which affects their growth and productivity. Stress caused by any living organism is called as **biotic stress**, which is generally affected by pathogens and insects. Unlike their animal counterparts, plants lack a circulating adaptive immune system to protect themselves against the invading pathogens. Plants are resistant to most pathogens in their environment, as they are not hosts for a particular pathogen or they could be, but harbor resistance genes that allows the induction of active defense mechanisms, which are based upon early detection and quick response to the invading organisms. In general, pathogens were classified into three basic types based on their mode of life style. They are necrotrophs (secrete toxins and feed on dead cells), biotrophs (live on living host and derive nutrients through a specialized organ were called haustorium). If it displays both the types of life style they are called hemibiotrophs (Fig. 1.1).

![Figure 1.1. Arabidopsis leaf infected with necrotroph (B. cinerea), Biotroph (H. arabidopsidis) and Hemibiotroph (P. syringae) (Pieterse et al. 2009).](image)

In order to respond to the pathogenic invaders, many early signaling events are triggered within the plant as a defensive response. It includes the fortification of cell wall through cellulose synthesis and lignin deposition, production of antimicrobial secondary metabolites such as phytoalexins and the accumulation of PR proteins such as glucanases and chitinases. Recognition of pathogen specific effectors leads to the production of ROS at the site of infection, which
develops a hypersensitive response (HR) that is followed by cell death (Glazebrook et al. 2005). This isolates the pathogens from further spread into the adjoining healthy tissues. This is, in fact, advantageous in the case of necrotrophs but not for biotrophs. Necrotrophic response is triggered by pathogenic toxins or Pathogen-Associated Molecular Patterns (PAMP), such as the breakdown product of plant cell wall by the activity of pathogen derived enzymes, which can stimulate the plant defensive responses (Schwessinger and Zipfel 2008; Nurnberger et al. 2009). Detection of these virulence proteins or other general elicitors triggers signaling events within the host cells that leads to diverse cellular responses, including changes in ion fluxes, synthesis of the stress related hormone such as ethylene, transcriptional reprogramming, production of reactive oxygen species (ROS), and often a localized form of programmed cell death, often referred to as the hypersensitive response (HR).

![Figure 1.2. Schematic representation of Systemic Acquired Resistance (SAR) and Induced Systemic Resistance (ISR) in plants (Pieterse et al. 2009)](image)

Additionally, activation of a local defense response can trigger the production of salicylic acid (SA) and the elicitation of broad-spectrum defense responses known as systemic acquired resistance (SAR). It is activated at the site of infection and the defense response is triggered in distal parts that are usually away from the infection site to protect the healthy tissues from the pathogenic
invaders (Fig. 1.2) and it is characterized by the co-ordinate activation of set of PR genes (Pathogenesis Related genes), many of which have antimicrobial activity (Van Loon et al. 2006).

Although some of these have been shown to have specific enzymatic activity, i.e., chitinases (PR-3) and β-(1, 3)-glucanases (PR-2), many have no known catalytic function and their mechanisms of action is not clearly understood (Yun et al. 1997). In some cases, the soil microorganism such as mycorhiza or rhizobacteria activates the defense responses especially against the necrotrophs at distant healthy tissue by producing the signaling components such as JA (Jasmonic acid) or ET (Ethylene). Such type of resistance is termed as Induced Systemic Resistance (ISR) (Pieterse et al. 2009) (Fig. 1.2).

Protection of crop from diseases can substantially improve the agricultural production. Although fungicides and pesticides have been used successfully to control diseases and pests, their continued and increasing use might lead to harmful effects on health and environment. In India, the amount of usage of chemical pesticides has increased five times from 1965-66 to 1990-91. Extensive usage instigates additional hazards like resistance to fungicides in fungal pathogens and resurgence in insect pests. So far, over 700 insect pests are reported to have developed resistance worldwide to a variety of insecticides, out of which 33 have been reported from India (Rai 2004).

Use of high-yielding crop varieties can improve the productivity, but carries the risk of devastating epidemics due to genetic uniformity. Conventional breeding utilizing the available gene pool of resistant traits has led to the development of a number of resistant plant varieties. However, there are some inherent problems associated with the conventional approach, such as, long gestation period in developing resistant hybrids. During these long years, even the pathogens might evolve and overcome the resistance, leading to a futile effort. Besides this, because of the differences in the ploidy level of the genome and other reasons, effective introgression of resistance genes into the susceptible cultivar from resistant wild species is often difficult in many crops. Additionally, transfer of desirable traits is often associated with transfer of undesirable genetic material because of linkage, known as linkage drag, which consequently reduces
the potential of the resulting introgressed material in subsequent crop improvement programs.

In this regard, perhaps the most promising field of genetic research has been genetically modified (GM) crops. Several GM crops with different altered traits e.g. herbicide tolerance (in canola, cotton, flax, maize, rice, sugar beet and carnation), improved insect resistance (Bt-cotton, maize, potato and tomato) and higher shelf life (tomato) are already under commercial use. Disease resistance through genetic modification has tremendous potential but requires a lot of patience and perseverance as the phytopathogens being microbial in nature might evolve resistance to the transgenes deployed against them. Transgenic modification also allows the introduction of genes for disease and pest control from unrelated plant species (Boller 1993) or organisms outside the plant kingdom (Rao 1995) to be incorporated into crops. Several current research efforts are aiming the development of disease resistance through recombinant gene technology, for example, transgenic modification, metabolic manipulation, defense pathway manipulation and knock out of genes.

However, the development of diseases and mechanism of disease resistance in plants are governed by the combined genotypes of the host and pathogen, and depends on a complex exchange of signals and responses. During the long process of host-pathogen co-evolution, plants have developed various mechanisms to ward off pathogen attack and the genes governing these intricate defense mechanisms could be used in developing genetically modified disease resistant crops.

In the past 20 years, genetic research has identified several plant genes involved in the defense signaling pathways and disease resistance. Antifungal genes like defensins, chitinases, glucanase, osmotin are a few potential genes that have been used in genetic transformation of important crop species by different research groups for achieving fungal disease resistance.

In plants, several signaling cascades get activated during pathogen infection and Salicylic acid (SA), Jasmonic acid (JA) and ethylene (ET) play a major role in activating the defense response genes. When a biotroph approaches the host, SA signaling cascade gets activated leading to the development of resistance by
expressing the PR genes such as PR-1, PR-2 and PR-5. Here, NPR1 (Nonexpressor of pathogenesis related proteins) plays a crucial role and acts as a positive activator for the expression of PR-1 gene. In contrast, any necrotroph that approaches a host, activates the JA and ET signaling cascade leading to the development of resistance by expressing the genes for defensin (PDF1.2), VSP2 (vacuolar sorting protein2), etc. Hence in the present study, we opted for two genes such as BjNPR1 from mustard (Brassica juncea) and a defensin (TvD1) gene from the weedy legume Tephrosia villosa. The gene BjNPR1 was characterized in our lab and identified as one of the candidate genes for plant transformation especially in crop plants. Hence, it has been utilized for mungbean transformation in the present study, and TvD1 was isolated and functionally characterized for biotic stress tolerance.

Thus, the thesis has been largely divided into two parts. They are as follows

**Part A (Chapter 4).** Heterologous expression of BjNPR1 in mungbean for amelioration of biotic stress tolerance.

**Part B (Chapter 5).** Characterization and heterologous expression of a legume defensin, TvD1 in tobacco for amelioration of biotic stress tolerance.