

## Discussion

### 5.1 Biochemical changes in the tea plant as a result of TMB feeding

This study noted a significant alteration in the biochemical configuration of the *C. sinensis* plant as a result of TMB attack. The role of plant phenolics in plant defense against biotic agents is well stated in literature and doesn't need any broad explanation. Phenolics serve as the most ubiquitous group of plant secondary metabolites. Inhibitors, digestibility reducers, antifeedants, natural animal poisons, insecticides, and protective chemicals against invasive invaders like phytophagous insects, herbivores and nematodes are only a few of their functions. Several factors affect the differential accumulation of phenolic compounds in plants. This study showed a marked decrease in the total phenolic content in all the clones when infested by the TMB. This decline, however, was not significant in TV1, TV22, TV26, TV9 and S3A3. Reduction in the total phenolic content of the tea plant when infested by TMB was also reported previously by a study (Chakraborty and Chakraborty, 2005). Decrease in phenolic contents in response to insect infestations have also been reported earlier in literature (Czerniewicz *et al.* 2017; Gantner *et al.* 2019). Differential induction of total flavonoids has been noticed in this study. The total flavonoid content was found to be less on the infested samples than the healthy ones in all of the clones studied. Flavonoids are important defense metabolites that possess cytotoxic properties against insects and other pathogens. Some insects also utilize flavonoids for their nutritional purpose. However, whether TMB utilizes tea plant flavonoids as food is still a question and needs to be explored. To further explain the decline in the total flavonoid content in the infested tea samples and to decipher the role of tea plant flavonoids in defense against TMB needs further experimental evidence. Biotic stress like insect herbivory leads to several enzymatic alterations in the host plant

body that facilitates host plant tolerance. Mechanical tissue damage primes the production of ROS in the plant body. Neutralization of these ROS is achieved by the action of antioxidative enzymes like POX, APX, CAT, PAL, PPO etc. Accumulation of antioxidative enzymes in response to insect herbivory is a common phenomenon. In this present study also, higher activities of POX, APX, CAT, PAL and PPO have been observed in TMB-infested samples as compared with the non-infested healthy ones. Thus, we may conclude that the tea plant might have increased its enzymatic antioxidant activities to defend against the feeding pathogen. Several authors have also previously reported the increase in antioxidant enzymatic activities in plants facing any biotic challenge. These enzymes might serve as notable defense regulators in tea plant's defense against TMB.

## **5.2 Change in expression pattern of genes/lncRNAs involved in primary metabolism**

### **5.2.1 DEGs related to primary metabolism of *C. sinensis* in response to TMB**

Unlike animals, plants cannot spurt out from herbivore attack and thereby they safeguard themselves by an induced build-up of numerous deterrent/insect-repelling/defensive molecules in their tissues that either impair herbivore growth and survival (War *et al.* 2018), or provide promising positive effects on plant immunity. Researches have shown that insect infestation on plants have profound effects on plant primary metabolism. Plant primary metabolites are believed to provide cellular energy requirements since energy is crucial during induction of multiple defensive pathways regulated by hundreds of genes (Schwachtje *et al.* 2018). In respect of primary metabolism, researches have mainly focused on metabolism of carbohydrates and amino acids. Insects derive nutrition from host plants which is the basic requirement for their survival and development. Therefore, in certain instances, plants limit nutrient availability to insect pathogens as a means of

defensive measure (Zhou *et al.* 2015). In this study, as many as 50 DEGs associated with amino acids biosynthesis were found to be repressed in response to TMB. Amino acids are one of the primary sources of nitrogen, hence suppression of genes associated with amino acids biosynthesis might be a defensive measure adapted by the tea plant to prevent the insect from gaining any fitness benefit. Some studies prove to support this hypothesis. For e.g., in a recent study conducted in *Cucumis melo*, decrease in amino acids content in the melon tissues due to elevated CO<sub>2</sub> resulted in population reduction of the aphid *Aphis gossypii* feeding on the plant (Moreno-Delafuente *et al.* 2021). In another study of *Aphis fabae* feeding on low amino-acid phloem sap content of *Lamium purpureum*, the aphids experienced poor growth and high densities of bacterial symbionts (Chandler *et al.* 2008). Amino acids may also serve as determinants for host preference by insect pathogens. Since, amino acid content determines insect growth, development and host preference, therefore it can be speculated that the *C. sinensis* plant might have modulated the amino acids biosynthesis machinery to prevent further infestation by TMBs, or to limit the already feeding TMB's access to nitrogen content. As a matter of fact, amino acids also have positive central role in plant's defense against insect pathogens as they serve as precursors for the synthesis of defensive compounds (Zeier, 2013). In light of this perspective, the observed suppression of genes in this study for amino acids biosynthesis might be a result of manipulation of *C. sinensis* primary metabolism by TMB. Therefore, determining whether these observed changes are actually a means of tea plant's defense response or is a manipulation by TMB for successful pathogenesis is a challenging task. The amino acid tryptophan (Trp) acts as a precursor for phytohormone auxin and several defense related metabolites including glucosinolates (Celenza, 2001; Halkier and Gershenzon, 2006). Many studies have portrayed auxin as a negative regulator of plant

defense against pathogens and decrease in auxin sensitivity/accumulation can effectively turn on plant defense mechanism (Navarro *et al.* 2006) against insects and other pathogens (Erb *et al.* 2012). High auxin production is linked with suppression of plant defense, especially SA mediated defense against phloem-feeding insects (Wang *et al.* 2007; Erb *et al.* 2012). Insects and other pathogens can also induce auxin accumulation to suppress plant defense (Erb *et al.* 2012). Here in this study, a significant set of genes involved in tryptophan biosynthesis were found to be upregulated in expression in response to TMB. This indicates that TMB might have reconfigured *C. sinensis* metabolism by manipulating auxin signaling pathway through induced synthesis of genes associated with tryptophan metabolism. Concomitantly, as mentioned above, tryptophan is also responsible for production of the defense metabolite glucosinolates in *Arabidopsis* and hence increased tryptophan metabolism observed in this study might indicate the involvement of tryptophan in production of defense metabolites in *C. sinensis*. In this study, upregulation of transcript levels responsible for accumulation of pentose sugars and concomitant decrease in transcript abundance responsible for production of other sugars like amino and nucleotide sugars was observed. This is in line with a study conducted in Rosaceae family where accumulation of tetrose and pentose sugar alcohols resulted in decreased availability of amino and nucleotide sugars when infected by a biotrophic pathogen *Gymnosporangium asiaticum* (Lee *et al.* 2016). Limiting the access of certain sugars might be a smart defense move exhibited by the host plant and the feeding insect might find it unfavourable for survival. The amino and nucleotide sugar metabolism pathway is mainly responsible for functions like lesion repair and cell wall biosynthesis, therefore from the other perspective, it can be inferred that suppression of this pathway might be attributed to successful pathogenesis by TMB. Riboflavin plays

essential role in plant defense responses and is reported to prime defenses against bacterial pathogen *Pseudomonas syringae* pv. Tomato in *Arabidopsis* (Zhang *et al.* 2009). Riboflavins can also enhance reproduction of sap-feeding insects *Laodelphax striatellus* and *Nilaparvata lugens* (Ju *et al.* 2020). TMB feeding induced expression of genes belonging to riboflavin metabolism, therefore whether this induction of increased gene expression is a host defense response or insect manipulation of host phytochemistry is still a question. However, more comprehensive studies will be necessary to validate these speculations.

### **5.2.2 LncRNA mediated regulation of genes related to primary metabolism of *C. sinensis* in response to TMB**

Advancements in Next Generation Sequencing technologies have proved to be helpful in the discovery of lncRNAs. They might be involved in regulation gene expression of plant primary metabolism. Several researches have focused on lncRNAs associated with regulating gene expression level throughout several biological processes in plants as well as animals (Bordoloi *et al.* 2021; Baruah *et al.* 2021). Along with physical changes, insect herbivory induces chemical changes in the plant body as well including alteration of primary metabolism and these changes might be regulated by lncRNAs. In this study, 80 TMB-responsive lncRNAs were observed to target 5804 genes. These target genes were identified by analysing the *cis-trans* activity of DELs and co-expression analysis of DELs and DEGs. GO and KEGG annotation and GSEA provided evidence regarding the involvement of lncRNAs in regulating genes associated with primary metabolism. Here also, a considerable set of lncRNA target genes involved in amino sugar and nucleotide sugar metabolism were seen to be suppressed in response to TMB infestation. This might explain the possible role of lncRNAs in the tea plant in reduction of the production of

certain sugars to limit the available nutrients to the invading pathogen. Some lncRNA target genes involved in the synthesis, utilization and degradation of chlorophyll were also seen to be suppressed during TMB infestation in this study. It is evident that chlorophyll is directly associated with photosynthesis and reduction in the rate of photosynthesis has also been reported in previous studies involving insect herbivory in plants (Attaran *et al.* 2014; Wei *et al.* 2009). Photosynthesis is an energy intensive process and therefore it may be compromised during insect herbivory as a means of trade-off for the synthesis of defensive compounds (Zhou *et al.* 2015). Since nitrogen is one of the principal nutrients of organisms, therefore suppression of transcriptional machinery involved in biosynthesis of amino acids or other amino acid conjugates like N-glycans might be a result of defense mechanism of the host plant to limit the available nutrients for the insect herbivores. We have noticed in this study that a significant set of genes associated with biosynthesis of N-glycans were downregulated. Since asparagine (Asn) is one of the four amino acids primarily involved in nitrogen assimilation, therefore suppression of production of N(Asn)-linked glycans is a clear indication of the plant's defense strategy to reduce nitrogen availability for TMB. Just like DEGs, a set of lncRNA target genes involved in Trp biosynthesis also showed increased expression during TMB infestation of this study. The vitamin ascorbate is a vital nutrient for insect herbivores (Barbehenn *et al.* 2008) and can also act as a determining factor for insects for host-selection (Goggin *et al.* 2010). Upregulation of genes belonging to ascorbate and aldarate metabolism likely corresponds to higher aphid diversity in crow creek plant (Goggin *et al.* 2010). LncRNA-target genes were upregulated in ascorbate and aldarate metabolism pathway in the present study which might indicate that the feeding insect might have modified the primary metabolism to derive nutritional benefit. Ascorbate and aldarate

metabolism pathway was seen to be enriched in other plant-insect interaction studies as well (Li *et al.* 2016; Dubey *et al.* 2013; Malka *et al.* 2020). The amino acid  $\beta$ -alanine has significant role in multiple plant stress responses. Elevated  $\beta$ -alanine levels have been observed during abiotic/biotic stresses in plants such as *Medicago truncatula* (Broeckling *et al.* 2005).  $\beta$ -alanine can also be converted to plant hormone ethylene under specific circumstances (Parthasarathy *et al.* 2019). Activation of lncRNA-target genes in  $\beta$ -alanine metabolism indicates a positive defense response of the tea plant. Target genes involved in metabolism of amino acids cysteine, methionine and tyrosine got activated in response to TMB. These amino acids are possible requirements for disease tolerance in plants like *Citrus* (Killiny and Hijaz, 2016). Tyrosine has antimicrobial properties (Mach, 2015) and it has been proven to have detrimental effects on a wide variety of insect pathogens as well (Coley *et al.* 2019). Cysteine acts as a precursor for several defense compounds and antioxidants like glutathione, plays role in cyanide detoxification and is an important regulator of plant immunity (Romero *et al.* 2014). Methionine is another important amino acid that serves as a precursor for plant hormone ethylene (Romero *et al.* 2014). Moreover a few genes associated with zeatin biosynthesis could also possibly act as potential lncRNA-targets. Zeatin is a growth hormone of the cytokinin family. Zeatins can induce expression of defense compounds against insects (Schäfer *et al.* 2015), can repair damaged tissue by initiating cell division (Akhtar *et al.* 2020). The identified lncRNAs of this study were also predicted to target genes involved in brassinosteroids biosynthesis and linoleic-acid metabolism. Brassinosteroids play important function in plant-insect interaction (Yu *et al.* 2018) and linoleic-acid is involved in JA biosynthesis, which is again an important defensive phytohormone. Taken together, these findings

indicate that lncRNAs might have significantly restructured *C. sinensis* primary metabolism in response to TMB infestation.

### **5.3 Change in expression pattern of genes/lncRNAs involved in secondary metabolism**

#### **5.3.1 DEGs related to secondary metabolism of *C. sinensis* in response to TMB**

Similar to primary metabolism, this study has also shown that *C. sinensis* has undergone altered expression of genes associated with secondary metabolism in response to TMB herbivory. The role of secondary metabolites in plant defense against insect herbivores is well-established. Plants derive fitness benefits alongside defending against insect pathogens through secondary metabolites. During stress, plants also go through an alteration of secondary metabolism. Secondary metabolites may be constitutively expressed in plants during normal conditions in dormant form and get activated only in response to stress (phytoanticipins) or they may be directly induced during pathogen attack (phytoalexins). Phytoalexins like terpenoids form an important group of herbivore-induced plant volatiles (HIPVs) and are reported to have toxic or repellent properties against pests and pathogens (War *et al.* 2012). Growing biochemical and genetic studies have unfolded the significance of terpenoids in plant resistance to insect herbivores. Terpenoids can execute interplant signaling to prime neighboring plants against pathogen attack and otherwise unaffected plant parts perceive these airborne signals to activate defense responses (Sharma *et al.* 2017). Emission of volatile terpenoids after insect feeding can also indirectly protect the host plant by facilitating the recruitment of insect parasitoids or natural enemies (Gill *et al.* 2010; Arimura *et al.* 2004; Ruther and Kleier, 2005). Insect oviposition stimulates terpenoids production which in turn attract egg-parasitizing insects (Hilker and Fatouros, 2015). A significant set of DEGs were seen to



get enriched in terpenoid backbone and monoterpenoid biosynthesis pathways in response to feeding by TMB in this study. This reveals that the tea plant might have defended against the insect through upregulation of genes associated with terpenoid biosynthesis pathways. Terpenoids emission in response to insect feeding has been well-documented (D' Alessandro and Turlings, 2006). Oxidation of phenols form quinones that are directly toxic to insects and can inhibit digestion of plant proteins in insects by covalently binding to leaf proteins (Bhonwong *et al.* 2009). We have observed an increase in expression of genes associated with ubiquinone and terpenoid quinone biosynthesis. This might suggest that it is an active defense response exerted by the plant upon TMB attack. Betalains form one of the major plant pigments alongside chlorophylls, carotenoids and anthocyanins (Tanaka *et al.* 2008). Although their role in abiotic stress response in plants is prominently known (Polturak and Aharoni, 2018), their possible involvement in biotic stress responses is poorly understood. Nevertheless, a few studies have suggested their probable involvement in providing defense against plant pathogens due to their radical scavenging ability (Polturak *et al.* 2017; Sepulveda-Jimenez *et al.* 2004). Induction of betalain synthesizing genes during TMB infestation indicates the possible defense mechanism of *C. sinensis* against TMB by use of betalains. Alkaloids are another group of important toxic chemicals that have several beneficial effects on plant health and they may be responsible for recruiting pollinators and providing protection against pests and pathogens (Sato and Kumagai, 2013). They can be unfavourable for insect diet and can reduce the palatability of plant tissues. Alkaloids pose poisonous effects on infesting insects thus serving as an efficient means of plant chemical defense. During TMB herbivory, the tea plant might have undergone induced alkaloids accumulation supported by upregulation of a set of lncRNA target genes in the

indole alkaloid and isoquinoline alkaloid biosynthesis pathways. Through all of the transcript level observations in our study, it may be inferred that TMB herbivory on *C. sinensis* plant has influenced the plant's secondary metabolism and further metabolomics studies might be necessary to reciprocate these findings.

### **5.3.2 LncRNA mediated regulation of genes related to secondary metabolism of *C. sinensis* in response to TMB**

The alteration of expression of genes related to secondary metabolism is regulated by lncRNAs and it is interesting to see that lncRNA-target genes were enriched in three terpenoid related secondary metabolic pathways viz. “biosynthesis of secondary metabolites”, “diterpenoid biosynthesis”, “terpenoid backbone biosynthesis”. This clearly implies the terpenoid mediated defense exerted by *C. sinensis* plant upon the feeding insect and it is regulated by lncRNAs. Terpenoids belonging to the group of VOCs (volatile organic compounds) are known to protect plants from pathogen damage in several aspects and can regulate defense in physiological concentrations (Erb and Kliebenstein, 2020). They can aid in priming hormonal defense signaling pathways and resistance in the plants of the neighbouring area and can also induce systemic acquired resistance in the attacked plant. In addition to having a vital role in plant immunity, terpenoids can also act as regulators of growth and development (Erb and Kliebenstein, 2020). In general, a considerable set of lncRNA-target genes were enriched in the pathway “biosynthesis of secondary metabolites”. Expression of two and six lncRNA-target genes belonging to flavonoid biosynthesis pathway got downregulated and upregulated respectively in the TMB-feeding experiment performed. It is already well-known that flavonoids have plant-protective properties and have detrimental effects against insects that can negatively influence insect behaviour, growth and development

(Mierziak *et al.* 2014). This clearly implies the undeniable role of lncRNAs in reshaping the molecular landscape associated with secondary metabolism. Regulation of genes related to biosynthesis of secondary metabolites by lncRNAs might provide novel insights into the mechanism of action of these lncRNAs and might provide opportunities for further research. These observations altogether infer notable significance of lncRNAs in reshaping of secondary metabolism as a response to TMB.

#### **5.4 Interaction between DEGs and their involvement in some important defense responsive biological pathways**

The SA and JA pathways play major role during biotic stress response in plants. The SA is mainly responsible for providing defense against phloem-feeding/sap-sucking insects whereas JA helps plants to defend against chewing insect herbivores and necrotrophic pathogens (Glazebrook, 2005; Laluk and Mengiste, 2010; Pieterse *et al.* 2012; Lazebnik *et al.* 2014). Fig. 5.1 shows a hypothetical, schematic representation of DEGs involved in signaling cascades and phytohormonal pathways including that of auxin, ethylene, SA and JA. The MYC2 transcription factor is responsible for inducing JA-mediated plant defense. Genes JAZ, AOC, ACX, LOX2 are associated in the regulation of JA-mediated defense response (Erb and Reymond, 2019). Our study has revealed the upregulation of PR1 protein, which is a defensive protein produced through SA pathway and provides disease resistance (Chandrashekar *et al.* 2018). The MAPK cascade signaling also induces PR1 protein (McNeece *et al.* 2019) and the significance of MAPKs in plant-insect interaction has already been discussed. The gene tryptophan decarboxylase (TDC), which is a DEG identified in this study, is responsible for the production of defensive alkaloids through a series of processes involving amino acids tryptophan and tyrosine. Furthermore, this study has revealed that TMB infestation has resulted in the

downregulation of transcriptions factors like WRKY33, ERF which are involved in plant defense response in various ways. Taking into account the DEGs identified in this study and the biological pathways they are associated with, we have constructed a schematic molecular network to depict the probable involvement of DEGs in defense metabolic pathways and their expression reprogramming during TMB infestation on tea plant. Defense response is a complex process, therefore bioinformatics coupled with biotechnological approaches will be necessary to decipher the exact molecular frameworks of defense.

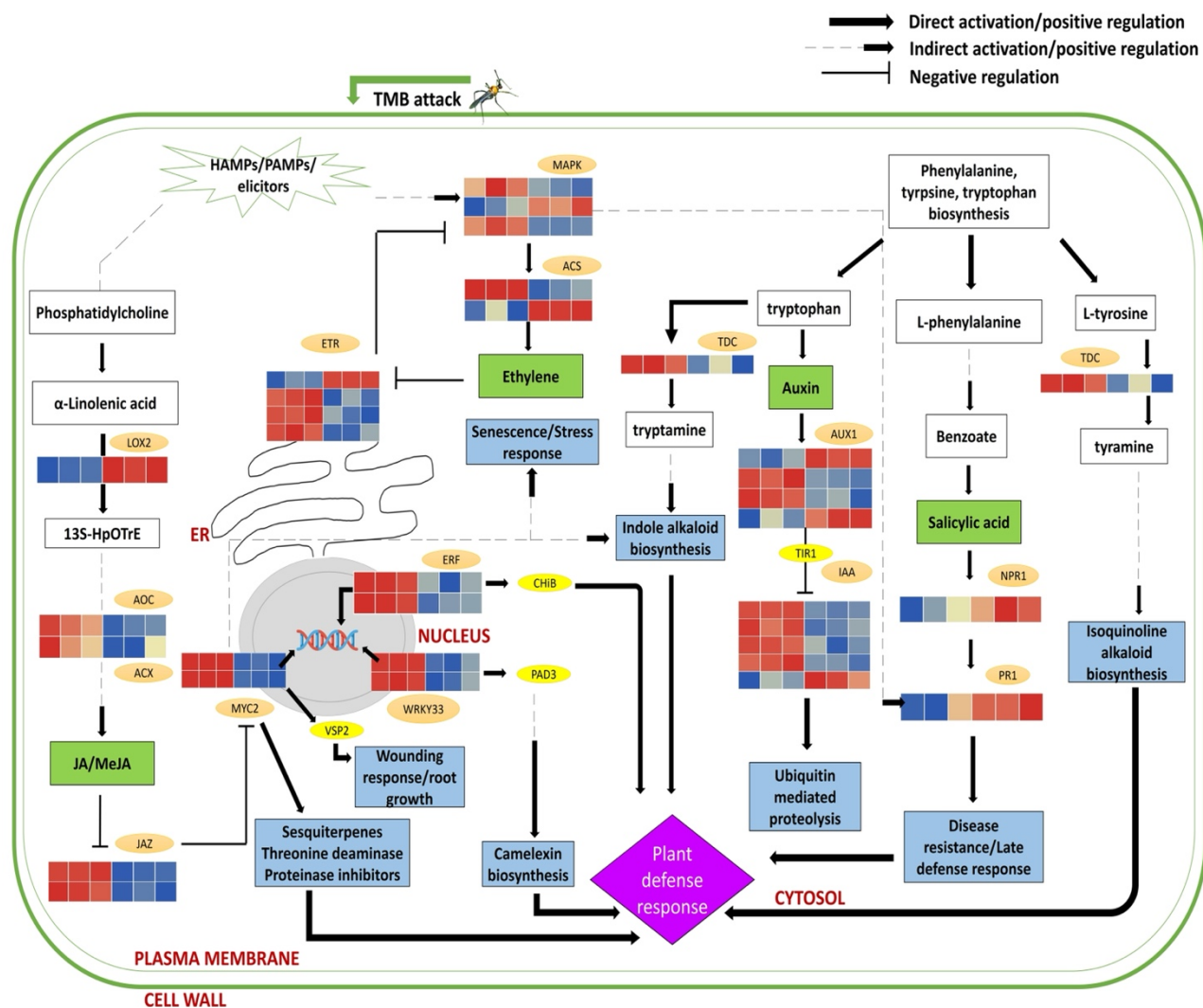


Fig. 5.1 A hypothetical schematic regulatory network representing involvement of genes in defense responsive biological pathways during TMB infestation in *C. sinensis*. Peach colored ellipses represent DEGs, yellow ellipses represent genes that are not differentially expressed during TMB infestation. Heatmaps represent expression pattern of DEGs in six RNA-seq libraries. Green rectangles represent plant hormones, blue rectangles represent plant's response to TMB feeding downstream of the pathway involved.

### 5.5 Involvement of circRNAs in tea-TMB interaction

CircRNAs were previously thought to be transcriptional noises or RNA-splicing errors in eukaryotes. However recently, high-throughput sequencing studies have provided evidence for the abundance of circRNAs as well as their functional roles in plant growth, development and response to stresses (Memczak *et al.* 2013). The availability of NGS technology coupled with bioinformatics approaches facilitates the genome-wide exploration of circRNAs in eukaryotes. Identification of circRNAs in plants subjected to various types of treatments have been reported in a number of studies (Ye *et al.* 2015; Pan *et al.* 2018; Liu *et al.* 2017; Lu *et al.* 2017; Zhao *et al.* 2017a; Lu *et al.* 2015; Zuo *et al.* 2016; Darbani *et al.* 2016; Wang *et al.* 2017b; Chen *et al.* 2017b). Nevertheless, as compared to studies performed in animals, circRNAs in plants have been less explored (Zhao *et al.* 2019). Also, a very few studies have been conducted regarding profiling of circRNAs during plant-insect interaction (Zhao *et al.* 2017b). Here we reported a very first survey concerning the identification of circRNAs in tea plant in relation to insect infestation. We obtained a total of 709 circRNAs in six RNA-seq libraries. The number of identified circRNAs in this study was lower than those identified in other plants such as *Oryza sativa* (12037) (Ye *et al.* 2015), *Pyrus betulifolia* (889) (Wang *et al.* 2018), *Arabidopsis* (6012) (Ye *et al.* 2015) and tomato (854) (Zuo *et al.* 2016). In this study, the CIRI tool identified hundreds of circRNAs in each sample. But, unlike other studies (Ren *et al.* 2018), where majority of the circRNAs belonged to the exonic category, most of the identified circRNAs of this study were categorized under intergenic group (62%) followed by exonic category (35%) and the circRNAs under the intronic category (3%) was the least (Fig.1). However, a similar proportion of intronic circRNAs was also

identified in other reports i.e., in Arabidopsis (3.8%) (Chen *et al.* 2017a), tomato (3.6%) (Zuo *et al.* 2016) and wheat (6.5%) (Ren *et al.* 2018).

NcRNAs like lncRNA and circRNAs are reported to function as eTMs, thereby sequestering miRNA active level (López-Urrutia *et al.* 2019). Thus, it has been well established that ncRNAs like circRNAs can have considerable roles in regulating gene expression pattern in eukaryotes. Studies have indicated that miRNAs involve genes and also other ncRNAs to actively take part in gene regulation in plant-insect interaction (Bordoloi and Agarwala, 2021). Analysis of miRNA sponging activity revealed that DECs Csi\_circRNA\_282, Csi\_circRNA\_219, Csi\_circRNA\_394 and Csi\_circRNA\_331 could possibly serve as mRNA decoys. These mRNAs/genes have been annotated to function as LecRLK, phospholipase, DNA helicase, aspartyl protease, tropinone reductase, condensin complex subunit. Lectins are glyco-proteins, act as pest-repellent agents and lectin receptors serve the function of recognizing and interacting with certain carbohydrates derived from wounded plant tissues or from invading pathogens (War *et al.* 2012; Lannoo and Damme, 2014). LecRLKs have recently emerged as another important member of the receptor-like kinases (RLK) family for their significant roles in plant innate immunity (Sun *et al.* 2017). LecRLKs may be able to identify a wide variety of ligands due to the variations in the lectin domain. A growing body of evidence suggests that LecRLKs are significant players in the interactions between plants and microbes, just as many other RLKs have been thoroughly defined for their roles in recognizing PAMPs and in providing plant defense. The perception of PAMP signals has been shown to activate a number of L-type LecRLKs, which then cause PTI with a series of signalling cascades involving protein phosphorylation. Along with providing response to bacterial and fungal pathogens, LecRLKs are also reported to be involved in perceiving insect

oviposition and feeding and play role in SA-JA crosstalk during insect herbivory (Sun *et al.* 2020c). Provided the noteworthy functions of LecRLKs in plants response to biotic stress, the identified DEC mimicking *C. sinensis* LecRLK might be indicative of the significant role played by circRNAs in tea plant's response to *H. theivora*. Aspartyl proteases are reported to provide defense to plants against pathogens by inducing the expression or activating defense associated genes in several plants like tomato, potato (Chen *et al.* 2014; Guevara *et al.* 2002; Figueiredo *et al.* 2021). In a study in tomato plant, the cleavage of a PR protein by aspartyl protease shown to release DAMPs which indicates the activation of the systemic acquired resistance (SAR) (Chen *et al.* 2014). Enzymes like phospholipase C are known to induce the accumulation of proteinase inhibitors (PI) in corn (Acevedo *et al.* 2018; Chen and Mao, 2020). Phospholipases trigger the ETI or PTI during defense responses in plants and their role in plant defense has been extensively reviewed (González-Mendoza *et al.* 2021). Alkaloids are well-known secondary metabolites that pose poisonous effects towards insect pests (War *et al.* 2012). Tropinone reductases are key enzymes associated with tropane alkaloid metabolism (Jirschitzka *et al.* 2012). But to have a clearer picture about whether circRNAs identified in this study are directly associated with regulating alkaloid biosynthesis in the plant in response to TMB infestation, further validation including metabolomics studies coupled with transgenic approaches will be necessary. Nevertheless, prediction of circRNAs acting as mimics of certain significant genes having efficient roles in defense against pest attacks, might be indicative of the importance of the still less understood/explored group of ncRNAs i.e., circRNAs in tea plant.

The tea plant's response to TMB is a complicated process which involves thousands of genes in several biological pathways. Genes associated with



nucleocytoplasmic transport include nucleoporins and nuclear transport receptors. Plant nucleocytoplasmic transport is an important process both for hormone signaling and response to foreign stimuli. Components of nucleocytoplasmic transport are directly/indirectly related to plant immunity (Tamura and Nishimura, 2014). Our study also predicted that the DEC-target genes were upregulated in the nucleocytoplasmic transport pathway. The role of terpenoids and other secondary metabolites in providing plant immunity and in defending against pests and pathogens has been well established (War *et al.* 2012). Secondary metabolites serve as defense compounds against herbivores as well as feeding insect pests. They are well-known to negatively influence insect survival and mortality (War *et al.* 2012; Sharma *et al.* 2017). Terpenoids belong to the group of VOCs (volatile organic compounds) and are well-known plant protectors. They are responsible for priming danger signals to neighbouring plants against a pathogen attack and they also possess plant-protective, insect-repellent and antioxidant properties that are crucial for defense response. Their involvement in plant immunity is undeniable and they can also regulate various plant metabolic processes like growth and development (Erb and Kliebenstein, 2020). Therefore, it is not surprising to see that several DEC-target genes belonging to pathways like diterpenoid biosynthesis were upregulated when TMB was allowed to infest upon tea plant. Tryptophan (Trp) is an essential amino acid and it serves as a precursor for the biosynthesis of plant hormone auxin and other defense metabolites (Celenza, 2001; Halkier and Gershenzon, 2006). We have seen that a significant number of DEC-target genes belonging to Trp metabolism pathway got activated. Several studies have described auxin as a negative role player in plant defense where the authors indicated that decreased sensitivity to auxin could possibly enhance defense response in plants (Navarro *et al.* 2006). Auxin accumulation can be correlated

with decreased SA-mediated plant defense response against insect herbivores (Erb *et al.* 2012). Insects can also manipulate host plant metabolism by increasing auxin production, thereby suppressing plant defense (Erb *et al.* 2012). A significant number of DEC-target genes belonging to pathways of N-glycan biosynthesis and porphyrin metabolism were seen to get suppressed during TMB infestation. The probable reduction in the production of N-glycans might be attributed to a kind of defense strategy adopted by the tea plant to limit the access of nitrogen to the feeding insect as because asparagine (N), is one of the primary amino acid requirements for nitrogen assimilation. Since porphyrin is directly associated with photosynthesis in plants (Mauzerall, 1977), therefore it might be speculated that TMB stress negatively influences the rate of photosynthesis in the plant. This might also be viewed as a kind of defensive response of the plant so as to save energy for the production of defensive metabolites by compromising an energy intensive process like photosynthesis. Reduction in the rate of photosynthesis has also been observed in certain plant-insect interaction studies as well (Attaran *et al.* 2014; Wei *et al.* 2009). This study has shown that genes targeted by DECs were also enriched and upregulated in pathway “ascorbate and aldarate metabolism”. Ascorbate is vital for insect performance and host-selection (Barbehenn *et al.* 2008; Goggin *et al.* 2010). Similar reports showing activation of "ascorbate and aldarate metabolism" pathway have been well documented in literature (Dubey *et al.* 2013; Malka *et al.* 2020). The identified DECs targeting genes involved in the aforementioned pathways might be an indication about how the tea plant uses non-coding RNAs like circRNAs to fine tune gene expression during insect feeding. However, a lot of work combining in silico and molecular approaches are needed to be done to clarify how circRNAs play such gene expression regulatory role.