Chapter 7: Summary
Plants are sessile organisms that are exposed to a constant barrage of environmental stresses which impact on growth, development and reproduction. Important traits such as yield and the resistance to biotic stress depend on internal physiological programs and their regulation by signal transduction pathways. Plants are the major source of food and biomaterials worldwide but their production is severely compromised by pathogens that cause disease and reduce yield and quality. Therefore, understanding how plants defend themselves against pathogens and herbivores, and how that may be manipulated, is therefore of critical importance for successful and sustainable agriculture.

Chickpea (*Cicer arietinum* L.), is a major source of high quality protein. Among temperate pulses, it is the most tolerant crop to heat and drought and is suitable for production in low fertility soils (Pande et al. 2005). Despite its economic importance, chickpea productivity has been low because of yield losses due to devastating foliar and soil-borne fungal diseases like *Ascochyta* Blight (AB), Fusarium wilt and Botrytis Grey mould, and insect pests like pod borer. Among these, AB caused by the ascomycete fungus *Ascochyta rabiei* (Pass.) Labrousse (teleomorph *Didymella rabiei* (Kovachevski) v. Arx) is the most important biotic constraint for chickpea production (Nene and Reddy 1987; Gaur and Singh 1996). The main objective of this work was to identify resistance-related genes by following one of the transcript profiling strategies. To understand quantitative disease resistance conferred by multiple genes, individual genetic factors determining disease resistance need to be elucidated. We decided to enrich genes that may contribute to constitutive resistance mechanism or by other defense system by screening for genes showing constitutively different expression levels between resistant and susceptible lines using transcript profiling. Once identified, the next objective would be to functionally characterize one of the selected genes. In addition, we also identified a set of genes that show significant induction upon *Helicoverpa* infestation, the other major factor that causes severe crop losses. These genes may also serve as ‘candidate genes’ for transformation and crop improvement in future.

**Identification of differentially expressed genes among *Ascochyta* resistant and susceptible lines of chickpea and their analysis**

To isolate chickpea genes involved in the resistance to the *Ascochyta* blight fungi, we used the suppression subtractive hybridization (SSH) method to generate cDNA libraries enriched in sequences expressed in chickpea plants during the early stages of infection and we also studied the differential behavior of their expression at basal level in resistant and susceptible germplasm lines. For focusing on genes strictly involved in the resistance, cDNA from resistant plants were subtracted with cDNA from the susceptible plants. As a result,
genes showing higher constitutive expression in the blight resistant germplasm line, FLIP84-92 C(2) compared to the blight susceptible line, PI359075 (1) and genes showing induction upon *Ascochyta* infection were identified. Genes with constitutive higher expression in the resistant lines are predicted to be directly involved in the resistance and genes showing no difference between resistant and susceptible line but showing induction after *Ascochyta* infection are predicted to be involved in basal defense. Higher accumulation of some of the transcripts at the basal level indicates that plants are already prepared for resisting against the fungus. In order to indicate their role in defense we also monitored the expression patterns of the isolated genes in response to exogenous application defense regulators. Involvement of both JA and SA together with some other unknown factors is implicated in the resistance mechanism against *Ascochyta*. These results provided novel insights to the molecular control of chickpea cellular processes, which may assist the understanding the chickpea defense mechanisms and allow enhanced development of disease resistant cultivars.

**Isolation and characterization of CaAr131 from chickpea**

A chickpea cDNA fragment, inducible by the *Ascochyta* blight and that shows high homology with *Hs1* Pro1 resistant gene was chosen for further characterization. Further its corresponding full-length cDNA clone was isolated from a chickpea cDNA library using *CaAr131* truncated primer as a probe. The full length clone obtained was completely sequenced and analyzed. The *CaAr131* cDNA is 1.3 kb long and encodes a predicted protein of 458 amino acid with an estimated mass of 52 kD. The *CaAr131* contain an imperfect LRR domain, phosphorylation sites and α-glycosylation sites. The phylogenetic analysis based on sequence alignment suggests that CaAr131 has a strong homology with *Hs1* Pro1 gene of beet root which confers resistance against a nematode. DNA gel blot hybridization strongly suggests that the chickpea genome contains a single copy of the gene. To further assess the expression pattern of *CaAr131* during fungal infection and various defense regulators, RNA gel blot analysis was performed which indicated that various plant defense regulators and osmotic stress conditions induce *CaAr131* expression. To further understand the mechanisms of regulation of *CaAr131* the functional characterization of its promoter was performed by isolation of 5'-upstream region and generation of deletion constructs and their analysis. To examine the spatial and temporal as well as tissue specific expression of this gene, its 5’-upstream sequences fusion construct (5’upstream of *CaAr131::GUS*) was generated and used to transform tobacco. The trichomes of transgenic tobacco plant showed strongest GUS activity.

To further demonstrate its functionality in plant defense we need to analyze its over-expression in transgenic plants and look for its response to pathogen infection.
Isolation, Identification and analysis of differentially expressed chickpea genes upon *Helicoverpa* infestation

Another major threat to chickpea successful production is the generalist herbivore, *Helicoverpa armigera*, which damages the aerial parts of the plant, including leaves and pods. Since most studies examining *Helicoverpa*-chickpea interactions have focused on specific gene or protein dynamics (Johnston *et al.* 1991; Jongsma *et al.* 1995; Giri *et al.*, 1998; Peng *et al.*, 2005; Srinivasan *et al.*, 2005), our aim was to identify target genes upregulated during mild insect infestation which may contribute to the defense response. To isolate *Helicoverpa*-induced genes, a subtractive cDNA library was constructed from chickpea seedlings under *Helicoverpa* mild infestation using SSH. In addition to known defense genes, we identified a number of genes and presumed biochemical functions that have not been previously associated with defense responses against insects. Using macroarray, we profiled and compared transcript patterns elicited by both herbivore and mechanical wounding. Comparative expression patterns on exogenous applications of various signaling compounds were obtained to evaluate the dynamics of regulatory pathways. In addition to investigating the effects of elicitation by mild insect infestation, induced plant defenses in chickpea were evaluated by examining signal compound elicitation on larval feeding behavior. In conclusion, this study shows that *Helicoverpa* attack triggers changes in transcript levels that are distinct from mechanical damage and are controlled mainly by MeJA and ET. Directly or indirectly, the majority of the genes identified as being *Helicoverpa*-activated, may have a significant effect on insects performance as it was depicted that elicitation with mild insect infestation, MeJA and ET affected larval feeding behavior. We expect that further functional characterization of these novel *Helicoverpa*-responsive genes which are regulated by MeJA and ET will extend our understanding about defense responses against insects and to develop new strategies for crop protection. Therefore, the results of this study advance the understanding of non-model plant-insect interactions on a broader scale.

Overall, this study isolated and characterized numerous defense related genes and their regulatory mechanisms that may be important in defense against various pests and pathogens, as well as other cellular functions. The findings of the present analysis can provide novel insights to the molecular control of chickpea cellular processes, which may assist the understanding of chickpea defense mechanisms and allow enhanced development of resistant cultivars. Further functional characterization of the novel *Ascochyta* and *Helicoverpa*-induced genes will extend our understanding about defense responses against the two important biotic factors which limits chickpea production and in developing new strategies for crop protection. The work embodied in this thesis would help improve our understanding of molecular mechanisms involved in resistance/defense in chickpea. In future,
development of *Ascochyta* and *Helicoverpa* resistant/tolerant chickpea varieties would also reduce the cost of disease control. Furthermore, the harmful impact on the environment incurred by the extensive use of antifungal chemicals and pesticides could be avoided by successful development of these varieties.