

A Seminar Paper  
On

**MicroRNA: An Essential Tool for Abiotic Stress Tolerance in Plants**

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## ABSTRACT

As climate is changing, plants facing different types of abiotic stresses which hinder their normal growth and development. Sometimes these abiotic stresses cause irreversible damages. Most of the cases some small RNAs called microRNA (miRNA) act as a defense mechanism which preserve the vitality of plants. They are the key molecules for regulatory network. After transcription of nuclear encoded MIR genes with help of RNA polymerase II, it gives rise to mature miRNA. They regulate different biological and metabolic process to induce tolerance level against stress. After the first discovery of miR gene in *Caenorhabditis elegans* researchers made it a research hotspot. All their researches aimed to find out expression profile of miRNA, their biogenesis, mode of action, making artificial miRNAs, roles in stress response and finally using this in modern agriculture for better output. By the advancement of technology different metabolic adjustment have gained in different cereals, legumes, fruit crops etc. This review is aimed to provide regulatory role of miRNAs in plant abiotic stresses as well as potential application of miRNAs as a new target for genetically improving plant tolerance to abiotic stress.

**Key Words:** miRNA, abiotic stress, stress response, application

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# CHAPTER 1

## INTRODUCTION

To meet the demand of a continuously increasing human population, production of food in sustainable manner is vital for our world (Mochida and Shinozaki, 2013). But as a sessile organism, plants always face adverse environmental perturbations. Because of pervasive nature and shocking impacts on plant growth, development and quality; abiotic stresses have become a major challenge nowadays (Wani *et al.*, 2016). Climate change, which has accelerated in recent decades, stands as a major cause of abiotic stress leading abnormalities in cellular homeostasis and has devastating impact on plants growth and development (Mickelbart *et al.*, 2015). Different abiotic stress conditions like drought, cold, extreme heat, heavy metals, salt and nutrition deficiencies are the major limiting factors in agricultural production (Akpınar *et al.*, 2013; Budak *et al.*, 2013; Budak *et al.*, 2015).

According to Ku *et al.*, (2015), plants preserve vitality against these abiotic stresses by triggering a network of genetic regulations that include altered expression of large proportion of genes by transcriptional and/or translational regulations. Recent researches indicate that, to attenuate plant growth and development under stress conditions plants allocate microRNA (miRNA) for critical post-transcriptional gene-expression regulators, though how this is achieved at molecular levels is yet to be understood with greater details (Zhang and Wang, 2015). Moreover abiotic stress conditions induce aberrant expressions of miRNAs in plants. Scientists have done computational approaches in recent years. High throughput sequencing has been done for identifying a large number of stress-related miRNAs. Nowadays miRNAs serve as a potential target for genetic manipulations. It also engineer abiotic stress tolerance in plants.

There are several types of sRNA and the microRNA (miRNA) is one of them which are small non-coding, (20–24 nt) sized, single-stranded riboregulator RNA. After transcription of primary miRNA (which is a double stranded stem loop like structure) (Voinnet, 2009) it give rise to mature miRNA. The primary miRNA is transcribed from nuclear encoded MIR genes with help of RNA polymerase II (Lee *et al.*, 2004). When After generating the mature miRNAs, they are loaded into the RNA-induced silencing complex, which contains an argonaute protein and then they bind to the mRNAs of targeted genes without functioning directly in plant growth and development or in plant response to environmental stress. Winter

and Diederichs, (2011); Iwakawa and Tomari, (2013) reported that, miRNAs participate in plant response to abiotic stresses by regulating key components of complex gene networks.

The majority of stress-responsive miRNAs target transcription factors which play an important role during plant response to different environmental stresses. If we compare between mRNA and miRNA, the miRNAs regulate gene expression either by targeting mRNAs for cleavage or by inhibiting protein translation (Jones-Rhoades and Bartel, 2004; Zhang *et al.*, 2014). MicroRNAs have been identified to work under biotic and abiotic stress conditions such as drought, cold, nutrient deficiency, salinity, bacterial infections, UV-B radiation, mechanical stress etc. Not only in stress condition but also they are involved in many biological and metabolic processes of plants like auxin signaling, leaf development and polarity, lateral root formation, meristem boundary formation and organ separation, transition from juvenile-to-adult vegetative phase and from vegetative-to-flowering phase, floral organ identity, reproduction and so on (Huang *et al.*, 2010). Besides controlling targets at post transcriptional level miRNAs control the gene expression causing epigenetic changes (Khraiwesh *et al.*, 2010; Wu *et al.*, 2010).

If we want to feed the growing human population, future agriculturists need to focus on quality breeding where we can produce new crop cultivars that have higher tolerance to different environmental stress conditions. Different types of genetical, biochemical, molecular and biotechnological approaches are now performed to make miRNAs in gene silencing, improving tolerancy, expression profiling etc. But more study and time is needed to make miRNAs become a real target for improving crop tolerance.

**Objectives:**

Based on the above discussion, the present study has been conducted aiming the following objectives-

- To review the regulatory actions of miRNAs in response to abiotic stress
- To discuss the recent success and potential applications of miRNAs for abiotic stress tolerance in plants.

## CHAPTER 2

### **MATERIALS AND METHODS**

This seminar paper is exclusively a review paper. Therefore, all the information was collected from secondary sources like various relevant books, journals, research articles etc. The title is selected with the consultation of my major professor. For collecting recent information, internet browsing was also practiced. Good suggestions, valuable information and kind consideration were taken from honorable seminar course instructors, major professor and other resource personnel to enrich this paper. After collecting all the available information, it has been compiled and arranged chronologically as per the objectives of this paper.

## CHAPTER 3

### REVIEW OF MAJOR FINDINGS AND DISCUSSION

In the entire plant life cycle, gene expression always regulates the morphological and physiological changes as well as adaptation in biotic and abiotic stresses. If we want to produce abiotic stress tolerant crops, we need a better understanding about gene-regulation mechanisms employed by the plants in response to environmental factors. Unprecedented attention has been gained by recent researches on non-protein coding small RNAs which block the specific messenger RNAs (mRNAs) and affect epigenetic modifications at the transcriptional level. According to Mittal *et al.*, 2016, microRNAs are the family members of these small ribonucleotide sequences which differ from each other on the basis of their size, biogenesis, mode of action and/or regulatory role. There are many miRNAs present in plants. They play an important role in post-transcriptional regulations. They start this with base-pairing through help of complementary mRNA targets, particularly transcription factors (TFs) (Li and Zhang, 2016).

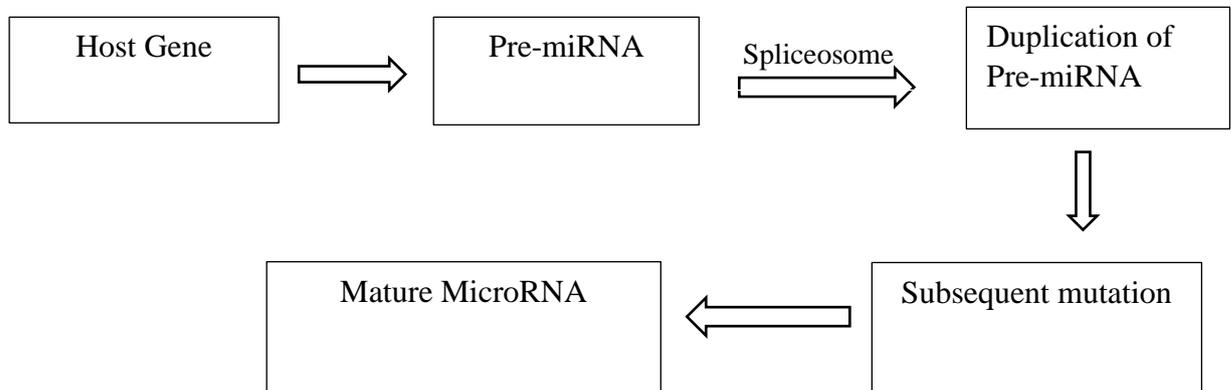
#### **3.1 Plant miRNAs: Brief Overview**

Many researchers like Axtell *et al.*, (2007); Zhu *et al.*, (2008); Klevebring *et al.*, (2009); Fahlgren *et al.*, (2007); Zhang *et al.*, (2009); Joshi *et al.*, (2010); Ma *et al.*, (2010); Dhandapani *et al.*, (2011) reported that, most plant species contain miRNA genes in their genome. It may be more than 100 in number. Till now plants have developed different stress linked survival mechanisms. But this small non-coding miRNA control gene expression via epigenetic changes (Wu *et al.*, 2010).

##### **3.1.1 miRNAs: Origin**

There are three different hypothesis proposed about origin and evolution of miRNA. The first hypothesis is about origin of miRNA genes was proposed by Allen *et al.*, (2004). According to them, newly emerged “young” miRNAs that always show extended sequence homology to their targets both within and outside the mature miRNA region. Secondly, transposable elements (TEs) transform into miRNA genes; as the miniature inverted-repeat transposable elements (MITEs) have the potentiality to become miRNA genes. Each has some kind of inverted repeats with a short internal sequence. These repeats can potentially turn into the hairpin structure of a miRNA gene. Different scholars suggested that dozens of miRNA genes has been originated from MITEs or other TEs in *Arabidopsis* and rice (Piriyaongsa and

Jordan, 2008). Thirdly and the most reliable one is that miRNA genes are generated by duplication of pre-existing miRNA genes through subsequent mutations (Li and Mao, 2007) (Figure 1).

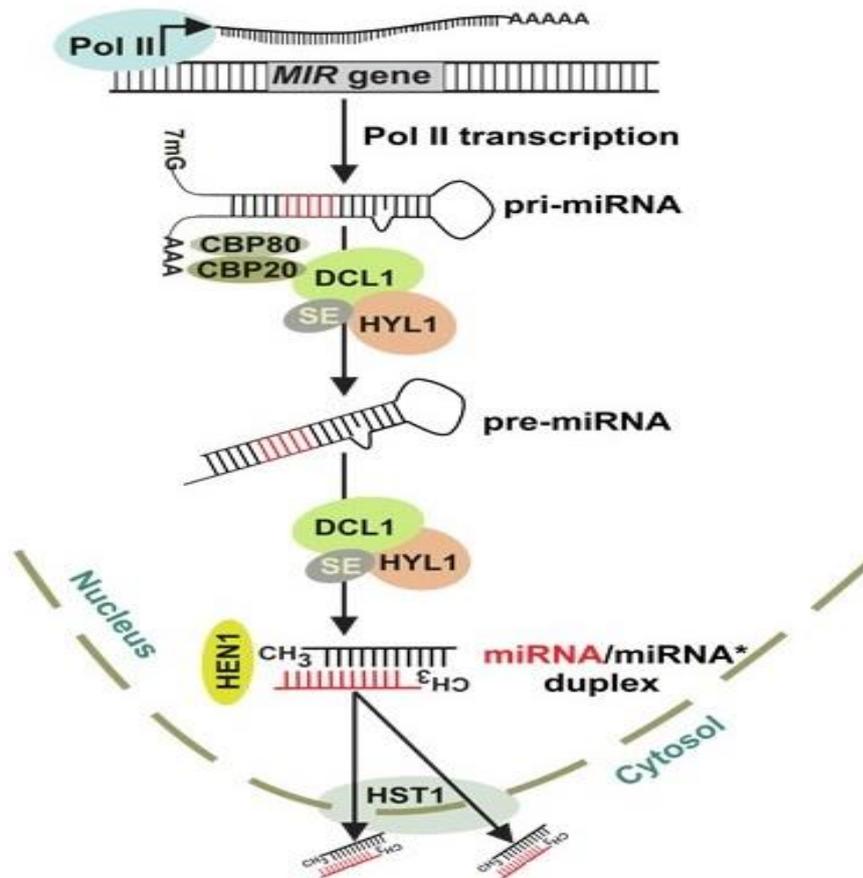


**Figure 1: miRNA Origin**

Source: Li and Mao, (2007)

### 3.1.2 miRNAs: Biogenesis

MicroRNAs are classified into two- “intergenic” or “intronic”. In both cases miRNA biogenesis begins in the nucleus. Independent mature miRNA comes from primary miRNA which is RNA-polymerase II-dependent (RNAPII) transcription. This is 20-22 nucleotides RNA strands which are coded by endogenous MIR genes. Initially primary miRNA (pri-miRNA) is synthesized which is folded into hairpin-like structures consisting of a terminal loop, an upper stem, the miRNA region, a lower stem, and two arms. These arms can be recognized and processed by Dicer-like RNase III endonucleases (DCLs). According to plant species DCL proteins differ in number. Simon *et al.*, published an article in springer on 2008 where they said that, there are four DCL proteins in *Arabidopsis thaliana*. Most miRNAs are catalyze through DCL1 protein where some accessory proteins including the double-stranded RNA-binding protein Hyponastic Leaves 1 (HYL1) and the zinc-finger protein Serrate (SE) act as assistants (Fang and Spector, 2007; Dong *et al.*, 2008) and after further processing mature miRNA generates (Figure 1). Then a bit of methylation by the enzyme HEN1, miRNAs are transported out of the nucleus through HASTY protein (HST) into the cytosol (Budak *et al.*, 2014, 2015; Sinha *et al.*, 2015; Kantar *et al.*, 2010; Gupta *et al.*, 2014; Ma *et al.*, 2015).



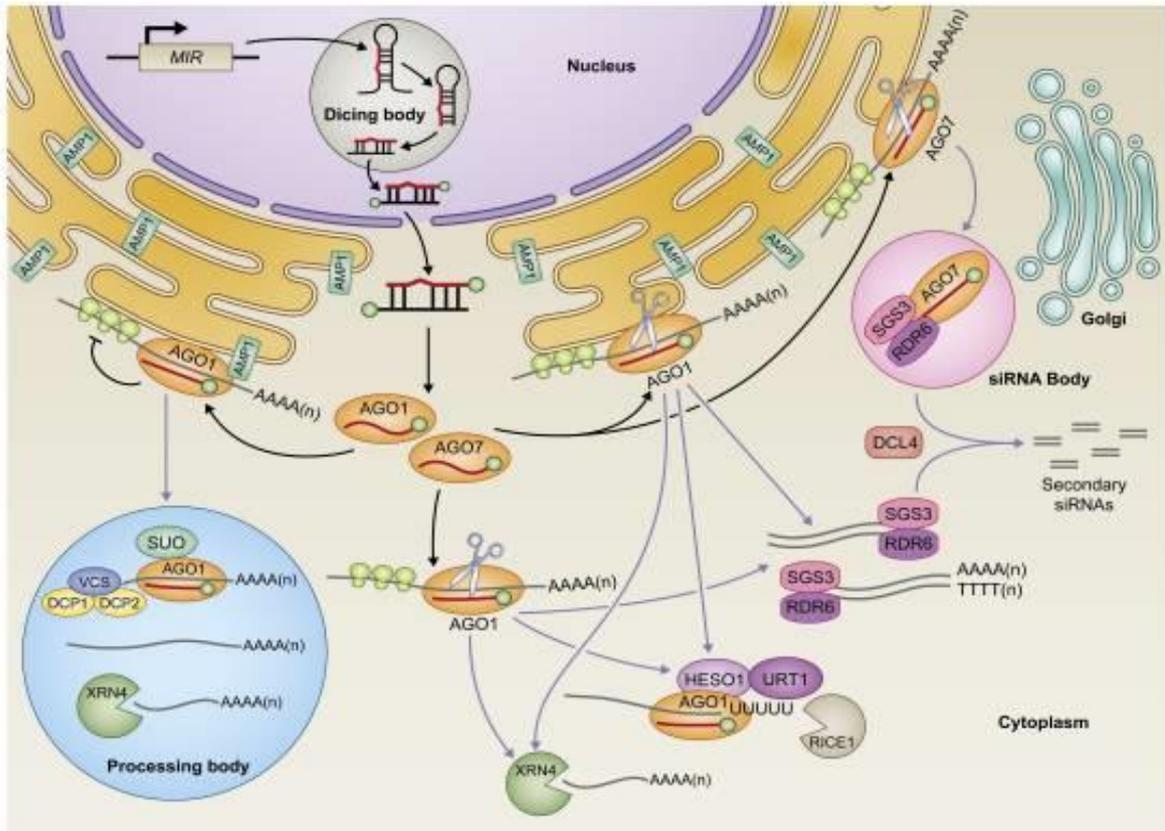
**Figure 2: miRNA Biogenesis Pathway**

Source: Khraiwesh *et al.*, (2012)

### 3.2 Plant miRNAs: Mode of Action

Transcription factors regulate the gene transcription as it is a key mechanism. miRNAs mediate gene expression regulations. This initiates after the generation of double stranded RNAs or single stranded RNAs. These RNAs are folded into stem-loop/hairpin structures in the cell (Jamalkandi and Masoudi-Nejad, 2009; Voinnet, 2009). Plant miRNAs regulate target genes at the post-transcriptional level via two major mechanisms (Chen, 2009).

1. transcript cleavage
2. translation repression (Figure 3)



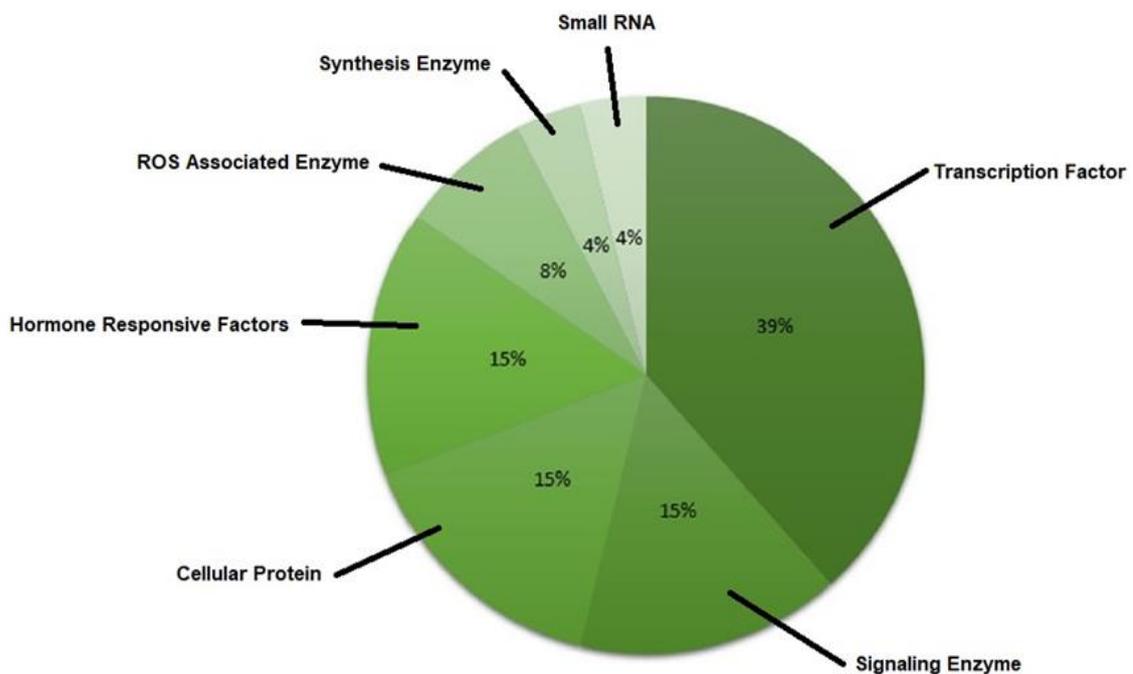
**Figure 3: Overview of microRNA (miRNA) Modes of Action in Plants**

Source: Chen, (2009)

Transcript cleavage is also known as slicing which occurs at a precise location in the target mRNA (Llave *et al.*, 2002). In case of plants, translation repression is less frequently observed than transcript cleavage. Overall, to direct post-transcriptional gene silencing through transcript cleavage or translation repression mature miRNAs are incorporated into ARGONAUTE proteins. ARGONAUTE 1 (AGO1) cleaves the miRNA target and then degrades the cleaved fragments. Here uridylation and turnover of 5' cleavage fragments take place. With help of endoplasmic reticulum (ER)-localized ALTERED MERISTEM PROGRAM 1 (AMP1), translation repression takes place on membrane-bound polysomes (MBPs). Then in association with MBPs SUPPRESSOR OF GENE SILENCING 3 (SGS3) and RNA-DEPENDENT RNA POLYMERASE 6 (RDR6), ARGONAUTE 7 (AGO7) cleaves miRNA targets and forms siRNA bodies. According to Budak and Akpinar, (2015), miRNAs bind to their target transcripts usually in complementary manner. By inhibiting their translation by cleavage and/or degradation of the target mRNA molecule, they fine-tune the target gene expression in higher eukaryotes.

### 3.3 Plant miRNAs: Roles in Abiotic Stress Response

All types of abiotic stresses are crucial environmental constraint limiting crop production. After the first discovery of miRNA genes in *Caenorhabditis elegans* (Lee *et al.*, 1993) plant miRNA become a research hotspot. After that a steady increase in identifying the miRNA families has been seen (Cao *et al.*, 2014). In case of stress conditions some miRNA always over or under-express to mitigate the stress. “The miRNAs fine-tune the target gene expression to degrade and/or inhibit protein production in higher eukaryotes” (Akdogan *et al.*, 2015). Scientists have found several types of stress regulated miRNA in model plants which cope with different biotic and abiotic stresses. It is acknowledged by Liu *et al.*, in 2008 that, about 117 miRNAs were analyzed under salinity, drought, and low-temperature conditions using miRNA chips representing nearly all known miRNAs identified in *Arabidopsis*. In figure 4, seven target sites for miRNA functions are shown which have been found till date. miRNAs dramatically affect the plants under abiotic stress conditions by regulating several coding genes (Reyes *et al.*, 2010; Sunkar, 2010). So we have to know how miRNAs regulate the gene expression and the coded genes response in stress conditions.



**Figure 4: Stress-responsive miRNAs Percentages with Respect to the Predicted Target**

Source: Alptekin *et al.*, (2017)

### 3.3.1 Regulatory Roles of Different miRNAs

The up and down-regulation of MIR gene sequence has confirmed the involvement of miRNAs in stress responses in different plant species. It is found by researchers that miR393 is one of the key miRNAs during stress responses (Sunkar and Zhu, 2004; Zhao *et al.*, 2007; Liu *et al.*, 2008; Arenas-Huertero *et al.*, 2009 & Trindade *et al.*, 2010). They found that miR393 showed altered expression in *A. thaliana*, cultivated rice, *Medicago truncatula*, *Phaseolus vulgaris* and other plants once they are under drought, salinity, coldness, and aluminium (Al) stress conditions. After setting a research Zhao *et al.*, (2009) reported that miR169 family respond differently to drought, salinity, low temperatures and aluminium in plants. They said in case of rice when it is under salinity and drought stress, the nuclear transcription factor YA (NF-YA) genes expression are modulated by miR169 family where it is more sensitive to drought (Li *et al.*, 2008) in case of *A. thaliana*. But in tomato plants, miR169 acts tolerant to drought (Zhang *et al.*, 2011a) as it targets a gene involved in the opening and closing of stomata. Sometimes they play up-regulation and sometimes they play down-regulation roles in same and different crops in their different developmental stages and different stresses. Table 1 is the illustrator of this thing.

**Table:1 Various miRNAs Aberrantly Expressed under Different Abiotic Stress Treatments in a Multiple Plant Species. (U indicates up-regulation, D indicates down-regulations)**

Crop Name	Stresses	Different Types of MiRNAs													
		miR 156	miR 157	miR 160	miR 161	miR 162	miR 165	miR 168	miR 393	miR 394	miR 396	miR 398	MiR 399	miR 408	
Maize	Drought	U				U		D				U	D		
	Salinity	D				U		U			D				
Rice	Drought	D		U				D	U			D			
	Salinity			D					U	D	D				
Wheat	Drought							D	U						
	Salinity	U		U			D		U			D	U	U	
Barley	Drought	U						U	D		U		U	U	
	Salinity														

Cotton	Drought	D	D	D	D	D	D	D	D	U	D	D	D	D
	Salinity	U	D	D	D	D	D	D	U	U	D	U	D	U
<i>Arabidopsis</i>	Drought	U						U	U		U			U
	Salinity	U						U	U		U	D		

One of the earliest papers of Jones-Rhoades and Bartel, (2004) reported that, inorganic sulphate assimilation was one of the targets of miR395. Their result shows that, miR395 is involved in sulphate uptake and metabolism in plants. At the same time, Sunkar and Zhu, (2004) in an experiment with 4 treatments (cold stress, drought stress, salt stress, hormones) in *Arabidopsis* seedling samples found miRNAs in all of them. miR393 was strongly induced in the samples by the stresses. In 2008 Lu et al., found miR1450 which was present in both monocot and dicots but the expression pattern was different. It down regulates under drought condition in case of *Populus trichocarpa* but up regulates in *Triticum dicoccoides*. Kantar *et al.*, in 2010 found some inverse correlation between miR156, miR166, miR171, miR408 and their targets in barley plants when they were treated by drought stress. In different tissues from different developmental stages in rice plants under drought conditions in soil miR156, miR171 and miR408 were also detected (Zhou *et al.*, 2010).

### 3.3.2 MiRNA: Target for Improving Plant Tolerance

Not only in drought and salinity conditions but also in biomass, nutrient homeostasis, heavy metal toxicity, oxidative stress, UV-radiation plants show effective performance with help of miRNAs in their cells. We all know about hypoxia. This hypoxia causes irreversible damage to crop plants. Scientists found that different types of miRNA in maize adjust plants morphological and physiological adaptations in root cell under hypoxia condition. Sunkar *et al.*, in (2006) found that the repression of some miRNAs and the up-regulation of SOD proteins have a crucial role in *Arabidopsis* plants under oxidative stress. If plants feel any mechanical stress miR408 up-regulates the tension and compression. Plant miRNAs do not function directly in growth and development of plants. They only regulate the key components of complex gene networks.

### 3.4 Applications of miRNA for Abiotic Stress Tolerance

MiRNAs always play a potential role in plant abiotic stress tolerance. Now we are using both miRNAs and artificial miRNAs (amiRNAs) to develop transgenic plants with high tolerance

to abiotic stress. The transference of one or more MIR genes between non crossable plant species (transgenesis) or between crossable of the same species (cisgenesis and intragenesis) has been successfully performed in several plant species (Table 2). These results are often explained by the involvement of miRNAs in diverse and sophisticated regulatory networks. Strong over-expression impairs the fine-tuning of several biological pathways, which could be overcome by using specific promoters (e.g. tissue-specific, stress-induced or developmental stage-specific promoters) (Basso *et al.*, 2019).

**Table 2: Recent Application of miRNAs through Genetics and Biotechnological Approaches**

Targeted miRNAs	Transgenic plants	Targeted stress
156	Switch grass	Increase of biomass (Fu <i>et al.</i> , 2012)
169	Arabidopsis	Sensitivity to nitrogen deficiency (Zhao <i>et al.</i> , 2011) and tolerance to UV radiation (Jia <i>et al.</i> , 2009)
	Tomato	Reduce transpiration rate( Zhang <i>et al.</i> , 2011)
319	Rice	Tolerance to chilling temperature, increase cell membrane integrity (Yang <i>et al.</i> , 2013)
395	Rapeseed	Enhanced tolerance to oxidative stress and heavy metal stress (Zhang <i>et al.</i> , 2013)
828	Sweet potato	Oxidative stress(Lin <i>et al.</i> , 2012)
394	Arabidopsis	restricted water loss (Ni <i>et al.</i> , 2012)
398	Arabidopsis	Increase tolerance to oxidative stress( Sunkar <i>et al.</i> , 2006)
	Brassica	Regulator of Cu homeostasis (Marschner and Minera, 1995)
390	Rice	Susceptibility to Cd ( Ding <i>et al.</i> , 2016)
396	Rice	larger grain size and enhanced grain yield (Li <i>et al.</i> , 2016)
397	Rice	Promotes panicle branching( Zhang <i>et al.</i> , 2013)
399	Tomato	Tolerance to cold and phosphorus deficiency(

Targeted miRNAs	Transgenic plants	Targeted stress
		Goa <i>et al.</i> , 2015)
482	Soybean	Increases of nodule numbers( Li <i>et al.</i> , 2010)

Source: Zhang *et al.*, (2015)

Moreover Holme *et al.*, (2013) reported that, any elements that can be used in T-DNA, can be engineered. It may enhance the transgene expression. This same native sequence can be used to maintain the original cis-gene features too. However, the manipulation of MIR genes using any of these strategies, especially overexpression driven by a strong promoter, has also resulted in undesirable traits, such as pleiotropic phenotypes.

### 3.5 Biotechnological Advances on miRNA (Artificial miRNAs)

Now we know the various aspects on biogenesis, function and mode of action of plant miRNAs in biochemical and molecular level. By the synthesis of synthetic miRNAs artificial MIR genes has opened a new prospect for pushing the boundaries. Now we are uncovering the specific functions of various genes through different strategies and these strategies are primarily based on the introduction of artificial MIR gene (amiRNAs) (Zhang *et al.*, 2018). These artificial miRNAs are putting into the host to produce specific miRNAs to silence target genes. Carlsbecker *et al.*, reported in 2010 that, the original miRNA sequence can be replaced by an engineered miRNA which can target a specific mRNA. Thus, amiRNAs are often engineered to focus on any mRNA with higher specificity. According to Manavella *et al.*, (2012), amiRNAs are stable and inheritable but the systemic movement of these mature amiRNAs is restricted in some cases. Carbonell *et al.*, in (2015) said that, the main drawbacks may be the selection pre-amiRNA sequences for effective silencing without any off-target effects. Moreover, amiRNA now successfully been used to knock out genes from insect pests, nematodes, viruses and other phytopathogens. It now controls the endogenous gene expression and study the role of new MIR genes (Kis *et al.*, 2016; Wagaba *et al.*, 2016).

## CHAPTER 4

### CONCLUSION

As world population is increasing, both the quantity and quality of agricultural production is necessary. Crops are challenging regularly by numerous adverse conditions like environmental stresses. Plants microRNAs improve tolerance against these stresses.

- ❖ MicroRNAs regulate gene expression in response these stresses via translational inhibition. Recently detailed study of miRNAs and their targets has expanded our understanding in regulatory systems. The fine-tuning role of miRNAs and overlapping roles in response to abiotic stress induced by drought, salinity, low temperatures, nutrient homeostasis, heavy metals etc. have broaden the research area. There are few reports that focus on validating miRNA function. One reason may be is because we have limited knowledge of the miRNA-regulated gene network that is involved in plant response to abiotic stress.
- ❖ Nowadays we are doing direct cloning and deep sequencing of small miRNA libraries. The use of artificial miRNAs (amiRNAs) which can target stress-responsive mechanism may solve the problem of reduce productivity due to abiotic stresses. Future studies in this field should switch from identifying miRNAs to validating the roles of individual miRNAs in plant. In order to achieve this goal, more new technologies must be developed so that it is able to screen and test multiple genes, including miRNAs at the same time. Furthermore, plant development and responses to stresses also requires the coordinated action of multiple mechanisms (Sorin *et al.*, 2005).

## CHAPTER 5

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