OPEN ACCESS Freely available online

Virology & Mycology

Review Article

Role of MicroRNAs in Biotic and Abiotic Stress Tolerance in Plants

Shweta Tripathia*, Pratyusha Boseb

Department of Biotechnology, Amity University Kolkata, Kolkata, India

ABSTRACT

MicroRNAs or miRNAs are small non-coding RNAs which are important regulators of various developmental and survival processes of the eukaryotic organisms. They regulate the expression of genes post-transcriptionally. Upregulation or downregulation of miRNA expression leads to alteration of many signalling and developmental processes which can help or harm the organism. Plants are no exception to that. In the last 30 years many miRNAs have been found to be deregulated under various abiotic and biotic stresses in plants and also to be closely associated in tolerance of the same. This review focuses on the involvement of miRNAs in stress tolerance in plants that have been identified so far.

Keywords: Mi-RNA; Abiotic stress; Biotic stress; Stress tolerance; Plants

INTRODUCTION

MicroRNAs (miRNAs) are a class of small endogenous RNAs that are non-coding in nature but regulate the expression of a wide variety of genes post-transcriptionally. They are present in both plants and animals. Many miRNAs and their precursors are evolutionarily conserved [1]. In plants, they have a length of 20 nucleotides-24 nucleotides whereas in animals they are 20 nucleotides-22 nucleotides in length [2,3]. They target single or multiple mRNAs and control their expression by inhibiting their translation or cleavage of that particular mRNA. Hence it is understandable that miRNAs play a critical role in various life-processes of an organism, including plants. This review focuses on the role of miRNAs in regulating the abiotic and biotic stress tolerance in plants and their recent developments.

MicroRNA was first discovered in C.elegans in 1993 by Rosalind Lee and her colleagues. They observed that the lin-4 gene, essential for postembryonic development of the C.elegans does not form any protein, rather it is cleaved into two transcripts of 22 and 61 nucleotides in length and the shorter transcript deregulates the expression of lin-14 by antisense complementarity to the 3' UTR of lin-14. Since then many advancements have been made in the field of miRNA and thousands of miRNAs have been identified and associated with various critical diseases like cancer. The occurrence of miRNAs in plants was first reported by Brenda J. Reinhert and colleagues in 2002 in Arabidopsis thaliana where they described 16 miRNAs out of which 8 were conserved and suggested that they might have key roles in the growth and development of the plant [4]. Since 2002, the presence of miRNAs has been documented in many plants including rice (Oryza sativa), potato (Solanum tuberosum), wheat (Triticum aestivum), etc.

Biogenesis and mode of action

Production of mi-RNA in plants: The miRNA biogenesis starts in the nucleus of plant cells. The miRNA encoding genes (MIR genes) are transcribed by RNA polymerase II (Pol II) enzymes and form the pri-miRNA which have 5' cap, 3' polyAtail and May or may not be spliced [5]. Pri-miRNAs form an imperfect double-stranded stem-loop structure with the help of proteins SERRATE (zinc finger domain protein), Hyponastic leaves 1 (HL1), Dicer-like RNase III endonuclease-1 (DCL1), and nuclear Cap-Binding Complex (CBC). The hairpin structure is cleaved by DCLs and forms the miRNA::miRNA* duplex which has 2-nt overhangs at 3' end on both ends [6]. Then the miRNA::miRNA* duplex is exported to the cytoplasm with the help of Hasty (an orthologue of animal Exportin 5) and assembled into an RNA-Induced Silencing Complex (RISC) where the guiding strand (miRNA) is included in the Argonaute Family of Proteins (AGO) while the passenger strand is degraded.

***Correspondence to:** Shweta Tripathia, Department of Biotechnology, Amity University Kolkata, Kolkata, India. Tel: 9007224665; E-Mail: pankhu77@gmail.com

Received: 25-May-2021, Manuscript No. VMID-21-10334; **Editor assigned:** 27-May-2021, PreQC No. VMID-21-10334 (PQ); **Reviewed:** 10-Jun-2021, QC No. VMID-21-10334; **Revised:** 10-Apr-2023, Manuscript No. VMID-21-10334 (R); **Published:** 17-Apr-2023, DOI: 10.35841/2168-9458.23.12.258

Citation: Tripathia S, Boseb P (2023), Role of microRNAs in biotic and abiotic stress tolerance in plants. Virol Mycol.12:258.

LITERATURE REVIEW

Mode of action of mi-RNA

The guiding strand guides the RISC to the target mRNA with the help of complete or partial complementarity of the bases present on the target mRNA and inhibits the gene expression. Plant cell mRNAs have only one complementary binding site for the miRNA-RISC complex which results in cleavage of that particular mRNA [7]. Mostly the miRNAs bind to their respective targets with full complementarity in plants and the complementary bases can exist anywhere along the length of the mRNA, which is not the case with animals. Deng, et al. have shown that the miRNA-RISC can also control gene expression by the formation of secondary siRNAs (phasiRNAs) and subsequent translational inhibition, although mRNA cleavage is the most common mode of action [8].

Identification of mi-RNAs in plants

Identification of potential miRNAs in plants is mainly done by two approaches the conventional reverse genetics approach and the non-conventional forward genetics approach [9]. The reverse genetics approach includes computational methods and experimental methods. Computational methods make use of the already known information about sequences and structures of identified miRNAs from databases such as miRBase (an online database that includes sequence, nomenclature, and target mRNA of the identified miRNAs) and search for homologous sequences or secondary structure alignments [10]. Expressed Sequence Tag (EST) analysis can also be done to predict orthologs of the identified miRNAs using EST databases. Some software tools used are miRBase, miRU, RNA mfold, findmiRNA, micro-HARVESTER, etc.

DISCUSSION

Experimental methods include cloning and sequencing of the small RNAs. It starts with isolation of total RNA from the plant tissue, followed by isolation of small RNAs from the pool, adaptor ligation, RT-PCR, cloning into libraries, and sequencing of the libraries. This is the traditional method of identifying miRNAs from plant tissues and has been used to identify mi-RNA from various plant species. But the experimental methods have certain drawbacks; these are very time-consuming, costly, and inefficient, the expression of the genes is tissue-specific, environmental stimuli-specific, and sometimes very low, the mi-RNAs might exist along with their cleaved target mRNAs and hence their purification is troublesome. Hence the computational methods are used mainly to identify new miRNAs and their target mRNAs in plants.

The forward genetics approach was used by Lee, et al. to identify miRNA in C.elegans. It is a traditional genetic screening method. It has been used only once in the case of plants by Baker, et al. This approach was very time-consuming, inefficient, and has limited applications, hence not a very popular method of identification of miRNAs.

The Plant Non-coding RNA Database (PNRD)

With the advancement of computational tools and databases and their advent in biological fields, more major regulatory networks are being studied and more miRNAs are being discovered. The Plant microRNA Database was constructed by Zhang, et al. in 2010 to meet the demand of information required for various plant mi-RNA researches. Since then many other databases have been developed such as PmiRKB, PMTED, etc. to analyze the functions and/or expression profiles of various plant miRNAs already identified. In 2014, Yi, et al. constructed the Plant Non-coding RNA Database (PNRD) by combining all of the scattered data, extensive literature-based data mining and annotation as well as data obtained from high throughput screening. It is a public, user-friendly resource, constantly updated for the study of various miRNAs identified to date and can be accessed at http://structuralbiology.cau.edu.cn/PNRD. As of May 2016, the database contains 28214 entries from 166 different plant species.

Role of mi-RNA in biotic stress tolerance in plants

Biotic stress in plants is caused by various pathogens like bacteria, fungi, viruses, nematodes leading to various challenges in the survival of the plant. MicroRNAs regulate the gene expression of plants by inhibiting the translation of their target mRNAs by the process called RNA interference or RNAi [53]. The plant immune system contains Pattern Recognition Receptors (PRRs) which recognize Pathogen-Associated Molecular Patterns (PAMP) or Microbe-Associated Molecular Patterns (MAMP) and provides PAMP-Triggered Immunity (PTI). But some pathogens can evade this PTI. In case of an attack by such pathogen (s), the expression of mi-RNAs in plants related to the immune system (targeting the synthesis of plant hormones e.g. auxin, abscisic acid, jasmonic acid) is either upregulated or downregulated in order to facilitate the infection of the host by the pathogens. During infection by Turnip Mosaic Virus, miR171 activity was found to be affected which results in defective RNAi and infection by the pathogen. In Arabidopsis sp. several mi-RNAs, such as miR393, miR319, miR159, miR160, miR165/166, and miR167 were upregulated, and miR825, miR398, miR390, and miR408 were found to be downregulated in bacterial infections. In Triticum sp. 24 mi-RNAs were found to be responsive against powdery mildew infection. But the gene regulation by mi-RNA is also helpful to plants in providing immunity to different biotic stresses by manipulation of auxin levels with the help of TIR1 (transporter inhibitor response 1). Many mi-RNAs miR160, miR166, miR167, miR171, and miR396 have been found to provide antiviral defense in rice plants against Rice Stripe Virus. The role of mi-RNAs in biotic stress responses opens up the possibility of producing artificial plant varieties which are resistant to specific pathogens. Transgenic Arabidopsis varieties expressing artificial mi-RNAs (amiR-P69159 and amiR-HC-Pro159) are found to be resistant to Turnip Yellow Mosaic Virus (TYMV) and Turnip Mosaic Virus (TuMV) respectively. Transgenic tobacco plants expressing miR2b are found to be resistant to Cucumber Mosaic Virus. Owing to this extraordinary potential of mi-RNAs to resist various diseases

caused due to such pathogens (bacteria, viruses, fungi, insects, etc.), mi-RNAs are being used for a new biological approach called "host-induced gene silencing" to protect the plants against pathogenic attacks.

Role of mi-RNA in abiotic stress tolerance in plants

Plants face numerous environmental adversities every day which affect them physiologically leading to delayed emergence of seeds, stunted plant growth, reduced rates of photosynthesis, oxidative stress, etc. Abiotic stress factors lead to alterations in expression profiles of the various genes. In plants, the microRNAs are significantly involved in the post-transcriptional regulation of genes related to various stress tolerance.

Drought stress tolerance

Drought stress is one of the most common stress factors encountered by plants which leads to reduced photosynthesis rates and reduced crop yields. Bhardwaj, et al. have identified 7 conserved miRNAs and 6 novel miRNAs which are upregulated in abiotic stress conditions out of which 2 are upregulated in drought conditions (Bju-N21 and Bju-N29). Barrera-Figueroa, et al. identified 11 miRNAs in cowpea that showed abnormal expression in drought conditions. Overexpression of various miRNAs (miR394, miR408, miR2118, etc.) has shown better drought tolerance in transgenic Arabidopsis, rice, creeping bentgrass, chickpea, tobacco, etc. Several tissue-specific miRNA analyses in rice have revealed the simultaneous upregulation and downregulation of various miRNAs in order to provide drought stress tolerance.

Salinity stress tolerance

Higher salt concentrations in the soil lead to reduced crop yields. Hypersalinity has been found to induce expressions of miR396 in Arabidopsis and maize. In rice, there was an abnormal expression of miR169 leading to deregulation of NF-YA transcription factor. miR395_2, miR390_1, miR172_2, Bju-N29, and Bju-N21 were found to show varied expressions in Brassica juncea. Populus trichocarpa showed reduced expression of miR530a, miR1445, miR1446a-e, miR1447 in response to salt stress. Salt-sensitive LM-6 varieties of cotton showed upregulation of miR156, miR169, miR535, and miR827 and downregulation of miR167, miR397, miR399. Arabidopsis plants with osaMIR393 overexpression have been found to be highly tolerant to salinity. Overexpression of mTIR1 (miR393-resistant TIR1 gene) and gma-miR172 in Arabidopsis, and Oryza sativa miR528 in transgenic Agrostis stolonifera have shown enhanced salt tolerance and higher germination rates. MiR172a has been shown to be responsible for providing salt tolerance to soybean plants.

Temperature stress tolerance

Constant cold stress hampers the growth and metabolism of the plant and destroys the structure of the cells. Many miRNAs responsive to heat and cold stresses have been identified to date. 18 varieties of miRNAs were found to be downregulated and miR812q was found to be upregulated in response to cold stress in rice. Bra-novel-miR3936-5p, miR319e, and miR166m^{-2} were found to be overexpressed in white turnip rape varieties. MiR396b has been found to regulate cold stress tolerance in trifoliate orange. Overexpression of OsmiR156 and ScmiR393 has been found to increase cold stress tolerance in Arabidopsis plants. Zhang, et al. identified 31 miRNAs that were upregulated and 43 miRNAs that were downregulated in tea plants. In tomato, 192 miRNAs were overexpressed and 205 miRNAs were found to have decreased expression in response to cold stress conditions.

High temperature also causes damage to the plants by increasing the rate of transpiration and water loss and decreasing enzymatic activities. 12 miRNAs have been identified in wheat which helps the plants to respond to heat stress. MiR396 in sunflower has been found to be downregulated in case of heat stress. 7 families of miRNA were downregulated in switchgrass in response to high temperatures.

Hypoxia and oxidative stress

Hypoxia is a condition when there is a prevalence of low oxygen concentration in the environment. It might happen due to waterlogging and scarcity of oxygen in the roots. This can alter the mode of respiration from aerobic to anaerobic and cause severe damage to the plants. Several miRNAs have been found to have upregulated levels in Arabidopsis in case of hypoxic stress (miR156 g, miR157d, miR158a, miR159a, miR172a, b, miR391, and miR775).

Oxidative stress is a condition in which there are overproduction and accumulation of Reactive Oxygen Species (ROS). These are extremely harmful because of their reactivity (as the name suggests) and can react with any biomolecule (DNA, RNA, proteins) and interfere with/alter their functions. These are converted to molecular oxygen or neutral molecules with the help of enzymes Superoxide Dismutase (SOD). In Arabidopsis, miR398 has been reported to be downregulated in oxidative stress conditions. In rice seedlings, some miRNAs (miR169, miR397, miR827, and miR1425) were found to be upregulated and some miRNAs (miR528) were found to be downregulated on treatment with H_2O_2.

Transgenic plants to combat biotic and abiotic stress

It is a well-established fact now that mi-RNAs play significant roles in controlling the expression of various genes at the post-transcriptional level. Hence they can control the normal growth and development of the plant and various signaling cascades. The expression of the desired gene can therefore be manipulated by altering the expression levels of either a mi-RNA or a target mRNA. This approach has been utilized in producing various transgenic plants which are tolerant to various abiotic and biotic stresses in order to achieve increased crop quality and better yields. Some of the mi-RNAs which have been targeted in transgenic plants to overcome problems related to virus attacks and abiotic stresses are summarized in Table 1 and Table 2 respectively.

Table 1: mi-RNAs targeted for production of abiotic stress-resistant transgenic plants.

Plant	mi-RNA targeted	Effect on plant
Arabidopsis	miR394	Tolerant to drought
	miR165/166	Tolerant to drought
	miR393	Sensitive to salinity
	miR396	Sensitive to salinity
	miR417	Sensitive to salt and ABA
	miR156	Tolerant to heat stress
	miR173	Tolerant to heat stress
	miR402	Tolerant to drought, salt and cold stress
Rice	miR159	Tolerant to drought
	miR166	Tolerant to drought
	miR319	Increased cold tolerance
	miR156	Reduced cold tolerance
Tomato	miR169	Tolerant to drought

Table 2: miRNAs used to induce resistance in plants against various viruses.

Plant	miRNA targeted	Resistant to virus
Arabidopsis thaliana	AthMIR159a	Cucumber Mosaic Virus (CMV)
		Turnip Mosaic Virus (TuMV)
		Turnip Yellow Mosaic Virus (TYMV)
Nicotiana tabacum	AthMIR159a	Cucumber Mosaic Virus (CMV)
		Potato Virus X (PVX)
		Potato Virus Y (PVY)
	AthMIR167b	Potato Virus X (PVX)
	AthMIR171a	Cucumber Mosaic Virus (CMV)
	AthMIR319a	Potato Virus Y (PVY)
		Tobacco Etch Virus (TEV)
Solanum lycopersicum	AthMIR159a	Cucumber Mosaic Virus (CMV)
	AthMIR319a	Tomato leaf curl New Delhi Virus (ToLCNDV)
	AthMIR390a	Tomato Spotted Wilt Virus (TSWV)
	SlyMIR159a	Tomato Leaf Curl New Delhi Virus (ToLCNDV)
Oryza sativa	OsaMIR528	Rice Black Streaked Dwarf Virus (RBSDV) +Rice Stripe Virus (RSV)
Triticum aestivum	OsaMIR395	Wheat streak mosaic virus
Zea mays	ZmaMIR159a	Rice Black Streaked Dwarf Virus (RBSDV)

CONCLUSION

Mi-RNAs and other small non-coding RNAs are now considered the key regulators of gene expression. With the availability of various online gene prediction tools, omics-based technologies, genome editing tools, and high throughput methodologies, more and more mi-RNAs with significant roles in plant growth, development, and survival are being identified, designed, and validated. The manipulation of gene expression by targeting the mi-RNA-mRNA couples has emerged as a feasible approach for producing various stress-tolerant transgenic plant productions. Because of the high specificity and efficacy of this approach, it is now being used to produce agronomically important crops with better yields. With the identification of more significant miRNAs, there is a potential for enhanced production of many other crops even under unfavorable conditions.

REFERENCES

1. Ai T, Zhang L, Gao Z, Zhu CX, Guo X. Highly efficient virus resistance mediated by artificial microRNAs that target the suppressor of PVX and PVY in plants. Plant Biol. 2011; 13(2):304-316.
2. Ali M, Javaid A, Naqvi SH, Batcho A, Kayani WK, Lal A, et al. Biotic stress triggered small RNA and RNAi defense response in plants. Mol Biol Rep. 2020; 47:5511-5522.
3. Baker CC, Sieber P, Wellmer F, Meyerowitz EM. The early extra petals1 mutant uncovers a role for microRNA miR164c in regulating petal number in Arabidopsis. Curr Biol. 2005; 15(4):303-315.
4. Barrera-Figueroa BE, Gao L, Diop NN, Wu Z, Ehlers JD, Roberts PA, et al. Identification and comparative analysis of drought-associated microRNAs in two cowpea genotypes. BMC Plant Biol. 2011;11:1-1.
5. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. cell. 2004; 116(2):281-297.
6. Berezikov E, Guryev V, van de Belt J, Wienholds E, Plasterk RH, Cuppen E. Phylogenetic shadowing and computational identification of human microRNA genes. Cell. 2005; 120(1):21-24.
7. Bhardwaj AR, Joshi G, Pandey R, Kukreja B, Goel S, Jagannath A, et al. A genome-wide perspective of miRNAome in response to high temperature, salinity and drought stresses in Brassica juncea (Czern) L. PloS one. 2014;9(3):e92456.
8. Cao X, Wu Z, Jiang F, Zhou R, Yang Z. Identification of chilling stress-responsive tomato microRNAs and their target genes by high-throughput sequencing and degradome analysis. BMC Genom. 2014; 15(1):1-6.
9. Carbonell A, Lisón P, Daròs JA. Multi-targeting of viral RNAs with synthetic trans-acting small interfering RNAs enhances plant antiviral resistance. Plant J. 2019; 100(4):720-737.
10. Chen L, Luan Y, Zhai J. Sp-miR396a-5p acts as a stress-responsive genes regulator by conferring tolerance to abiotic stresses and susceptibility to Phytophthora nicotianae infection in transgenic tobacco. Plant Cell Rep. 2015; 34:2013-2025.