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RESEARCH PAPER

Exploring the role of tiny, potent player microRNAs in tea plant (*Camellia sinensis*) under the influence of pathogen attacks: Review

Samina Kausar[1*], Rana Badar Aziz[2], Muhammad Shahbaz[3], Muhammad Adeel Ghani[4], Abdullah bin shaikh[4], Muhammad Shah Nawaz[3], Mansoor Hameed[1*], Muhammad Zia Shahid[5], Asad Nawab[6], Muhammad Usman Shoukat[7]

[1*] *Department of Botany, University of Agriculture Faisalabad, Pakistan*
[2] *Department of Horticulture, Bahauddin Zakariya University Multan, Pakistan*
[3] *Department of Department of Plant Pathology Bahauddin Zakariya University Multan, Pakistan*
[4] *Institute of Food Science and Nutrition Bahauddin Zakariya University, Multan, Pakistan*
[5] *Department of Soil Science, University of Agriculture Faisalabad, Sub-Campus Burewala, Pakistan*
[6] *Department of Entomology Bahauddin Zakariya University, Multan, Pakistan*
[7] *School of Automotive Engineering, Wuhan University of Technology, Wuhan, 430070, China*

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Abstract

Tea is cultivated as a cash crop all over the world and is consumed by more than 2 billion people throughout 125 countries nowadays. It is a nutrient-dense beverage with therapeutic characteristics that has a long list of health advantages. Pathogenic attacks are becoming the main dilemma in tea plants because of the continuous and adventurous development of plant pathogens, including insect pests, fungi, bacteria, viruses. Biogenesis pathways of miRNA are vital for the development, improvement, and protection of tea plants. MiRNAs of plants play key roles in a variety of regulating networks relating to plant growth, metabolic signaling, and environmental stress reactions. Various techniques, i.e., Transcriptional gene silencing, virus-induced gene silencing, Hairpin gene silencing, co-suppression, and artificial miRNAs, all these techniques are RNA interference (RNAi) strategies that have been used to shield tea cultivars from various stressed conditions. In this review paper, we summarized recent findings of miRNA-mediated regulation, features, and the defensive system of tea cultivar's responses to plant pathogens, particularly fungal pathogenic attacks and insect herbivory. Eventually, we concluded the novel functions of miRNA-mediated gene silencing for future research and how it can be utilized for the improvement of pathogenic attacks tolerance in transgenic tea plants.

* **Corresponding Author:** Samina Kausar ✉ sameenakausar888@gmail.com

Introduction Tea and Coffee are the most well-known refreshments in the world, as well as the most manufactured and used drinks (Liu *et al.,* 2017; Wambulwa *et al.,* 2016). Tea has an extensive history of use in the Guizhou Plateau, but nowadays, it is grown all over the world (Chen *et al.,* 2012; Wei *et al.,* 2018; Niu *et al.,* 2020). It is consumed by more than 2 billion people throughout 125 countries now a day (Tai *et al.,* 2018; Zhang *et al.,* 2018). Tea is made by infusing extracted young shoots of a tea plant (*Camellia sinensis*) from the genus Thea of the Theaceae family (Ma *et al.,* 2018a, b, c). Commercially cultivated tea comes from the Tea plants (*Camellia sinensis*) (Xia *et al.*, 2017). It is attained from the young leaves of the tea plant and is a nutrient-dense beverage with therapeutic characteristics that has a long list of health advantages (Naghma and Hasan, 2013). The use of tea on a daily basis lowers the chances of many malignancies, obesity-related disorders, cardiac and neurological disorders (Hayat *et al.,* 2015). Tea contains 700 biologically active components such as theanine, catechins, volatiles, and caffeine which give its distinctive flavor as well as a variety of nutritional properties (Xia *et al.,* 2017; Niu *et al.,* 2020).

Leaves of the tea plant have been known as an excellent source for a variety of teas, which include black tea, which is fermented; oolong tea, which is semi-fermented; and green tea, which is non-fermented (Khan *et al.,* 2013). Green tea is China's most famous and frequently utilized drink. It is made from young leaves of the tea plant and retailed without fermentation, subsequently wither steam, dry, and grad. There are some bioactive compounds, i.e., caffeine, fluorine, theanine, Catechins, γ-aminobutyric acid, and saponins (Chacko *et al.,* 2010). Black tea is an energizing beverage made by the oxidization and fermentation of tea leaves. The oxidation and fermentation procedures used in black manufacturing deteriorate the polyphenolic compounds in leaves of tea, resulting in the formation of various stimulants such as caffeine, theaflavins, proanthocyanidins, and thearubigins (Waugh *et al.,* 2017). Oolong tea is an outcome of partial oxidation of tea leaves that falls somewhere in between green and black tea production. Theanine, theaflavins, catechins, thearubigins, and caffeine are all present in high concentrations (Boros *et al.,* 2016). Phenolics, as well as other useful compounds, are found in many varieties of tea. They have therapeutic benefits on fitness by lowering the chance of chronic disorders such as heart disease, osteoarthritis, diabetic disease, and cancers (Khan *et al.,* 2013). Tea plants, as sessile creatures, are subjected to a variety of abiotic and biotic stresses in their natural environment, affecting healthy growth and development, which results in significant losses of tea yield around the world (Zhang *et al.,* 2019). Farm owners utilize chemical pesticides significantly for the management of pests and pathogens under biotic stress, which has resulted in the establishment of stress tolerance by such biotic agents over pesticides. To satisfy the demands of the rising population, the establishment of disease and pest-resistant agricultural crops with enhanced yields using unique technological ways is becoming a high priority. The utilization of various plant resistance to adverse biotic stressors is required for the sustainable growth of tea plants within similar fields (Zhou *et al.,* 2017). Tea plants produce a variety of functional gene outputs, including non-protein-coding regulating components in exposure to pathogen attack, including ribosomal RNA, transfer RNA, and small nuclear RNAs (Xia *et al.,* 2017).

Small nuclear RNAs (snRNAs) are tiny non-coding RNAs of 20 to 24 nucleotides that govern the transcriptional and post-transcriptional genes expressions of resistance sensitive and protein-coding. Depending on its biogenesis as well as its roles, small RNAs (sRNAs) in plants are divided into two groups: microRNAs (miRNAs) and short interfering RNAs (siRNAs). SiRNA is made of an excellent double-stranded RNA transcript, whereas miRNA is made through single-stranded stem-loop precursors components (Prabu *et al.,* 2010). MiRNAs of plants play key roles in a variety of regulating networks relating to plant growth, metabolic signaling, and environmental stress reactions (Chen *et al.,* 2010). The defensive mechanism of plants is mostly dependent on the expression of defense responding genes under various environmental stresses. MiRNAs had been discovered to be significant regulators in the reprogramming of the expressions of genes under stressful situations, according to research. RNA interference-based techniques will be utilized to manipulate miRNAs plus their target genes in order to generate stress-tolerant crops (Qi *et al.,* 2019). Hairpin gene silencing, Transcriptional gene silencing co-suppression, and artificial miRNAs. All these techniques are RNA interference (RNAi) strategies that have been used to shield various agronomic cultivars from several stressed conditions (Zheng *et al.,* 2015; eyaraj *et al.,* 2020). RNAi-dependent pathogen prevention strategies, such as spray-induced gene silencing (SIGS) and host-induced gene silencing (HIGS), were established currently to evaluate pathogenic genetic functioning in crops utilizing small RNAs (sRNAs) and double-stranded RNAs (dsRNAs) (Wang *et al.,* 2017).

In this review paper, we explored tiny, potent player microRNA biogenesis and its functions in crops, including the miRNAs that have been discovered and their attacks in the tea cultivars. After that, we summarized our recent findings of miRNA-mediated regulation, features, and defensive systems in tea cultivar's responses to plant pathogens, particularly fungal pathogenic attacks and insect herbivory.

Biogenesis and diverse functions of plant microRNAs

The microRNAs of Plants have been primarily detected inside intergenic regions, where they are transcribed into primary transcripts through RNA polymerase II by non-coding nuclear genes, i.e., pri-miRNAs. There is a cap on the 5′ end of pri-miRNA while the 3′ end is polyadenylated. as well as inadequate fold-back structures are formed in this process (Voinnet *et al.,* 2009). This pri-miRNAs are therefore processing into the stem-loop precursors miRNAs (Pre-miRNAs) through nuclear-processed centers known as D-bodies and SmD3/SmB-bodies from enzymatic complexes that include double-stranded RNA (dsRNA) binding protein HYPONASTIC LEAVES1 (HYL1), DICER-LIKE-1 RNase III endonucleases (DCL1) as well as C2H2-zinc finger protein (Kurihara *et al.,* 2006; Fang *et al.,* 2007) DCL1 converts this stem-loop-structured pre-miRNAs into the miRNA-miRNA* duplex. The miRNA-miRNA* duplex is methylated at the 3′ terminal nucleotides of every strand through the S-adenosyl methionine-dependent methyltransferase HUA ENHANCER 1 (HEN1) for the protection of uridylation as well as its exonuclease activities (Jeyaraj *et al.,* 2020; Li *et al.,* 2005).

HASTY, which is called HST, is an Exportin-5 homology protein. It facilitates the export of the miRNA-miRNA* duplex after methylation in the cytoplasm. A small RNA degrading nuclease (SDN) class of exonucleases splits the methylated miRNA-miRNA* duplex into miRNA called guide strand as well as miRNA* called passenger strand within the cytoplasm (Yang *et al.,* 2006). The RNA-induced silencing complex (RISC) is then loaded with the duplex's guide strands and miRNA. The Arganoute (AGO1) associated proteins, including the RNA binding domains, are the significant and essential constituent of a miRNA-RISC complex. If one miRNA is integrated into the cytoplasm RISC, they control the appearance of their particular desired genes through cleaving mRNA just on 10th and 11th nucleotide by the 5′ end of miRNA and through repressing translation of the targeted mRNAs (Jones *et al.,* 2013; Jeyaraj *et al.,* 2020).

Potent player MicroRNAs and its target genes identification in tea plant

To the identification and categorization of miRNAs as well as their mRNA targets in the tea plants, there are two techniques as experimental methodology and bioinformatics are used.

The computational methodology for the identification of conserved miRNAs is based on the discovery of homologous sequences of recognized, mature precursor miRNAs by other species. Prabu and Mandal (2010) used EST-based comparative genomics to identify 4 candidate tea miRNAs by

4 families as well as thirty potential targeting genes to eleven miRNA families in the tea plant for the 1st time.

Following that, Zhu and Luo (2013) used a similar approach to discover fourteen new tea miRNAs and their fifty potential targeting genes. For a better understanding of the functions of miRNA in tea plants, such predicted targeted genes are additionally investigated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and gene ontology (GO) pathways (Zhu *et al.,* 2013). 6 novel sRNAs have been discovered in young and old leaves of tea under a dormant or non-dormant phase of growth by using an experimental technique in 2012 (Mohanpuria *et al.,* 2012; Jeyaraj *et al.,* 2017; Liu *et al.,* 2019; Zhao *et al.,* 2019).

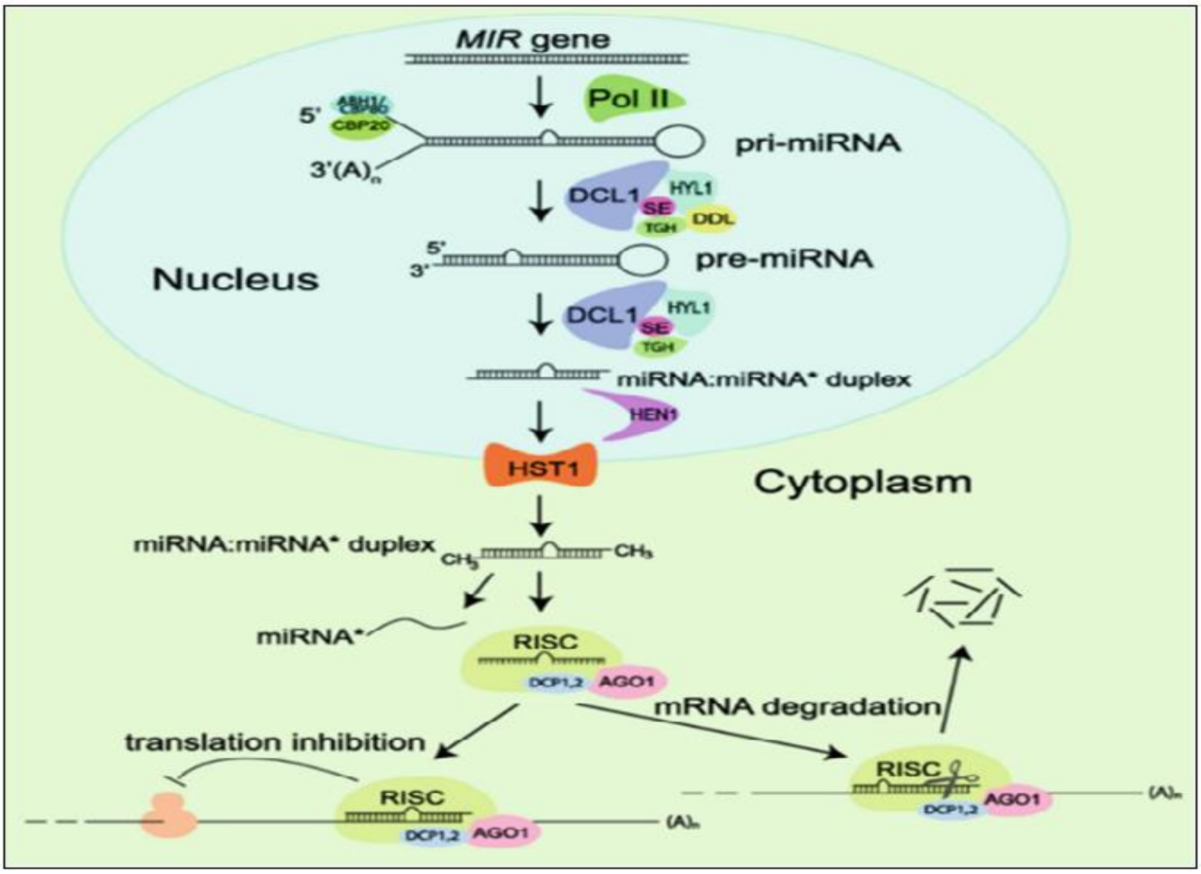

Fig. 1. Biogenesis pathway of microRNAs.

Tea plants showed chilling stressed responses (Zhang *et al.,* 2014), cold and freezing stresses, drought stress (Liu *et al.,* 2016; Guo *et al.,* 2017). It showed responses to *Ectropis oblique* attacks and also to *Colletotrichum gloeosporioides* inflammation (Jeyaraj *et al.,* 2017), including the synthesis of catechins (Sun *et al.,* 2017) as well as biosynthesis of terpenoids (Zhao *et al.,* 2018). Targeted genes of miRNA in the site of cleavage were recognized through the mRNA degradome sequences along with functional genomic annotations for the determination of intricate also energetic networks of miRNAs participating in regulatory responses of stressors and development in tea plants (Jeyaraj *et al.,* 2019). Through the regulatory network of transcriptional genes, the majority of identified miRNAs have already reported playing important roles in the improvement and development of plants as well as their resistance under abiotic and biotic stressed conditions.

Biotic stress responses in tea plants

Various pathogens such as fungi, bacteria, nematodes, weeds, and arthropod pests, are the most common causes of biotic stresses. Tea mosquito bugs, tea geometrid, red spider mites, and shot hole borer are just a few of the main tea pest species that wreak havoc on tea cultivars (Tewari *et al.,* 2016). Plant disorders are among the most popular biotic stressors, affecting photosynthetic processes and the rate of transpiration that affect the quality of tea (Ponmurugan *et al.,* 2007). The tea plant has found to harbor 400 disease-producing pathogenic organisms, the majority of them are fungi, with some of the viruses, bacteria, and phytoplankton are thrown in for good measure (Tewari *et al.,* 2016).

The diseases caused by fungi are sorted into three significant classes in view of the plant parts impacted by microorganisms, like stem, root, and leaf infections. Since the tea plant is planted for its young, tender leaves, leaf sicknesses are more serious issues. Anthracnose, brown blight, black rot, blister blight, red rust bird's eyespot, and grey blight are some of the major common foliar illnesses that affect tea plants (Ponmurugan *et al.,* 2016). Tea plants can withstand biotic stress by activating molecular systems involved in stress signaling perceptions, signal transduction, upregulation of precise stress-induced genes, and metabolites biosynthesis (Huang *et al.,* 2012). Stress inducive miRNAs in plants are currently identified as crucial genetic regulation of small RNA molecules in a variety of developmental processes. It is also associated with biotic stress and abiotic stress resistance (Khraiwesh *et al.,* 2012).

The role of tiny, potent player microRNAs in tea plant under pathogenic attacks

Tea is a valuable beverage cultivar that is frequently subjected to pathogenic attacks caused by insect communities (Wang *et al.,* 2016). Tea geometrid (*Ectropis oblique*), which is called a chewing insect, is a popular insects pest in China (Chen *et al.,* 2012). In general, plants react with insect-induced stress in two ways: directly and indirectly. Plant traits that enable mechanical protection included hairs, thick leaves, trichomes, spines, and thorns. They create toxicants such as anthocyanin, terpenoid, phenolics, alkaloids, and quinines. Which destroy or delay herbivores' growth are referred to as direct defense mechanisms (Hanley *et al.,* 2007).

The production of a mixture of volatile compounds which attract natural herbivores predators through giving food (extrafloral nectar) as well as a refuge for predators is referred to as indirect defenses (Arimura *et al.,* 2009). MiRNAs produced from the hosts are essential regulators of plant resistance against pathogenic attacks. Jeyaraj and coworkers (2017) discovered 130 recognized miRNAs as well as 512 different miRNAs by means of a high throughput sequencing-based miRNAs recognition technique. Out of them, 80 conserved and 356 novel miRNAs are found in the mechanical wounds geometrid attacks. The discovery of miRNA targets, on the other hand, is necessary for studying the activities of miRNAs under stress-responsive regulatory systems. *Ectropis oblique's* oral secretions include elicitors that penetrate tea plants through pest herbivory. Such elicitors operate as chemical signals to tea plant defense because they fool the invader's surveillance system in tea cultivars. The potential of tea plants to recognize these elicitors as well as quickly activate defense responses seems to be critical to their herbivory resistance. In plant-pathogenic interactions, the recognition of receptor sites participating in defense induced by elicitors is critical for responding to stresses (Nurnberger *et al.,* 2006). Tea miRNAs regulate such receptor kinases, and its identification is pivotal for plant-insect interaction defense (Wang *et al.,* 2016). Jeyaraj *et al.* (2017), in tea plants, revealed that *Ectropis oblique* had been strongly identified by numerous plant receptors such as serine and threonine-protein kinase (Ser and Thr kinase), Cysteine-rich receptor-like protein kinase, as well as mitogen-activated protein kinases (MAPKs) for signaling transduction pathway activations (Artico *et al.,* 2014; Hill *et al.,* 2015). Furthermore, it is found that OS-responsive miRNAs influenced the expression of receptors and kinases such as Ser and Thr kinase, and MAPKs. Three novel miRNAs (csn-miRn19, csn-miRn57, and csn-miRn421) target LRR-RLKs, whereas csn-miRn387 and csn-miRn421 target CRR-RLKs (Jeyaraj *et al.,* 2017). Tea miRNAs have been potential targets LRR-RLKs as well as Ser/Thr-kinase receptors that influenced its biotic impacts by provoking Ca^{2+} influx and Ca^{2+} binding protein initiation via MAPK cascades, accompanied with the stimulation of several phytohormones synthesizing and ROS biosynthetic pathway. The defense system of Plants under the attacks of pests is modulated by ROS-related enzymes and Ca2+-related proteins (Kiep *et al.,* 2015; Pavani *et al.,* 2015).

Specifically, enzymes glutathione S transferase or calcium-binding protein have been reported as a possible target for csn-miRn335 and csn-miRn57, accordingly (Jeyaraj *et al.,* 2017). Overall, such miRNAs have been engaged with protein kinase-induced signal cascades, prompting miRNA-mediated defense responses in tea plants versus OS-induced stressors. Transcriptional factors binding to the promoter region of a particular gene in DNA assist in the conversion of gene evidence from DNA to mRNA. Ethylene-responsive transcription factors (ERFs) basic helix-loop-helix transcriptional factors are some of the most important transcriptional factors involved in abiotic or biotic stresses response in plants (Das *et al.,* 2019).

For both plants and animals, transcriptional factors have been a good target of miRNAs (Kumar *et al.,* 2014). All csn-miRn301, csn-miRn19, csn-miRn421 and csn-miRn392 are obviously engaged in the production of bHLHs transcription factor after OS-induced stresses in the tea plants (Jeyaraj *et al.,* 2020). The transcription factor bHLH is involved in a variety of processes in plants, including growth and defense responses. Certain miRNAs might control the interaction among MYBs, including bHLHs, and thus the generation of defense-related secondary metabolites in tea plants. Various enzymatic steps in the pathway of flavonoid production, phytochrome A (phyA) signaling, and induction of ABA-inducible gene expressions are controlled by this relationship. This also controls the generation of secondary metabolites involved in defense, which includes glucosinolate (Nakata *et al.,* 2013; Xu *et al.,* 2015). These findings suggest that miRNAs played important functions in the tea plant's resistance against herbivory attacks, and it's worth investigating its possible biological activities in the face of OS-induced stressors. During the attacks of herbivory, 2 tea miRNAs, csn-miRn335 and csn-miRn57 were recognized as possible targets for ERFs. Ethylene seems to be a critical modulator for plant biotic stress response and therefore is required during numerous developmental stages. ERFs may be generated via ethylene or pathogenic stress as downstream constituents of the ethylene signal responding system. ERFs operate as a crucial regulating center in plants responding to stressors via attaching with sequences containing AGCCGCC motifs (the GCC box) that is a cis-acting component (Berrocal *et al.,* 2002). Plant growth regulators, i.e., gibberellin, Auxin, salicylic acid, brassinosteroid, and cytokinin, interact with ERFs, enhancing stress adaptability (Chen *et al.,* 2005). As a result, by stimulating ERF, tea miRNAs effectively counteract OS-induced stressors. To overcome these insect stress responses, distinguished up-regulated OS-induced miRNAs were found to be appreciably engaged in the regulation of 4 major pathways, particularly regarding signal perception, receptor recognition, signal transduction, and activation of defensive genes in plant-insect interface in tea plants.

Strategies for plants protection based on potent miRNA-mediated gene silencing

Usually, as a strategy of genetic control, including natural resistance measures towards numerous biotic and abiotic stressors, plants utilize RNA-mediated gene silencing (RNAi). Small RNAs, including miRNAs and siRNAs, are well-studied and primarily responsible for such mechanisms. In 2 kinds of pathogenic stressors, i.e., *Ectropis oblique*, and *C. gloeosporioides* attacked, a huge proportion of target or miRNAs in tea plants associated with various biotic functions have been discovered. Furthermore, these researches emphasized the various regulation patterns of the targeted gene expressions. Various findings have consistently revealed the importance of miRNAs for plants in a wide range of growth and signaling mechanisms. Stress-induced changes in the expression profiles among these small molecules could indicate that they could be used as genetically improved targets (Zhang *et al.,* 2015). Recent advancements in genome-wide high-throughput sequence data for several major plant species, as well as miRNA's evolving impact in regulatory target genes associated in diverse stages of a life cycle of plants, have made miRNA a crucial player in the transgenic field (Sun *et al.,* 2012; Zhou *et al.,* 2013; Zhang *et al.,* 2016).

Plants use miRNA-mediated gene silencing as a positive or negative immune reaction controller, as well as an inducer for a positive or negative immune defense regulator. Several agronomic plant species have used miRNA-mediated gene silencing to protect themselves from a variety of biotic stressors (Tiwari *et al.,* 2014; Khalid *et al.,* 2017). Research revealed that the overexpression of miRNA had caused pleiotropic phenotypic traits and other unwanted attributes. Stronger overexpression negatively affects the fine-tuning of various biological processes, but it can be avoided by utilizing precise promoters, such as developmental stage promoters, stress-induced, and tissue-specific (Yang *et al.,* 2013; Niu *et al.,* 2016). MiRNAs overexpression alters the expressions of targeted mRNAs and has been implicated in response to oxidative stress (Naya *et al.,* 2014), degradation of pollens (Ma *et al.,* 2017), development of flowers and fruits (Yao *et al.,* 2016; Liu *et al.,* 2017) infrastructure of plants (Tang *et al.,* 2018), growth of roots (Zhang *et al.,* 2016), Osa-miR7696 overexpression is responsible for resistance against rice blast infections, according to research (Campo *et al.,* 2013). The bacterial flagellin-derived peptide of *Pseudomonas syringae* (*P. syringae*) has been observed to stimulate miR393 in Arabidopsis, which causes enhanced tolerance against P. syringae by unintentionally regulating mRNAs expression F-box auxin receptors. Overexpression of miR393 minimizes the bacterial titer of plants about 5 folds, offering tools for disease resistance in plants (Navarro *et al.,* 2006). MAMP-induced callose deposition has been instantly regulated by miR160a overexpression, whilst MAMP-induced callose deposition. Bacterial inflammation resistances are negatively regulated by miR398b and miR773 (Li *et al.,* 2010).

Over-expression of 2 miRNAs, i.e., miR319 g & miRStv 11, in *Stevia rebaudiana* leaf increased the levels of *steviol glycoside* via biogenesis of *steviol glycosides* (Saifi *et al.,* 2019). Mishra *et al.* (2018) discovered the overexpression of csn-miRn37a in vulnerable Capsicum annuum L. promoting the expressions of the defense-related gene. It improves tolerance against Colletotrichum truncatum via suppressing ethylene response factors (ERFs) as well as limiting colonization and growth of fungus. Cotton's miRNAs, i.e., miR398 as well as miR2950 were overexpressed in *Gossypium hirsutum*. Through targeted various ORFs (genes) of the cotton leaf curl multan virus (CLCuMuV), including related beta-satellite genes (CLCuMB), HS6 was able to successfully create tolerance towards cotton leaf curl disease (CLCuD) (Akmal *et al.,* 2017). In *Nicotiana benthamiana* leaf attacked with Sclerotinia sclerotiorum, transitory overexpressing of miR398b resulted in increased fungal infections (Naya *et al.,* 2014). While transgenic techniques were used to increase the level of expressions of various genes in order to enhance crop resistance against salinity and drought stresses. The bulk of such transgenic crops showed very little improvement in crop resistance towards salinity and drought stressors (Bartels *et al.,* 2005; Sunkar *et al.,* 2012). For example, through the regulation of nuclear factor Y subunit genes and Sly-miR169 overexpressed to provides greater resistance to drought stress in tomatoes (Zhang *et al.,* 2011), with the regulation of its multiple target genes, osa-miR396c overexpression reduces alkali and salt stress resistance (Gao *et al.,* 2010). GhmiRNA157 precursors Overexpression targets 5 SQUAMOSA promoter-binding protein-like (SPL) genes in cotton, by the auxin signal transduction and regulation of MADS-box genes, it influences shape creation and flower development (Liu *et al.,* 2017). Ethylene is a key hormone for plant growth and stress tolerance.

In Arabidopsis, Zhang *et al.* (2016) found that the overexpression of miR319b reduced the levels of MYB33 transcription and improved the ethylene-responsive root traits. Ding and fellows (2016) found the members of miR169 (pag-miR169a, n, r) attack the precise dormancy genes Heme Activator Protein2 transcriptional factor (PagHAP2-6) in *Populus alba*. This research found PagHAP2- 6 overexpression in Arabidopsis with transitory co-expressional analyses in *Nicotiana benthamiana*, implying both miR169 as well as their targets PagHAP2-6 are implicated during the dormancy of Populus alba cambium, exogenous abscisic acid regulating them.

Exogenous abscisic acid increases the expressions of miR394 whilst decreasing their targeted expression in Leaf Curling Responsiveness (LCR). The drought and salinity stress response in Arabidopsis, might be mediated via MiR394 or LCR, which are abscisic acid-regulated (Song *et al.,* 2013). Overexpression of bra-miR158a1 & bra-miR158a2 induces pollen mass destruction with diminishing pollen grains in *Brassica campestris* (Ma *et al.,* 2017). MiR172 Overexpressed in transgenic tomatoes changes fruits and flowers growth through modulating Apetala2 (AP2) gene expression, as revealed by Yao *et al.* (2016).

According to Zhu *et al.* (2009), miR172 over-expressed in *Oryza sativa* provokes spikelet determinacy damage results in abnormalities of flower organs. According to Wang *et al.* (2016), the GIGANTEA ortholog (GmGIa) stimulates gma-miR172a metabolic activity while suppressing Glyma03g33347. Glycine max transgenic lines overexpressing gma-miR172a exhibited an earlier floral trait as well as enhanced floral-related gene expression. In Arabidopsis, Shi and colleagues (2015) discovered the overexpression of Tristetraproline distresses the auxin response factor (ARF17) expression, miR160 targeted genes, causes male infertility. MicroRNA171 overexpressing in barley influences transition period and determinacy of floral meristem. OsmiR396d overexpressed in rice regulating architecture of plants via suppressing growth-regulating factors OsGRF4 & OsGRF6 expressions through the signaling pathways of gibberellin brassinosteroid (Curaba *et al.,* 2013).

Due to the greater selectivity and lesser unfavorable off-target impacts of amiRNA-based techniques, establishing resistance in plants against biotic stress is the most effective technique for knocking down/out specifically targeted gene expression (Yogindran *et al.,* 2015). In general, artificial miRNA may cause 1000-fold inductions, which results in incorrect outcomes, due to its binding with any mRNA at the binding sites in the 3′-UTRs and randomly down-regulation of that gene. Greater levels of mimic disrupt the functions of other miRNAs, which normally target these genes by competing for binding sites in the machinery of endogenous processes.

Anti-miR, short tandem target mimic, i.e., STTM is a technology for understanding the activation or inhibition targeted mRNA that can be used to inhibit a specific miRNA. STTM used in crops for the suppression and blockage of the activities of precise endogenous mature miRNAs (Wang *et al.,* 2019). STTMs were shown to effectively blockage of miRNA functions in a variety of dicot and monocot species (Teotia *et al.,* 2017). Current researches suggest that these techniques, such as over-expression of miRNA, anti-miRNAs, and miRNA mimic to target specific genes in the host plant associated with plant protection, could be beneficial for plant development and defense.

Conclusion and future perspectives

Tea plants are regarded as the most enticing area of research owing to their lots of benefits for human health. MiRNAs have been identified as a tiny, potent player in tea plants responding to pathogen attacks. Therefore, understanding its vital functions and regulating its expression has the ability to enhance plant resistance against biotic stressors. In this review paper, we summarize the implications of pathogen attack in tea plants, biogenesis of miRNA, and its vital roles, as well as the recognition of the tea plant's biotic stress-responsive miRNAs. The exploration of tea miRNAs that induce miRNA mediated gene silencing into cells of pathogens because it adds a new regulatory layer to the plant-pathogen interaction.

Three techniques are used for this purpose. (1) Overexpressing the specific miRNA in order to silence or reduce particular target gene expression. (2) Producing artificial miRNAs (amiRNAs) to silence a specific gene of interest. (3) For the suppression of the functions of a specific miRNA, scientists create artificial miRNA mimics. They can be utilized for miRNA-based genes manipulation to control the gene expression in vivo investigation of specific miRNA activities. It is critical to understand the transport and molecular pathways of miRNA-mediated gene silencing in a pathogenic cell. MiRNA's concise activity was demonstrated by genome-wide expression assessment, indicating that it is a precise approach for gene silencing.

The introduction of targeting miRNAs in plants offers new visions to the vital function of miRNAs that is utilized for the development of stress-tolerant crops as a substitute to current methods. Lastly, we conclude that the above findings will provide novel clues for the intervention of tiny potent player miRNAs that has the ability to produce biotic and abiotic stress-resistant, disease tolerant, genetically improved, high yield tea plants.

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Author's contribution

Samina Kausar, conceptualization, methodology, analysis, manuscript writing, original draft writing, editing, and reviewing the manuscript. Rana Badar Aziz gave the main idea and helped in the literature review, writing, and editing of the manuscript. Muhammad Shahbaz helped in writing the Biogenesis and diverse functions of plant microRNAs Muhammad Adeel Ghani helped in writing Biotic stress responses in tea plants. Abdullah bin shaikh helped in manuscript editing and proofreading. Muhammad Shah Nawaz helped in abstract writing and proofreading. Asad Nawab helped in writing potent player MicroRNAs and their target genes identification in the Tea plant. Mansoor Hameed, assisted in the finalization of the research idea, supported in writing, proofread, and approved the final manuscript. Muhammad Usman Shoukat, software and help in the drafting of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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