Chapter 1

Introduction
Plants being the primary producers, because of their capacity to convert inorganic molecules into organic energy through photosynthesis, are always engaged in complex dialogue with other organisms. Plant-Biotic interactions are mostly hostile but some organisms have developed neutral or symbiotic relationships to maintain energy flow towards them. From the start of agricultural practices plant diseases have concerned humans as it limits the food supply to them and with the growing population this concern as elevated. Diseased plants may be toxic for humans and animals, and they reduce food quality and production. Breeding of high-yielding varieties, use of synthetic fertilizers, and improved irrigation has contributed to double the world food production within the last 40 years to match the demands of an increasing human population. Owing to limitations in resources such as yield potential of crops and availability of arable land, water and climate change, sustainability of production at elevated levels is only possible with adequate disease control. Understanding the molecular & biochemical complexities of plant-biotic interactions and applying obtained knowledge for sustainable agriculture is the aim of research projects around the globe.

The resistance against most non-adapted pathogens (non-host resistance) and the ability to reduce the disease severity of an adapted pathogen (basal resistance) lets a plant to complete its life cycle. Preformed structural and biochemical barriers and induced defense both contribute to tolerance or resistance against pathogens. Like animals plants also have the capacity to recognize a potential pathogen and mount defense responses against it. Most of the pathogens are recognized by their conserved structural or chemical components and plants mount defense responses but pathogens have also evolved to suppress this basal defense strategy by their virulence effectors. These effectors are recognized in resistant plant through resistance (R) genes (Flor, 1971). Thus, throughout the co-evolution of plant and microbes this 'tug of war' is going on. Between recognition of a pathogen to the mounting of defenses, various complex-signaling cascades, plant hormones, transcriptional changes are involved. In order to better visualize and understand the complexities of plant-biotic interactions, scientists have classified the interactions and defense mechanisms of plants based upon presence and absence of hypersensitive response (HR) and major hormone pathways involved in defense against a particular pathogen. Pathogens are also classified based upon their life-style inside host plant like biotrophic, hemi-biotrophic and necrotrophic.

Chickpea (Cicer arietinum L.) is an important pulse crop, contributing enormously in supplying dietary protein and soil nitrogen fixation. It accounts for about 15% of the
world's total pulse production and India contributes to 64% of the world production of this legume crop (FAO, 2008). The yield potential of this crop is affected by various biotic and abiotic stresses. The necrotrophic foliar fungal disease Ascochyta blight (caused by *Ascochyta rabiei* (Pass.) Labrousse) and the soil-borne necrotrophic fungal disease Fusarium wilt (caused by *Fusarium oxysporum* f. sp. *ciceris*) are considered the most serious biotic stresses. Other diseases are more geographically localized like pod borer (*Helicoverpa armigera*) in Australia and India. In many areas of production, the chickpea crop is affected by the Ascochyta blight (AB) and in case of severe infection 100% crop loss is reported (Singh and Reddy, 1993; Chang et al., 2007). Use of resistant cultivars is considered the most viable option for long-term management of AB. However, only partial resistance is available among the cultivated chickpea germplasm and the improved cultivars are only moderately resistant to AB (Anbessa et al., 2009). Despite many reports on Quantitative Trait Loci (QTLs) for resistance to AB, the use of marker-assisted selection in breeding for resistance to AB in chickpea has been limited. Therefore, the other option is to increase basal resistance against AB for durable tolerance. This could be done by identifying important defense regulators and use them in chickpea through biotechnological tools for improved resistance.

To identify the genes involved in chickpea defense against AB, three forward Suppression Subtractive Hybridization (SSH) cDNA libraries from early stages of chickpea infected tissues were generated and sequenced earlier in the laboratory. Many genes related to transcription factors were present in these libraries. Our interest focuses on regulatory mechanisms controlling the defense response of plants to invading pathogens. Reprogramming of cellular functions in response to external stimuli involves complex changes in gene expression. The activities of a multitude of genes are subject to up- or down regulation and follow defined temporal programmes. The perception of an external stimulus immediately leads to the activation of primary-response genes. Activation of these genes is mediated by pre-existing signaling components including transcription factors. Since, the temporal and spatial expression of genes is controlled by the transcription factors; they may be utilized for the generation of broad-based pathogen tolerant plants. In present study, we aim for the functional characterization of a WRKY transcription factor gene of chickpea. The WRKY family members bind to W-box (TGACC/T) motifs which are present in the promoters of several defense-related genes. This gene also showed induction at mRNA levels shortly after pathogen infection revealed by macroarray experiments.
The objectives selected for this study are:

1. To isolate the full-length gene of a WRKY family transcription factor and its expression analyses.

2. To elucidate its functional role in plant defense by overexpression in transgenic tobacco.

3. To isolate and analyse the 5'-upstream sequences of this gene in transgenic tobacco.

4. To isolate the interacting partners of this protein using yeast two-hybrid or related strategy.