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Introduction

“Tea” was first introduced to the world in written form in a Chinese dictionary as “a beverage made from boiled leaves”. Chinese people consumed “tea” to treat various ailments. The tremendous increase in demand of tea ruled out its supply from wild trees and eventually various regions started growing tea as a major beverage crop of the world (Weatherstone, 1992). India harbours three distinct tea growing regions- Darjeeling, Assam and Nilgiri. Tea is grown in altitudes ranging upto 2000 meters in Darjeeling with its cool and humid climate where a combination of a number of natural factors like rainfall, the altitude and the sloppy land gives Darjeeling tea its unique flavour and aroma. Contrastingly, Assam, possessing vibrant climate with adequate rainfall (100 to 150 inches annually) produces tea with strong and bright liquor. Assam has also made its recognition as the largest tea exporter of the country bearing 40% of the country’s tea exports. The Nilgiris, with its hilly landscapes receives rainfall ranging upto 100 inches per year. Tea here is grown in heights of about 2500 meters providing the liquor its exclusive taste.

The genus *Camellia* belongs to the family Theaceae with about 82 species. The tea plant bearing the botanical name *Camellia sinensis* (L) O. Kuntze possess two distinct and well-recognized intra-specific varieties namely *Camellia sinensis* var. *sinensis* (L.) (the China variety) and *Camellia sinensis* var. *assamica* (Masters) Kitamura (the Assam variety), mainly characterized based on leaf morphology. The two varieties are morphologically distinct from one another, the former is characterized by relatively narrow and erect leaves, unlike the latter with broad leaves. However, in 1962, Wight

categorized the tea plant based on the morphology of styles into three forms viz. *C. sinensis* (L.), *C. assamica* (Masters) and *C. assamica* ssp. *Lasiocalyx* (a sub-species or popularly known as Cambod tea). Tea is a heterogenous plant and the cultivated tea clones exhibit clone-specific morphological and biochemical differences. All of the studied clones and varieties of *C. sinensis* bear chromosome number of 30 (n=15) (Bezbaruah, 1971; Kondo, 1977).

Tea, *Camellia sinensis* (L) O. Kuntze, being the most popular non-alcoholic drink, preceded by only water, has always been on high demand among the people worldwide. Therefore, in order to match the rising demand of people, tea plant cultivation has been increased extensively in the recent decades. India recorded an approximate of 1 million tons of tea production in 2022, second only to China with an approximate of 2.8 million tons of tea production in 2022. The gradual rise in production of tea can be attributed to the improved technology, introduction and selection of clones based on yield efficiency and better nutrition and fertility facilities. However, the extensive and widespread cultivation of tea have drawn the attention of insect and mite pests to the plant posing as a major disbalance in the commercial tea cultivation. This has encouraged the occurrence of several diseases thus limiting tea production (Bora and Bora, 2022). Tea plant is a home for more than 300 insects, mites and disease causing microbes that attack various parts of the plant including buds, stems and leaves, thus resulting in 11 to 55% loss in yield (Roy *et al.* 2015).

However, among the biotic agents causing stress in tea plants, the tea mosquito bug (TMB), *Helopeltis theivora* Waterhouse (Hemiptera: Miridae) has emerged as the most prominent and difficult to control pest of tea plant in recent years and its management has also become an inevitable problem amongst tea growers. Large scale

and indiscriminate use of pesticides to manage TMB, is not only adversely affecting the natural ecosystem but also creating dangers of pesticide residue in made tea (Roy *et al.* 2009). The genus *Helopeltis* has about 41 species. But only three of them namely *H. antonii*, *H. theivora* and *H. bradyi* are reported in India. In India, significant damages to the tea crop has been observed to be caused by *H. theivora* alias TMB. TMB belongs to the group of phloem feeders or phloem sap sucking mirids of the order Hemiptera. Unlike chewing insects and cell content feeders, phloem suckers cause less tissue damage which trigger cell signalling response and activation of defense hormones in the host cell. North East India reports a significant loss of 15-20 lakh kgs of commercial tea per year due to TMB infestation. The TMB normally feeds on young shoots and new flushes. During feeding the insect inserts its salivary enzymes including proteases and lipases into the tea plant tissues. Feeding of TMB results in the formation of a circular ring around the feeding spot which slowly turns into light brown in colour. As time progresses, the ring dries up and many such dried feeding spots in a single leaf makes it unsuitable for plucking and subsequent processing (Das, 1984; Sundararaju and Sundara Babu 1999; Stonedahl, 1991). Pesticides are eventually found to be ineffective against the pest may be due to the adaptation mechanism of the pest. However, it is worthy to mention that relying on biological methods of pest control is highly recommended so as to maintain the quality of made tea in the long run without disturbing the ecological equilibrium. Initially, the TMB was considered as a minor pest of tea but later on it became a significant threat to the tea plantations. As such, reports claim that almost 80% damage to the pluckable shoots of tea plant has been caused by this notorious and devastating pest resulting in large scale crop loss (Bora and Gurusubramanian, 2007; Roy *et al.* 2009). TMB has been now considered as a severe risk to tea plant life due to its polyphagous

nature and rapid multiplication capacity (Roy *et al.* 2009). TMB infestation on tea plant occurs almost throughout the year but infestation peaks from months of May to November every year. The use of insecticides has till date been the primary means of controlling measure. But pesticide use hasn't been found to be profoundly effective against eliminating the pest fully, rather its regular use has only worsened the scenario due to the development of resistance in the TMB against the commonly applied insecticides, thus contributing to its reoccurrence (Bora and Gurusubramanian, 2007; Rahman *et al.* 2006; Sarker and Mukhopadhyay, 2003, 2006; Roy *et al.* 2008; Mukhopadhyay and Roy, 2009). Presence of alternate hosts of TMB has also contributed to the resurgence of this pest.



Fig. 1.1 Photographs of *H. theivora* infested tea plants

Plant-herbivore perception is the starting point of the entire plant defense tactic. Defense, overall, is a complicated strategy and it is not surprising to state that plants have evolved a sophisticated machinery to battle with disease causing pests. Unlike constitutive defense mechanism, induced defense, which is defined as the response

generated by the plant after the onset of a pest attack, facilitates the deployment of particular defense molecules specific to the type of feeding organism. Defense strategy involves multiple components ranging from physical barriers like trichomes and thorns to chemical defensive traits including primary and secondary metabolites. Defense can be attributed as direct i.e., secretion of components to defend against the feeding pathogen, as well as indirect i.e., secretion of components like green leaf volatiles and volatile organic compounds (VOCs) to attract the natural enemies or predators of the feeding insect. Plants are highly skilled for proper energy utilization in normal metabolic processes including growth and development and other environmentally induced responses through regulatory channels. Plants perception of herbivory involves recognition of HAMPs (herbivore associated molecular patterns) and PAMPs (pathogen associated molecular patterns), which are molecules of pathogen origin and DAMPs (damage associated molecular patterns), which are secreted in the plants in response to wounding by chewing herbivores or pathogen attack. This recognition, achieved by pattern recognition receptors (PRRs) of plant origin, activates the defense signalling cascades leading to systemic acquired resistance in the plant. However, defense induction may also occur without the involvement of PRRs (Erb and Reymond, 2019). Events associated with early response signalling include membrane depolarization, rise in cytosolic Ca^{2+} concentration, production of reactive oxygen species (ROS), activation of mitogen-activated protein kinases (MAPK) cascade signalling in the target plant. Ca^{2+} is considered as a major secondary messenger associated with defense signalling. Changes in the cytosolic Ca^{2+} concentration, as mentioned above, is associated with stress response as well as regulates growth and development. Various Ca^{2+} sensors like calcium-dependent protein kinases (CDPKs), calmodulins (CaMs), calmodulin-like proteins

(CMLs) come into action to initiate the Ca^{2+} mediated defense signalling which further induce the production of the defensive phytohormone jasmonic acid (JA) in the host plant. Perception of herbivory initiates local and systemic ROS burst which is dependent on the RBOH protein D (RBOHD). ROS accumulation is an important indicator of plant oxidative stress. Ca^{2+} influx and CDPKs are responsible for the activation of RBOHD, hence accumulation of Ca^{2+} in wounded cells is directly associated with ROS burst during early defense response (Erb and Reymond, 2019). The role of MAPKs in plant defense and stress response is critical. MAPKs regulate the expression of several defense associated genes. Transcriptional activation of several herbivore-induced/oral secreted genes are dependent on the MAPK pathway. Study involving silencing of salicylic acid induced protein kinase (SIPK) and wound induced protein kinase (WIPK) showed that mutants were unable to biosynthesize JA and therefore failed to resist against the feeding insect *Manduca sexta* (Kandoth *et al.* 2007). JA has been well recognized as a vital component of the plant immunity. Its role in defense against necrotrophic pathogens and chewing herbivores/insects is inevitable. Studies have revealed that JA mutant plants, or plants impaired in the JA biosynthesis machinery, showed weak resistance to chewing insects. The JA pathway involves a network of molecular components that regulate the synthesis of the canonical bioactive form of JA i.e., Jasmonoyl-L-isoleucine (JA-Ile). This JA-Ile induces the downstream cascades resulting into the activation of transcription factors like MYC and expression of defense genes thus leading to resistance against a wide range of herbivores (Fernández-Calvo *et al.* 2011; Song *et al.* 2017; Erb and Reymond, 2019). The antagonism between JA and salicylic acid (SA) has been well reviewed. SA is an important plant defense hormone mainly involved in providing defense against sap-sucking feeders and biotrophic pathogens. Just

like JA, the SA pathway also encompasses several molecules like NPR1, a key element of the SA pathway. Role of other phytohormones like ethylene (ET) in plant stress response is worth mentioning. Along with defense response, ET is associated with a wide range of physiological processes in plants. Herbivore attack and recognition of pathogen elicitors rapidly induce ET accumulation in plants. ET is responsible for fine-tuning and regulation of JA-mediated signalling such as accumulation of protease inhibitor (PI) proteins (O'Donnell *et al.* 1996), emission of herbivore-induced plant volatiles (HIPVs) (Schmelz *et al.* 2006), production of nicotine (Winz and Baldwin, 2001). Systemic acquired resistance (SAR) is achieved in plants by means of a communicating signal that alerts the plant against a herbivore attack. Several studies are indicative of the role of the plant vascular system in transmitting this signal to various parts of the plant body (Orians *et al.* 2000, Schittko *et al.* 2000). Since it further came to light that JA perception is required for developing SAR, therefore it was evident that this JA-induced compound or signal is responsible for transmitting the alert signal and eliciting defenses. However, the mechanism by which this alert signal gets spread across the plant body is still unclear. Another important aspect to be mentioned regarding plant-herbivore interaction, is the carbohydrate-binding proteins called lectins. Lectins are well-studied for their insecticidal properties (Sharma *et al.* 2004; Van Damme, 2008). Reduction in insect performance and survival has been observed in case of insects belonging to orders Lepidoptera, Hemiptera, Coleoptera after they were allowed to feed on diet incorporated with plant lectins (Vandenborre *et al.* 2009).

Non-coding RNAs (ncRNAs) are RNAs lacking protein-coding potential and having essential role in functional biology. They are well-known to regulate the expression of protein-coding genes in both pre and post-transcriptional scenarios. Lately,

high-throughput next-generation sequencing (NGS) has opened doors for the discovery, characterization and analysis of several types of ncRNAs. This includes the microRNAs (miRNA), small interfering RNA (siRNA), trans-acting siRNA (tasiRNA), long noncoding RNA (lncRNA), and circular RNA (circRNA) in plants (Wang *et al.* 2021; Zhou *et al.* 2020; Fan *et al.* 2020; Sun *et al.* 2020a, 2020b; Wang *et al.* 2019a, 2019b; Jeyaraj *et al.* 2017a, 2017b; Theibaut *et al.* 2014; Cervera-Seco *et al.* 2019). During the last decade, it was revealed that lncRNAs are master regulators of gene expression and are considered as one of the fundamental molecules to regulate several aspects of plant development and stress response. LncRNAs can regulate gene expression in a variety of ways. They can act as targets of miRNAs and regulate the corresponding miRNA target genes by forming competing endogenous RNA (ceRNA) networks, thereby acting as miRNA sponges (Sun *et al.* 2020a, 2020b). They also serve as miRNA precursors and take part in the biogenesis of miRNAs (Mercer *et al.* 2009). Recent reports suggest the involvement of complex biological network in eukaryotic gene regulation comprising of lncRNAs, miRNAs and mRNAs, where lncRNAs and mRNAs compete against each other to bind to miRNA response element (MRE) of miRNAs. LncRNAs can also act as signalling molecules to take part in various signalling pathways, as guides that localizes specific protein complexes and also as scaffolds that brings two or more proteins into discrete complexes. LncRNAs also serve a crucial functional purpose in plants' growth and development. A small number of experimentally discovered lncRNAs in plants have been described, including Enod40, COLDAIR, COOLAIR, IPS1, HID1, LDMAR, LAIR, TL, DRIR. According to reports, these lncRNAs play a role in a number of processes, including nodule development, male sterility, phosphate uptake and homeostasis, nutrient deficiency, vernalization, photomorphogenesis, yield enhancement and pathogen

infection. In view of the above, it can be said that it has become necessary to explore the lncRNA-miRNA-mRNA regulatory pathway during *H. theivora* infestation in tea, which will help to unravel lncRNA associated defense responses in tea plant during TMB infestation. Although, there are a number of studies relating to role of lncRNAs in plant physiology and metabolism, abiotic and biotic stress, growth and development of plants, yet only a few researches on lncRNAs have been made in the field of plant-insect interaction study. There are a very few studies associated with decoding the regulatory role of ncRNAs in plant-herbivore or plant-insect interaction in general. Though much have been explored regarding circRNAs and their role in plant biology, yet there is less contribution towards research involving circRNAs in plant's response to feeding insects. Till date, there is only one study that is reported for role of lncRNAs in plant-insect interaction (Li *et al.* 2021) and though Bandyopadhyay *et al.* (2015) carried out transcriptome profiling of tea plant during TMB infestation through suppression subtractive hybridization (Bandyopadhyay *et al.* 2015), yet reports for high-throughput transcriptomic evaluation of TMB responsive defense in the tea plant is lacking. Here, a transcriptome-wide study has been provided through which it has been endeavoured to provide a detailed account of *C. sinensis* transcriptomic reprogramming as a result of TMB infestation and also to highlight the role of tea plant lncRNAs and circRNAs in the defense regulatory network in tea plant against TMB. The present study has three proposed objectives:

1. Biochemical analysis of *H. theivora* infested and non-infested tea samples
2. Identification of differentially expressed lncRNAs and genes during *H. theivora* infestation
3. Prediction of lncRNA-mRNA-miRNA network during *H. theivora* infestation