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Review of literature

2.1 History, taxonomy, cultivation of tea and its challenges

The first authentic account of tea dates back to 780 A.D., when Lo-Yu, a Chinese author, first described tea's manufacture in his book "Cha Ching". The people of China have cultivated the tea plant for more than 2000 years on numerous small plots of land as they were quite aware of the tea plant's importance and that it could be of great commercial success and could serve as an appetizing drink after processing. Though it was first used as a medicine by the people of Burma and Siam, but as time passed, this drink made from boiling leaves functioned as a palatable drink for the people. It was not until the end of the 6th century that the Chinese treated tea as a beverage rather than a mere medicinal drink. Over the centuries, the tea plant got spread to other parts of the world from its original site to Assam, Yunan, Siam and Burma (Weatherstone, 1992). The China variety, which is characterized by small and narrow leaf is reported to be true-bred that underwent minimal cross-breeding whilst the Assam variety was subjected to hybridization whilst the tea found growing in the Shan States of Burma and Siam has been the most hybridized. The tea plant grows till a height of 30 to 40 feet if left undisturbed and is native throughout the South-east Asian forests. Major Robert Bruce, the then resident of the province of Assam, was made known about the existence of tea in the year 1823. He then conveyed the information to Charles Bruce, under whose leadership, the first tea cultivation was performed in the upper Assam region. North-east India happens to be the pioneering region where the government conducted the experiments for cultivation of both Assam and China teas. In the year 1837, the first tea, manufactured in the upper Assam region, was exported to Calcutta in bottles down the

river. Reportedly, the first tea plantation in Darjeeling could be dated back to the early 1850s where seeds of the China variety were planted. This China variety was found to be suitably grown in Darjeeling's hills. Subsequently, the first tea gardens were opened in 1857 in Terai and in 1874 in the Dooars (Weatherstone, 1992).

The genus *Camellia* comprises of about 82 species and they are native mainly to South-east India (Sealy, 1958). Out of all the reported species of *Camellia*, tea is considered to be the most commercially imperative of all. However, tea taxonomy is well-debated throughout the decades. Tea plant bears diverse morphological, biochemical and physiological features (Wickremasinghe, 1979; Banerjee, 1988). Originally, tea was described by Linnaeus (1752) as *Thea sinensis*. Further exploration and collection of tea led to the discovery of two distinctive forms of the plant- the narrow and small-leaved China variety originally described as *T. sinensis*, and the broad-leaved Assam variety designated originally as *T. assamica* (Masters, 1844). Sealy (1937) assigned the mostly cultivated varieties under the genus *Camellia*, but due to the great deal of morphological, anatomical and biochemical similarities between *Thea* and *Camellia*, it was later on decided to retain the single generic name *Camellia* (Wight, 1962). Kitamura (1950) and Sealy (1958) classified tea plant based on leaf structure and growth habitat into intra-specific forms of *C. sinensis* (L.), viz., the China variety, *Camellia sinensis* var. *sinensis* (L.) and the Assam variety, *Camellia sinensis* var. *assamica* (Masters) Kitamura. But later on, Wight (1962) categorized tea plant based on the characteristics of styles to differentiate between species and sub-species. He considered the nomenclature *C. sinensis* (L.) for *C. sinensis* var. *sinensis* (L.) and *C. assamica* for *C. sinensis* var. *assamica* (Masters). In addition, Wight (1962) recognized a third form of tea plant, originally described as the southern form of tea (Roberts *et al.* 1958) or Cambod race

(Kingdon-Ward, 1950), which resembles the Planchon's *Thea lasiocalyx* (Barua, 1963) to be a sub-species of *C. assamica* and therefore named it to be *C. assamica* ssp. lasiocalyx (Planchon ex Watt). However, the present-day tea is botanically named as *Camellia sinensis* (L.) O. Kuntze, regardless of species-specific dissimilarities.

With the advent of time, tea, an important industrial crop, has received extensive cultivation across a dozen countries with its top 10 countries including India, China, Kenya, Argentina, Sri Lanka, Turkey, Indonesia, Vietnam, Japan and Iran. India bags the second position in the amount of tea production, preceded by China. India alone produced 1.2 billion kilograms of tea in the fiscal year 2022 (www.statista.com). A survey conducted in the year 2018 showed that 6.37 lakh hectares of land was cultivated for tea production in India. The availability of improved nutrients, technology, fertility management and introduction of clones having high yielding capacity have favoured this extensive rise in tea cultivation across the world. This widespread cultivation of tea has grabbed the attention of many disease-causing microorganisms and insect pests. Like any other agricultural crop, tea poses as a potential target crop for pests as well as diseases, as it affords a suitable micro-climate and consistent food supply for several pests and insects. Tea is known to be a target crop for more than 300 insect species (Hazarika *et al*. 2009). Although majority of them are seasonal, yet the leaf chewing pests are the most serious as feeding by such insects cause severe tissue loss. Tea diseases are mostly fungal in origin, though a few have also been reported to occur from algae, viruses and bacteria. Its commercial cultivation in monocultural conditions and the alterations made in the habit of the plant to maintain it in a low height for easy pruning and plucking have invited the occurrence of diseases in the plant and hence tea plant cultivation has long been a challenge for the tea growers all around the globe.

2.2 Plant-insect interaction – an overview

Natural interactions between plants and insects display distinctive genetic, biochemical, and molecular network patterns that cover several levels of biological organization (Schoonhoven *et al*. 2005), from cytological and genetic subcellular mechanisms to molecular and biochemical activities (Kessler and Baldwin 2002) at the level of the ecological community (Kessler and Halitschke 2007). The cell surface receptors or pattern recognition receptors (PRRs) are responsible for the first level of pathogen recognition (referred to as PAMPs- triggered immunity or PTI) by sensing herbivore and pathogen-associated molecular patterns (HAMPs and PAMPs) originating from microbial pathogens or damage-associated molecular patterns (DAMPs). PTI or basal resistance is accompanied by a number of intracellular reactions, including accelerated ion fluxes across the plasma membrane, generation of reactive oxygen species (ROS), stimulation of calcium-dependent mitogen-activated protein (MAP) kinases, accelerated expression of genes involved in defense (Thomma *et al*. 2001). Certain pathogens have successfully evolved to circumvent basal resistance, often by virulence effectors that decrease PTI. In response, plants have developed a second line of defense through resistance (R) proteins that can efficiently detect these pathogen effectors either directly or indirectly by means of effector-triggered immunity (ETI) (Zipfel, 2008). ETI is frequently complemented by the hypersensitive response and is linked to added local defense responses that stop the spread of pathogens (De Wit, 2007).

Lately, isolation of many HAMPs from insect pests has contributed to the growing knowledge regarding plant-insect interaction. Presence of a peptide fragment, inceptin, in *Spodoptera frugiperda* oral secretions (OSs), is highly indicative of it being an efficient defensive inducer in maize and cowpea (Schmelz *et al*. 2006). *Spodoptera exigua* OSs

containing radiolabeled volicitin (17-hydroxylinolenoyl-L-Gln) binds to an undiscovered putative PRR on the maize plasma membrane with great affinity (Truitt *et al*. 2004). The brown planthopper (BPH) (*Nilaparvata lugens*) is reported to contain a mucin-like protein that is responsible for inducing defense responses in rice (Schweizer *et al*. 2013). The role of a leucine-rich repeat receptor kinase (LRR-RK) in rice has been emerging to be an effective molecule for plant perception and defense against the striped stemborer (SSB), *Chilo suppressalis* (Hu *et al*. 2018). Certain components present in insect OSs can liberate defense response without the involvement of PRRs. For example, glucose oxidase, an active ingredient of salivary secretions of caterpillars, can oxidize glucose to form the defense molecule hydrogen peroxide (H_2O_2) that can effectively produce defense response by diffusing through membranes (Acevedo *et al*. 2015). Glucose oxidase has also been reported to induce defense response in tomato (Louis *et al*. 2013). Morever, *Schistocera gregaria* OSs can induce 12-oxo-phytodienoic acid (OPDA) which in turn is responsible in releasing defense hormone from membrane lipids (Schäfer *et al*. 2011). Mechanical injuries resulting from chewing herbivores lead to the release of DAMPs that induce defense responses. Some sensors like THESEUS1 and FERONIA have been reported from plants that sense the cell-wall integrity and therefore help in responding against any cell-wall modifications (Hématy *et al*. 2007; Stegmann *et al*. 2017). However, further research is necessary to describe their probable role in defense against herbivores. Mechanical damage in tissues induces the accumulation of several molecules into the apoplastic space that are further perceived to activate the defense responses in the plant. Wounding prompts the production of certain signals referred to as secondary danger signals in the plant body that migrates and induces defense activities throughout the plant. Systemin, an 18 amino acid long peptide, has been identified as a

secondary danger signal from tomato plant that causes the accumulation of JA-responsive proteinase inhibitors in the plant upon wounding, thus negatively impacting chewing herbivores (Orozco-Cardenas *et al*. 1993). Certain receptors like SYR1 have binding affinity with systemin, and mutants lacking SYR1 showed decreased resistance to *Spodoptera littoralis* (Wang *et al*. 2018). Among the other molecules released apoplastically upon mechanical injuries are ATP and NAD(P). Exogenously applied ATP induces an increase in Ca^{2+} concentration in the cytosol and the activation of JA-responsive genes, indicating that ATP perception is associated with the downstream JA signalling (Choi *et al*. 2014; Tripathi *et al*. 2018). A plasma membrane bound receptor named DORN1 (DOES NOT RESPOND TO NUCLEOTIDES 1) has been found to bind and perceive ATP in damaged cells (Choi *et al*. 2014). NAD(P) has been identified in the extracellular space of wounded *Arabidopsis* leaves. Discovery of LecRK-I.8 has shown that it binds with high affinity with its ligand NAD^+ that gets released by wounded leaves of *Arabidopsis* plant, thus activating defense response. LecRK-1.8 has also been recognized as a defense inducer against *Pieris brassicae* infestation in *Arabidopsis* and *Manduca sexta* feeding on *N. attenuata*, but the answer for the ligands in question remains unknown (Gouhier-Darimont *et al*. 2013; Erb and Reymond, 2019).

Membrane depolarization and rise in cytosolic Ca^{2+} concentration are some of the early signaling events that take place within minutes of mechanical damage. This increase in cytosolic Ca^{2+} concentration is driven by certain membrane channels that encourages the passage of Ca^{2+} into the cytosol, followed by sensing of these Ca^{2+} ions by various known Ca^{2+} sensors to activate defense (Erb and Reymond, 2019). Among the known ion channels are the TWO-PORE CHANNEL1 (TPC1), the gain-of-function mutation of which exhibits an enhanced resistance to insect herbivores like *S. littoralis* (Bonaventure

et al. 2007). Another ion channel called the glutamate receptor-like (GLR) ion channels are also seen to take part in *Arabidopsis-S. littoralis* interaction, as mutants deficient in GLR channels showed increased susceptibility to the feeding insect (Nguyen *et al*. 2018). Various sensors like calcium-dependent protein kinases (CDPKs), calmodulins (CaMs), calmodulin-like proteins (CMLs) decrypt these Ca^{2+} signals. These Ca^{2+} sensors are reported to affect plant defense in both positive and negative ways. For example, a CML (CML37) positively affects defense in *Arabidopsis* against *S. littoralis* through enhanced activation of the JA-pathway (Scholz *et al*. 2014). However, another CML (CML42) negatively affects resistance to *S. littoralis* (Vadassery *et al*. 2012). CDPKs positively regulate ROS burst by activating respiratory burst oxidase homologs (RBOHs) proteins that are responsible for production of ROS. This herbivory induced local and systemic oxidative burst can influence plant resistance in both positive and negative ways. As for instance, rbohD/F mutants of *Arabidopsis* exhibited increased resistance to *Spodoptera exigua* (Block *et al*. 2018). Contrastingly, rbohD mutants were seen to be more susceptible to *Myzus persicae* and *S. littoralis* feeding (Miller *et al*. 2009; Wu *et al*. 2013). Another group of protein kinases called the Mitogen-Activated Protein Kinases (MAPKs) also get stimulated upon insect herbivory and mechanical injury. *C. suppressalis* feeding on rice triggers MPK3 and MPK6 that in turn activate the defense hormone JA pathway and subsequent JA accumulation. mpk1 and mpk2 mutants exhibited susceptibility to *M. sexta* and *Macrosiphum euphorbiae* (Kandoth *et al*. 2006; Li *et al*. 2007).

Plant responses to insect herbivory is a complicated process and the idea of numerous hormones synchronically playing together forming a hormonal crosstalk cannot be ruled out. Additionally, induced plant secondary metabolites have been emerging as significant defense regulators that can implement defense action in the plant

body. Phytohormones are well-recognized modulators of plant defense and their effects on the defensive response of the plant against insect pathogens is favorably context dependent. SA is known to mainly provide defense against sap-sucking or phloem-feeding insects whereas JA facilitates defense against chewing herbivores. The SA-JA antagonism has been a well-talked topic in plant-insect relation. SA has been recognized as a negative regulator of JA signaling, thus implying that SA accumulation in the plant suppresses JA-mediated plant defense. In certain instances, phytohormone *Et al*so has been seen to antagonize JA by suppressing JA responsive defense pathway (Song *et al*. 2014). ABA, a well-known regulator of plant abiotic stress response, is also responsible for activating the downstream JA signaling and for reinforcing resistance to chewing insect herbivores. Mutants deficient in HERBIVORE ELICITOR REGULATED 1 (HER1), an inhibitor of ABA catabolism, showed less JA accumulation and increased susceptibility to *M. sexta* (Dinh *et al*. 2013). It can thus be concluded that the impression of phytohormones SA, JA, ET and ABA differs among different plant species and herbivore feeding guilds. Effect of other growth hormones like gibberellins (GAs) on plant defense response is also noteworthy. GAs negatively regulate downstream JA-signaling by degrading DELLA repressors, which are positive regulators of JA signaling. A study shows that GA accumulation leads to increased resistance to BPH in rice, implying that GAs positively impact defense against phloem-feeders (Li *et al*. 2015).

2.3 Plant biochemistry during plant-insect interaction

Both direct and indirect defenses are used to mediate the highly dynamic biochemical systems of defense against herbivores. The defensive chemicals have an impact on herbivore eating, growth, and survival and are either produced constitutively or in reaction to plant harm. Additionally, plants exude volatile chemical substances that

draw the herbivores' natural foes. These tactics are used separately or in combination with one another. As a result of evolutionary competition between plants and insects, plants have evolved a sophisticated defense mechanism that can identify nonself molecules or signals from injured cells, much like animals can, and thereafter initiates the plant immune response. By influencing host plant selection, survival, and reproductive success, plants directly compete with herbivores. Indirectly, they do so by influencing other species, such as natural enemies of insect pests (War *et al*. 2012). The synthesis of toxic compounds such terpenoids, alkaloids, quinones and phenols that either kill or delay the development of herbivores are examples of direct defenses that have an impact on the biology of the herbivore. Plant mediated indirect defenses can come into action by releasing a combination of volatiles that attracts the herbivores' natural enemies particularly, as well as by giving them food and shelter to increase their efficiency, and as such pathogenic insects can be thwarted. Plant phenols are one of the most prevalent and frequent groups of defensive chemicals among secondary metabolites, and they play a significant role in defense against herbivorous pathogens. Phenols serve as a defense strategy for plants not just against herbivores but also against competitive plants and microbes. It is a common occurrence for phenols to change both qualitatively and quantitatively and for oxidative enzyme activities to increase in response to insect attack. Phenols are crucial for the cyclic reduction of Reactive Oxygen Species (ROS), which in turn triggers a series of events that activate defense-related enzymes. One possible defense mechanism used by plants to fend off herbivorous insects is the oxidation of phenols, which is mediated by enzymes polyphenol oxidase (PPO) and peroxidase (POD). Quinones, which are created when phenols are oxidised, bond covalently to the proteins in leaves and prevent herbivores from digesting them (Bhonwong *et al*. 2009).

Additionally, quinones are directly poisonous to insects. Flavonoids have cytotoxic abilities and by affecting an insect's behaviour, growth, and development, flavonoids defend plants against insect pests. Furthermore, flavonoids chelate the metals and scavenge free radicals, particularly ROS, to lessen their production (Treutter, 2006). Detoxification of ROS activity can be obtained by antioxidative enzymes. Defending enzymes such peroxidase (POX), polyphenol oxidase (PPO), phenylalanine amino lyase (PAL), ascorbate peroxidase (APX), and catalase (CAT) catalyse chemical processes to detoxify ROS generated in plants. Insect feeding is directly discouraged by the PODs' production of phenoxy and other oxidative radicals in conjunction with phenols. These PODs also produce toxins that lessen tissue palatability and digestibility, which in turn causes nutrient deficiencies in insects that severely impact their growth and development. Additionally, it has been found that PODs are directly poisonous to herbivores' stomachs (Zhu-Salzman *et al*. 2008). PPOs are responsible for catalyzing the conversion of phenols to quinones that suppress the nutritional quality of the insect's food (War *et al*. 2012). APX, PAL and CAT are important enzymes of the plant antioxidative system and are influenced by biotic and abiotic factors affecting plants.

2.4 Transcription factors involved in plant-insect interaction

The role/involvement of transcription factors (TFs) in plant-insect communicatory network is definite and they are known to play key roles in modulating plant immunity. The MYC TFs contain a basic helix-loop-helix (bHLH) domain and are well-studied in the area of stress biology. Role of MYC TFs is prominent in defense responsive pathway involving JA and in the downstream signaling of genes for synthesis of proteinase inhibitor proteins, sesquiterpenes as defense metabolites (Erb and Reymond, 2019). MYCs function in regulation of defense against herbivory in plants like

Arabidopsis. Herbivory triggers the activation of JA pathway leading to degradation of JAZ, that are MYC repressors and are normally in action in absence of herbivory (Erb and Reymond, 2019). MYC TFs regulate JA-mediated plant defense response and mutants deficient in MYC2, MYC3, MYC4, and MYC5 exhibit susceptibility to attack from insects like *S. littoralis* and *S. exigua* (Fernández-Calvo *et al*. 2011; Song *et al*. 2017). Glucosinolates (GS) are a class of defensive molecules of plants belonging to Brassicaceae and they have inevitable roles in plant protection and immunity. Biosynthesis of GS requires the contribution of MYC and MYB TFs (Gigolashvili *et al*. 2009; Schweizer *et al*. 2013). The MYB-MYC unit is an efficient regulatory system controlling the biosynthesis of GS in *Arabidopsis*. MYBs (MYB28, MYB29, MYB34, MYB51, MYB76 and MYB122) directly associate with MYCs (MYC2, MYC3, and MYC4) and control the biosynthesis of tryptophan and methionine-derive GS in *Arabidopsis*. MYCs can also directly regulate biosynthesis of defense metabolites and expression of antiherbivore genes. Expression of genes coding for defensive antiherbivore proteins like threonine deaminase, PI proteins are also known to be regulated by MYC TFs. They can effectively bind with promoters of GS biosynthetic genes in *Arabidopsis* and TPS genes that are important for response to environment stimuli as well as regulation of synthesis of sesquiterpenes (Hong *et al*. 2012). Lately, MYC2-LIKE and WRKY TFs also have been proved to negatively impact plant immunity. Studies show that JA-ASSOCIATED MYC2-LIKE mutants exhibited improved resistance to *S. exigua* (Sasaki-Sekimoto *et al*. 2013; Song *et al*. 2013). JAV1 and JAZ are negative regulators of JA-mediated plant defense. WRKY51 associates with JAV1 and JAZ molecules to repress JA pathway. WRKY TFs are also responsible for shutting down excessive JA production by interacting with AOS (Allene oxide synthase)

when there is no attack or herbivory (Yan *et al*. 2018). WRKY TFs also take part in the negative feedback loop of expression of MAPKs in rice. As discussed earlier, MAPKs have significant role in response to herbivory, but their over-production might not be a useful strategy. Direct phosphorylation of WRKY53 by rice MPK3 and MPK6 can in turn activate WRKY53 for inhibiting their activity, thus forming the negative feedback loop (Hu *et al*. 2015).

2.5 Taxonomy, life cycle of *H. theivora* and its infestation on tea

A number of phenotypic characters like the presence of pale antennal segment I, long and erect setae at most parts of segment II and III, readily distinguish the *H. theivora* from its relatives (Stonedahl 1991). This sap feeding insect bears many common names viz. mosquito bug, tea bug or tea mosquito bug (TMB). *H. theivora* is characterized by a broad lateral stripe at the head, pale pronotum and lateral edges of abdominal sterna I-IV (Stonedahl 1991). Molecular classification of *Helopeltis* spp. based on the mitochondrial cytochrome oxidase-I (COX-1) DNA sequence was successfully conducted by Rebijith *et al*. (2012). Schuh (2002-2013) reported *H. febriculosa Bergroth, H. oryx* Distant*, H. theobromae* Miller*, H. theivora theobromae* Miller *and Afropeltis theivora* to be synonymous with *H. theivora* Waterhouse. The genus is geographically distributed across both the hemispheres. TMB arises in all tea-growing regions of the world and has been identified as a devastating pest of tea as well as cashew plants. Tea is the primary and favourable host of TMB, but apart from tea, it also feeds on a wide range of plant species and causes significant devastation like cashew, *cocoa*, *Acacia*, pepper. TMBs can breed throughout the year and survive pesticides or adverse environmental conditions even during unavailability of host by living on some noncrop hosts until the favourable host is available. TMB can subsist on a number of major and alternate hosts, yet, its functioning

and survival is found to be better only on tea plant. Evidences also show that tea is the most suitable host for TMB as because its growth and survival rate, fecundity etc. were reported to be higher on tea plant when compared with other hosts. Gope and Handique (1991) provided an account of lifecycle and biology of TMB on Assam tea and Roy *et al*. (2009) from Dooars tea plantation. Typically, a TMB takes two weeks to complete its life cycle during summer season, and approximately 5 to 6 weeks during winter season (Das, 1984). The adult male and female are to some extent morphologically different. The female is larger than the male with an orange pronotum and green or white abdomen in contrast with the thin and smaller male with black pronotum and bluish abdomen. The females lay oblong-shaped eggs preferably on petioles, stems, midribs of leaves and soft parts of the bush. The nymphs emerge after a brief incubation period, which varies according to seasons, less than a week in summers and more than two weeks in winters. The nymphal stage lasts for about approximately 16 days in general (Das, 1957, 1965). Climatic conditions can greatly influence TMB's performance. Humid weather and precipitation are key climatic factors that highly favour the growth, reproduction and performance of TMBs. The adults and nymphs suck the phloem juice from young and succulent parts of the shoots like buds, young leaves, tender stems through the stylet. Release of their toxic salivary secretions trigger tissue damage and within 24 hrs of feeding, the punctured area becomes gradually turns brownish in colour forming brown spots all over the feeding area. Subsequently, the attacked part becomes unsuitable for plucking and processing. TMB exhibits high levels of resistance to commonly employed insecticides like endosulfan, cypermethrin, deltamethrin, quinalphos. This pest has brilliantly developed the capability to withstand these toxic pesticides by enhancing the detoxifying activity of its enzymes (Roy *et al*. 2015).

Substantial loss in quantity as well as quality of tea has been a result of TMB incidence on tea plantations. As mentioned earlier, the nymphs and adults use their mouthparts to suck the sap juice of the plant from tender and young shoots producing brown feeding spots within few hours of sucking. As a consequence, serious commercial loss of the crop occurs as because the feeding shoots curl up and dry making it unsuitable for further harvesting and processing. Moreover, oviposition results in over-callusing and stem cracking, which also stunts development and promotes stem dieback. (Das 1965; Roy 2008; Sudhakaran 2000). Technically, TMB feeding results into two types of damages- damage of pluckable shoots and severe exhaustion of the bushes as an outcome of dieback thus rendering the bushes to delay flushing and to provide poor harvests (Rao 1970). Being the nastiest enemy of tea plant, TMB feeding has resulted in about 55% crop loss in Africa (Rattan 1992) and about 11-100% loss in Asia (Muraleedharan 1992a, 1992b). Previously, DDT use proved to manage this pest effectively but since its discontinuation, the TMB re-established itself as the most serious pest of tea. (Banerjee 1983; Das 1984). TMB has gained the nation's importance as the most devastating pest and it has been concluded that India accounts for about 50% of the crop loss due to TMB attack on almost 80% of its tea growing regions (Bora and Gurusubramanian 2007; Roy *et al*. 2008; Roy and Gurusubramanian 2013). Bangladesh reports for about 100% of crop loss due to TMB in certain cases (Ahmed *et al*. 2011).

2.6 Non-coding RNAs – their potential in plant developmental processes and stress response

While 90% of the eukaryotic genome gets transcribed into RNA, only a little (2%) proportion of the transcribed RNA undergo translation and form proteins (Pauli *et al*. 2011, Rai *et al*. 2019). The rest can be designated as noncoding RNAs (ncRNAs) that

serve numerous molecular functions ranging from signal transduction, metabolic regulation, development as well as stress responses. Previously, these noncoding transcripts were thought to be transcriptional noise due to their poor protein-coding potential (Ariel *et al*. 2015, Pauli *et al*. 2011). But the advent of next-generation sequencing technologies coupled with efficient tools and techniques facilitated the understanding of noncoding RNA biology and their functional value and significance. NcRNAs arise from different locations of the genome such as intergenic regions, transposons etc. NcRNAs can be divided into a few classes viz. (a) small RNAs that are typically 18-30 nucleotides long. This includes miRNAs, small interfering RNAs (siRNAs), natural antisense transcript-derived small interfering RNAs (nat-siRNAs), repeat-associated small interfering RNAs (ra-siRNAs), trans-acting small interfering RNAs (ta-siRNAs), heterochromatic small interfering RNAs (hc-siRNAs), secondary transitive siRNA and long small interfering RNAs (lsiRNAs) (b) medium sized ncRNAs (31-200 nt long) (c) long non-coding RNAs (lncRNAs) that are identified to be more than 200 nt in length (d) circular RNAs (circRNAs) that are formed by a non-canonical form of alternative backsplicing resulting in the covalent circularization of a 3′ downstream donor and the 5′ upstream acceptor. These set of ncRNAs are associated with diverse cellular processes and serve undeniable roles during the life cycle of eukaryotes. A brief description of different types of ncRNAs and their functional roles have been summarized below-

2.6.1 miRNA

MicroRNAs (miRNAs) are a class of endogenous small noncoding RNAs of 19-24 nt in length that negatively regulate gene expression. Biosynthesis of miRNAs in plants, just like in animals, includes multiple steps. Transcription of the miRNA genes to form

primary miRNA (pri-miRNA) by RNA polymerase II is the first step of miRNA biogenesis. This is followed by processing of pri-miRNA to form precursor mRNA (pre-mRNA) by the enzyme DICER-LIKE1 (DCL1) in assistance with HYPONASTIC LEAVES 1 (HYL1) and SERRATE (SE). The pre-mRNA is further processed into the miRNA/miRNA∗ duplex. This duplex then undergoes methylation catalyzed by HUA ENHANCER 1 (HEN1). The mature miRNA then in association with ARGONAUTE 1 (AGO1) regulate gene silencing through the RNA interference (RNAi) mechanism (Song *et al*. 2019). Plant miRNAs recognize target mRNAs through sequence complementarity and silence these genes either by target mRNA cleavage or by translational repression (Chen 2009, Yu *et al*. 2017). miRNAs play definite roles in development and stress response. They significantly function in temperature stress response in plants. Arabidopsis miR398 is a well-known heat stress regulator. Expression of miR398 is induced by two heat shock TFs and enhanced expression, miR398 silences genes COPPER/ZINC SUPEROXIDE DISMUTASE 1 (CSD1), CSD2, and COPPER CHAPERONE OF CSD thus promoting the expression of HSPs and improving heat tolerance in plants. However, transgenic plants that contained miR398-resistant CSD1, CSD2, and COPPER CHAPERONE OF CSD showed increased sensitivity to heat and decreased heat tolerance (Guan *et al*. 2013). One of the evolutionarily conserved miRNAs miR319 positively regulates cold tolerance. miR319 targets genes TEOSINTE BRANCHED1, CYCLOIDEA, PROLIFERATING CELL NUCLEAR ANTIGEN BINDING FACTOR and therefore its overexpression improves cold tolerance in sugarcane and rice plants (Thiebaut *et al*. 2012; Yang *et al*. 2013). HD-ZIP III is a target for miR165/166 and the miR165/166-HD-ZIP III module is a regulator of drought tolerance in *Arabidopsis* and rice. Decreased expression of miR165/166 results in

enhanced drought tolerance in these plants (Yan *et al*. 2016; Zhang *et al*. 2018). miRNAs are important players that mediate plant immune responses to biotic stresses. In *Arabidopsis*, PAMP flagellin (flg22) induces upregulation of miR393 which in turn downregulates its targets F-box auxin receptors TIR1, AFB2, and AFB3. This results in enhanced defense against the viral pathogen *Pseudomonas syringae* pv. tomato (Navarro *et al*. 2006). In rice, transcription of miR398b is induced in response to the blast fungus *Magnaporthe oryzae* (Li *et al*. 2014). Several conserved miRNAs and their target genes have been identified in plant-insect interaction studies. Aphid (*Macrosiphoniella sanbourni*) feeding in *Chrysanthemum morifolium* induced miR159a upregulation which targets GAMYB-like 2 gene (Gibberellic acid myeloblastosis) responsible for coding MYB (Myeloblastosis) transcription factors (TFs) involved in the synthesis of gibberellic acid (GA) (Xia *et al*. 2015). The MYBs are a large number of TFs known for their role in plant growth and development like stamen, petal and anther development, lateral organ formation, root development and embryogenesis in *Arabidopsis*, anthocyanin synthesis in Petunia and *Zea mays*, GA signalling in *Oryza* and *Hordeum*, heat, cold and drought tolerance in *Oryza, Arabidopsis, Saccharum* etc. (Ambawat *et al*. 2013; Mandaokar *et al*. 2006; Solano *et al*. 1995; Gubler *et al*. 1997; Agarwal *et al*. 2006; El-kereamy *et al*. 2012; Prabu and Prasad, 2012; Sattar *et al*. 2012; Bozorov *et al*. 2012; Stief *et al*. 2014; Olsen *et al*. 2005). In *Chrysanthemum* and *Cucumis melo*, miR160a, miR167 and miR393a were found to be downregulated in response to aphid feeding. Potential targets of these miRNAs were Auxin Response Factors (ARFs) and Transport Inhibitor Response 1 (TIR1), which were highly upregulated during aphid infestation and repressed the actions of proteins involved in suppressing auxin signalling (Xia *et al*. 2015; Sattar *et al*. 2012). Synthesis of auxin negatively regulates plant defense against pathogens by involving in a

complex hormonal cross-talk that is discussed in the later part of this review. Accumulation of auxin in the plant body enhances plants susceptibility to pathogens (Denanc´e *et al*. 2013). In contrast, miR167 showed elevated levels during *Manduca sexta* feeding on to- bacco plants indicating decrease in transcript abundance of ARF6 and ARF8 (Bozorov *et al*. 2012), thus suppressing auxin signalling. In a study involving two tomato species being infected by a whitefly species (*Bemisia tabaci*), a number of miRNAs have been found to be differentially expressed and targeted genes belonging to different TFs and receptor proteins. Whitefly and *M. sexta* infestation on tomato and tobacco respectively induced expression levels of several miRNAs like miR156 and miR157. As a consequence, expressions of SPL TFs (squamosa-promoter binding like proteins) were reduced that usually function in leaf initiation and developmental transition in plants (Stief *et al*. 2014). SPL TFs are important regulators of plant growth and development and ac- cumulates in the plant body as age progresses (Mao *et al*. 2017). The GRAS family TFs are known to be repressors of phytohormone GA which are known players in mediating growth and development in plants but play ambiguous roles in plant defense (De Bruyne *et al*. 2014). The same study also showed overexpression of miR170 and miR171, which were seen to target GRAS family TFs (Stief *et al*. 2014). In *Arabidopsis*, overexpression of miR164 regulated NAC domain proteins responsible for embryonic, vegetative and floral development in plants (Bozorov *et al*. 2012; Olsen *et al*. 2005; Wang *et al*. 2018). Furthermore, accumulation of miR164 and subsequent targeting of NAC TFs is reported in aphid-melon interaction study (Sattar *et al*. 2012).

2.6.2 siRNA

For the very first time, the involvement of siRNAs in abiotic stress response was reported by Sunkar and Zhu. According to the report, nat-siRNAs derived from the natural

antisense transcript of the SRO5 gene plays an important role in salt stress management in *Arabidopsis.* The SRO5-P5CDH-nat-siRNA module is an effective osmotic stress response regulatory system (Borsani *et al*. 2005). The involvement of siRNAs in biotic stress response in plants has also been uncovered. An effector of the bacterial pathogen *Pseudomonas syringae* pv. tomato (pst) induces the expression of nat-siRNAATGB2 that effectively suppresses its target PPRL. PPRL is a negative regulator of ETI against the pathogen (Katiyar-Agarwal *et al*. 2007). Some other classes of siRNAs that are 30-40 nt long, commonly described as lsiRNAs, also get induced by the same bacterial pathogen (Katiyar-Agarwal *et al*. 2006). Differential abundance of siRNAs in response to nematode (*H. schachtii*) infection was also reported (Hewezi *et al*. 2008).

2.6.3 circRNA

Circular RNAs (CircRNAs) have recently emerged as definite role players belonging to the non-coding RNA world. Their biogenesis requires a covalent circularization of a 3′ downstream donor and the 5′ upstream acceptor through an alternate splicing called backsplicing (Szabo and Salzman, 2016) process. The mechanisms governing biogenesis, export, degradation and the functional importance of circRNAs, however, are either unknown or the subject of unproven speculations. Reports on occurrence of circRNAs have proved that circRNAs serve an inevitable part of eukayotes. Much evidence has emphasized the putative role of circRNAs in gene regulation. CircRNAs have been proven to possess the ability to bind to miRNAs and repress their action, a process known as miRNA sponging. The ciRS-7-miR-7 module is an example of such sponging activity of circRNAs. Imperfect binding of circRNA ciRS-7 to miR-7 makes the miR-7 unavailable for binding to its target mRNAs involved in certain types of cancers (Kefas *et al*. 2008; Reddy *et al*. 2008). While still being done, the search for circRNAs in plants

is moving more slowly than it does in mammals. Only a small portion of the more than 100,000 circRNAs that have been discovered from various plants and are reported in the plant circRNA database (Chu *et al*. 2017) have been validated. CircRNAs have been identified and characterized in plants in a number of studies (Bai *et al*. 2021; Bao *et al.* 2019; Bian *et al.* 2021; Chen *et al.* 2017, 2022; Darbani *et al.* 2016; Dong *et al.* 2021; Eisenberg and Levanon, 2018; Fu *et al.* 2019, 2020; Ghorbani *et al.* 2018; Han *et al.* 2021; He *et al.* 2020; Hong *et al.* 2020; Jiang *et al.* 2021; Medina *et al.* 2021; Meng *et al.* 2018; Pan *et al.* 2018; Qin *et al.* 2018; Ren *et al.* 2018; Salih *et al.* 2021; Tong *et al.* 2018; Zeng *et al.* 2018; Zhan *et al.* 2021). A handful of circRNA functions have been confirmed through studies involving transgenics. Overexpression of the ciRNA lariat41 in A. thaliana has been seen to cause a number of consequences like delayed flowering, decreased fertility, altered phyllotaxy (Li *et al.* 2016; Cheng *et al.* 2018). Yellowing of tomatoes in transgenic lines overexpressing PSY1-circ1, a circRNA derived from its parental gene Phytoene Synthase 1 (PSY1), is observed due to reduction in buildup of lycopene and β-carotene. Similarly, overexpression of PSD-circ1, a circRNA derivative of Phytoene Desaturase (PDS) gene, leads to the formation of yellow tomatoes along with photo-bleached leaves, petals, and sepals (Tan *et al.* 2017). Another overexpression study conducted in studying the function of circRNAs is that of the circRNA derived from the 6^{th} exon of SEPALLATA3 (SEP3) gene. Increased expression of the said circRNA produces flowers with decreased stamen numbers and surplus petals in Arabidopsis plant (Conn *et al.* 2017). The circRNA circATS1, a derivative of glycerol-3-P acyltransferase gene, is responsible for providing cold tolerance in *A. thaliana* (Gao *et al.* 2019). In terms of functional importance, plant circRNAs can regulate different cellular processes by a variety of mechanisms by inhibiting the expression of parental genes by forming R-loops,

by acting as miRNA sponges, by interacting with RNA-binding proteins (RBPs), or sometimes they might also get translated to peptides (Misir *et al*. 2022; Liu *et al*. 2022).

2.6.4 Long non-coding RNAs – undeniable mediators of plant biotic stress response

Transcriptomic studies in eukaryotes revealed that > 90% of the genome gets transcribed, out of which only a small percentage of the transcripts get translated to peptides. The remaining untranslated transcripts correspond to ncRNAs, including the long non-coding RNAs (lncRNAs). These lncRNAs are generally defined as transcripts that are of more than 200 nucleotides in length and that lack any apparent protein coding ability (Quinn and Chang 2016; Budak *et al.* 2020). Tens of thousands of lncRNAs have been discovered both in animals and plants through genome-wide studies but their functional significance has been a subject of debate. The genome of plants synthesizes thousands of lncRNAs from intergenic, intronic, coding regions or even from antisense strands. Most lncRNAs are transcribed by RNA Pol II, while Pol IV and Pol V also contribute towards the transcription of certain lncRNAs (Wierzbicki *et al*. 2008; Li *et al*. 2014). Most of the plant lncRNAs are reported to be polyadenylated, whereas those of mammals are non-polyadenylated (Andersson *et al*. 2014). With the advent of high-throughput sequencing technologies, significant progress has been made in the field of lncRNA research. Publicly available databases like the Plant Long non-coding RNA Database version 2.0 (PLncDB V2.0) has been designed to incorporate as many as 1,246,372 lncRNAs from more than 80 plant species (Jin *et al*. 2020). In addition, NONCODEV6, another database for lncRNAs, consists of 94,697 lncRNAs from 23 plant species (Zhao *et al*. 2020). EVLncRNAs2.0 is a database of lncRNAs consisting of experimentally validated functional lncRNAs and comprises of 506 lncRNAs (Zhou *et al*. 2020). LncRNAs frequently serve as regulatory, catalytic, or structural agents in the context of gene

expression (Wierzbicki *et al*. 2021). LncRNAs can regulate gene expression at different phases including transcription, splicing, and translation as they can associate with different regions of the genome like promoter, introns, exons, UTRs (Fatica *et al*. 2014; Gao *et al*. 2016; Ben *et al*. 2009; Caley *et al*. 2010). LncRNAs also play major role in safeguarding the genome integrity along with getting engaged in responses to adverse environmental conditions including abiotic and biotic stresses (Zou *et al*. 2016; Quinn and Chang, 2016; Hong *et al*. 2022). Gene expression is a complex phenomenon and lncRNAs can regulate gene expression through a variety of mechanisms. They can associate with DNA and RNA by complementary base-pairing and with proteins by interaction mechanism. LncRNAs can act as miRNA sponges and inhibits the miRNA-target mRNA binding process for gene silencing, as enhancers of transcription, as guides to specific proteins and as scaffolds to provide a structural platform for proteins to aggregate and form complexes. LncRNAs serve as key players in plant's growth and development. Certain number of functionally validated lncRNAs have been characterized in plants. These include COOLAIR, COLDAIR, ELENA1, Enod40, LDMAR, IPS1, HID1 etc. Numerous processes, including the development of nodules, vernalization, photo-sensitive male sterility, and photomorphogenesis, are said to be influenced by these lncRNAs. The FLOWERING LOCUS (FLC) gene is an important gene for maintaining the process of vernalization. During cold temperature, lncRNAs COOLAIR (Cold-induced antisense intragenic RNA) and COLDAIR (Cold-assisted intronic noncoding RNA), that are derived from the locus of FLC gene, are reported to repress FLC gene expression and maintain reduced accumulation of FLC gene product through epigenetic modification and promoter interference (Heo and Sung 2011). In *Arabidopsis*, the lncRNA ELF18-INDUCED LONG-NONCODING RNA1 (ELENA1) induces the

expression of PR1 gene by disintegrating FIB2/MED19a complex and discharging the FIB2 from PR1 promoter (Seo *et al*. 2017, 2019). A nat-lncRNA MSTRG.19915, a derivative of a MAPK gene, was reported to regulate susceptibility to downy mildew causing pathogen in Chinese cabbage (Zhang *et al*. 2021). The role of lncRNAs in JA-mediated resistance to herbivory in *N. attenuata* is also evident as reported by Li and his co-workers (Li *et al*. 2021). The miRNA-sponging or eTM activity of lncRNAs was for the first time discovered in 2007 during the study of phosphate starvation response in plants. The miRNA miR399 targets the PHO2 gene that encodes ubiquitin-conjugating E2 enzyme and is significantly involved in phosphate starvation in plants. However, during phosphate deficiency, the expression of lncRNA IPS1 gets induced and this in turn mimics the PHO2 gene, thereby sequestering the miR399 active level. As a result, PHO2 gene product accumulates and serve the appropriate response to phosphate starvation stress (Franco-Zorrilla *et al*. 2007). These studies deepen our understanding of how lncRNAs act as universal regulators in response to a variety of stimuli.