Abstract

The two commercially cultivated coffee (Coffea) species, *C. arabica* (arabica) and *C. canephora* (robusta), respond differently to the coleopteran insect pest *Xylotrechus quadripes*, commonly known as coffee white stem borer (CWSB). CWSB is the most destructive insect pest of arabica coffee plants in India. It is also distributed in other Asian countries like Bangladesh, Myanmar, Nepal, China, Thailand, Vietnam, Indonesia and Sri Lanka. The adults of *X. quadripes* have distinct flight periods and the females lay eggs in cracks and crevices on the bark of woody stems. After initial tunneling & feeding in the bark, the larvae enter the wood and make extensive galleries in the main stem and thick primary branches. Tunneling may also continue to some extent in the main roots. CWSB larvae block the tunnels with frass behind them to deter predators. Apart from affecting the quality and quantity of the produce, severe infestation can heavily damage the main stem up to roots and kill the plants. Young plants of up to 7–8 year old are killed easily, whereas, older plants may survive for a few more seasons. Conventional breeding has not been successful in developing arabica varieties resistant to CWSB. Currently available control measures have not been able to control the pest effectively. As a consequence, CWSB has become major factor contributing to the continuous decline of area under arabica plantations in India. The severity of the pest has increased in recent years possibly due to changing climatic conditions caused by global warming. The pest causes substantial capital loss and heavy financial distress to the arabica coffee farmers as millions of severely infested arabica plants are killed or have to be uprooted every year to prevent further spread of the pest. As a consequence arabica is getting replaced by robusta. While *C. arabica* is highly susceptible to CWSB, *C. canephora* and other species like *C. liberica* are resistant to CWSB. It is essential to understand the genetic mechanisms of defense mounted by the resistant species against CWSB to come out with strategies to breed arabica varieties resistant to CWSB.

Application of genomics and related areas was thought to be helpful to unravel the genetic basis of coffee plants resistance against CWSB and this idea formed the basis for this study. Profiling of transcriptional changes in the bark tissue of robusta due to
CWSB infestation and related work was planned to get an insight into the genetic basis of
resistance against the pest. Specific objectives and work plan were drawn up and study
was carried out accordingly. The results obtained in the study form the basis of this
thesis. The background for carrying out the study is described in Chapter 1
(Introduction). The literature survey done in this regard is given in Chapter 2. The results
obtained are described in the following 6 chapters.

Using Suppression Subtractive Hybridization (SSH) technique forward subtracted
cDNA library was constructed and analyzed from the robusta (C. canephora Cv CXR)
bark tissue artificially infested with CWSB larvae (Chapter 3). The SSH library has
provided a valuable EST database for the genes expressed in the bark tissue and possibly
modulated by CWSB infestation. The library provided 217 unigenes which matched 203
C. canephora genes in Coffee Genome Hub (CGH) database. Sequence similarity
searches in the public databases like CGH/SGN (Sol Genomics Network) and GenBank
(NCBI) revealed that the transcripts broadly function in direct defence, defence
signalling, ROS scavenging, transport, cell wall modification, photosynthesis and abiotic
stress. Several of these transcripts exhibited polymorphism compared to C. canephora
sequences in CGH indicating their usefulness for DNA markers. The SSH library
revealed several transcripts that may be involved in defense response against herbivory.
Interestingly transcripts of a cytochrome p450 gene matching isoleucine N-
monooxygenase 2 gene were encountered abundantly in the SSH library. In the next step
attempt was made to profile changes in expression levels of some of the putative defense
related genes due to CWSB infestation by quantitative real time PCR (qRT PCR)
(Chapter 4). Expression levels of caffeine synthase, lipoxygenase, a zinc finger protein, a
MYB domain transcription factor, transcription factor LHW and a pectate lyase gene of
C. arabica and C. canephora were assessed at different time points up to 96 hrs. The
analysis revealed interesting variation in expression levels of these genes possibly
modulated by CWSB infestation. Attempts were made to obtain and analyze complete
cDNA sequences for some putative defense-related genes from partial EST sequences
using RACE technique (Chapter 5). RACE efforts were made on pectate lyase, PEBP,
CBL-interacting protein kinase, phospholipase A2, transcription factor LHW, RING H2
zinc finger protein, UDP glucosyl transferase, GAGA binding factor with different levels
of success. Interestingly the RACE efforts also resulted in some transcripts of new defense-related genes not found in the SSH library. Attempt was made to get the promoter sequence of abundantly expressed isoleucine N-monooxygenase 2 gene by Genome Walking technique (Chapter 6). The promoter elements of this gene may be responsible for its specific expression. The study generated 593 bp upstream sequence in which a TATA Box and TSS (transcription start site) were identified. The TATA box is at 33 bases upstream from TSS (-33 position). The sequence also contained several transcription binding sites. Jasmonates (JAs) are involved in plant defense signaling against herbivores. Attempt was made to identify the *C. canephora* genes involved in JA biosynthesis pathway (Chapter 7). The complete genome sequence of *C. canephora* is available in the web-based database of Coffee Genome Hub, with the help of this database and NCBI database the genes of all the enzymes involved in the JA synthesis were identified in *C. canephora* confirming the conserved nature of the pathway in coffee. Caffeine is thought to be a defense chemical in several plants and coffee plants constitutively store it in beans and leaves. This study discovered the insecticidal activity of caffeine on CWSB in artificial diet based bioassay (Chapter 8).

Overall, the results described in the thesis conform to the basic objective of the study of throwing light on the genetic basis of CWSB resistance coffee. Some results of the study are published in one article and another article is accepted for publication in another peer reviewed journal.