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# Plant microRNA: Biogenesis and role of plant mi-RNA in molecular breeding

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## Abstract

MicroRNAs are extensive, small, non-protein-coding RNA molecule and endogenous in small regulator's RNAs which consist of 20-24 nucleotide and that have been important for many physiological and developmental processes. miRNAs are found in plants, animals and as well as in some viruses also. As the population is increasing so that the food production is need to be doubled by year 2050. It is necessary to improve the crop requirements or their quality and quantity. In this review paper we will discussed the dynamic pathway of biogenesis in miRNAs and the possibility of using these miRNAs for crop improvement through genome editing or through negative regulation that expressed the gene involved in post transcriptional regulation either by promoting the degradation of target mRNA in mRNA cleavage by perfect complementary or in translation Suppression by partial complementary. A common fuction of miRNA is control of gene expression by regulating some features that are - Transcription factor; stress response protein; proteins that impact development, growth and physiology of plant. Capitalized miRNA is a mature form which denote as "miR", Ex- miR156 in switchgrass, regulate the increased biomass, starch and digestibility; while the uncapitalized "mir" refers to the pre-miRNA and pri-miRNA which is in immature form in the nucleus. As the miRNA play a very crucial role in the plant growth and development thereby, it is also responsible for the various stresses like biotic stresses and abiotic stresses, disease resistance, heat tolerance, hormonal signal transduction etc. In this review we discuss the importance and role of molecular breeding in plants.

**Keywords:** miRNA; dynamic pathway; post transcription regulation; transcription factor; translation suppression; environmental stresses; crop breeding

## Introduction

MicroRNAs is typically 21 or 22 nucleotides in length which is non-protein-coding RNA molecule, found in both animals and plants. Animal and Plant microRNAs are differ from each other, animal miRNAs mainly act by translational inhibition using targets at the 3'-UTR while in plant miRNAs regulate target genes at the post-transcriptional level through two main mechanisms: transcript cleavage and translation repression. In this review paper we will discussed briefly about the plant miRNAs biogenesis, function and role in the field of molecular breeding.

By 2050, the world population will rise to 9 billion and food production needs to be almost doubled to meet the rising demand for food [Nogoy 2018] [25]. Growing the world's population, the shortage of arable land and water, global changes in environment and the need for biofuels as alternative sources of energy have created serious questions about the potential adequacy of global crop plant food output (Rosegrant and cline, 2003; Brown and Funk, 2008) [29, 5, 6]. Various crop scientist and breeders in whole world are dedicated themselves for finding the cure for the challenges the threaten food nutrition and security. To solve these problems, new agricultural technology, in addition to water and land conservation efforts, will be required to ensure global food supply and stability.

However, natural non-coding miRNA was discovered in metazoans around 26 years ago, and since then scientists have been exploring its biogenesis and function and then crop scientists have begun harnessing their possible uses to increase crop quantities.

At Harvard University, scientist Dr. Victor ambros and his colleagues (in1993) discovered a small molecule of RNA, called *Lin-4*, with around 22 nucleotides in *C. Elegans* (Lee *et al*., 1993) [19] this is the first miRNA identified in history [zhang 2014].

Reinhart and his fellow mates discovered in 2002 that miRNAs are also present in plants, 16 miRNAs of Arabidopsis were cloned in their study, and 8 of them are preserved in the genome of rice. To date, through base pairing with their complementary mRNA targets, plant miRNAs play a crucial or dominant role in post transcriptional gene regulation [In 2002 Reinhart *et al.*] [28].

A large number of miRNAs were also found in various plant species in the following year, including aradiopsis and rice, and many of these miRNAs are differentially expressed as plant growth and morphology [zhang 2014].

In order to enhance their qualitative and quantitative characteristics, including resistance to biotic and biotic stresses, plant molecular breeding appears to be an effective and economic way to tailor crops. Recent studies have begun to reveal powerful and unexpected miRNA roles in regulating native gene silencing plant growth [liu2012]. In almost all aspects of normal plant growth and development, and biotic stress, microRNAs play crucial roles (Budak *et al*. 2015; Shriram *et al*., 2016; LiS. J. *et al*., 2017; Brant and Budak, 2018) [7, 8, 32, 21, 4].

## MiRNAs biogenesis

MiRNAs, a new class of endogenous small regulatory RNAs with 20-24 nucleotides, are small, non-protein-coding RNA molecules (Reinhart *et al*. 2002; Bartel, 2007; Zhang *et al*., 2006c) [28]. The number of miRNAs is varied among the plant species; it is from less than 100 to several hundreds. For example- 378 miRNA genes in rice genome while 72 miRNA gene in papaya **(**Nozawa *et al*, 2012) [26]. They are expressed by genes that are intended to provide miRNAs, but control genes (mRNAs) with the exception of those that express them (Mack 2007) [22]. We are inclined to bear in mind that miRNAs is just only one in each of the classifications of non-coding RNAs. There are other classification are also exist such as small interfering RNA (siRNA), PIWI-Interacting RNA (piRNA, found in animals only), small RNA derived tansferd RNA (tsRNAs) and long non coding RNAs (LncRNAs) (Borgers ad Marteinssen, 2015; Czech *et al*., 2018; Zhu *et al*., 2018; Treier *et al*., 2019). These are classified on the basis of their length, structure and function. The single important factor which separating these non-coding RNAs from each other is the mode of biogenesis. The miRNAs region unit is graded as either 'intergenic' or 'intronic' depending on their position within the order. The area unit of intergenic miRNAs settled between two protein-coding genes and the are transcribed by DNA-dependent RNA enzyme II (Pol II) as separate units, while the area unit of intronic miRNAs processed from their host transcript introns (Millar and Waterhouse, 2005; Budak and Akpinar, 2015) [7, 8].

The biogenesis of miRNA will be seen (Fig.1). The biogenesis of miRNA is regulated at several levels, including:-

- MiRNA transcription,
- Its processing in the nucleus,
- Modification process in the nucleus RNA editing, RNA methylation and uridylation,
- Nuclear export and
- Argonaute loading during cytoplasmic process.

For the production of miRNA, primary miRNA (pri-miRNA) (i.e. hairpin structured precursor) is transcribed by RNA polymerase II (Lee *et al*. 2004) [20]. The mediator, elongation, and transcription factors such as NEGATIVE ON TATA LESS2 (NOT2) and CELL DIVISION CYCLE5 (CDC5) promote this process (Fang *et al*. 2015; Wang *et al*. 2019). Now the stem loop precursor (Pre-miRNA) is processed by Pri-miRNA. In the nucleus, miRNA is unstable and processed into miRNA-miRNA* duplex by DCL1 (Dicer Like enzyme1) (Kim 2005) [17] and stabilized by S-adenosyl methionine-dependant methyltransferase. HUA ENHANCER1 (HEN1), a reaction that prevents degradation by small RNA degrading nuclease (Voinnet 2009) [34]. Methyl group that is present on the 3' terminal nucleotide of each strand that prevent the uridylation and following degradation by the SMALL RNA DEGRADING NUCLEASE(SDN) (Li *et al*., 2005). HASTY transport protein (an ortholog of exportin-5 protein) helps in the nuclear transports of modified miRNA-miRNA*(miRNA is guide strand and miRNA*is degraded strand) into cytoplasm and then processed into mature miRNA (Park *et al*. 2005) [27]. The guide miRNA strand is then incorporated into argonaute proteins (AGO1) to hold out the silencing reactions. The destiny of miRNA is depending on its level of complementarity with similar miRNA sequence (Alikelayeh *et al*. 2016). An almost complete or perfect complementarity leads to total degradation of the target mRNA (mRNA cleavage), while partial complementarity leads the repression of protein translation. Moreover, we can view that miRNA biogenesis is a dynamic pathway with a tight interconnection and co-regulation of co-factors, genes, and proteins (Achkar *et al*. 2016).

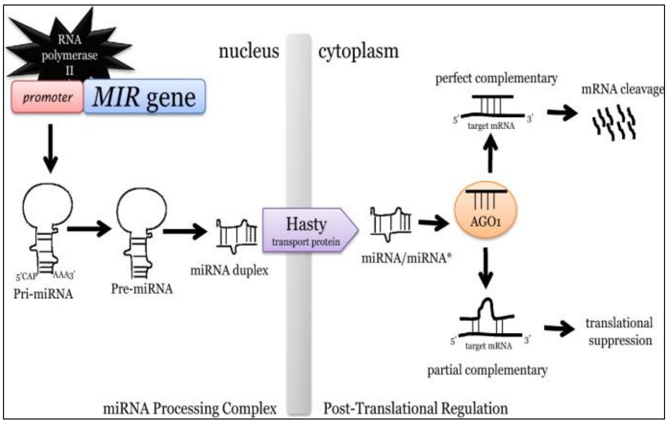

**Fig 1:** miRNA biogenesis in plant.

As shown in the fig.1, hairpin structure precursor that is pri-miRNA in transcripted by RNA polymerase II from plant mRNA gene which then converted into pre-miRNA (stemloop precursor). The interaction of the RNA-binding protein DAWDLE (DDL) with DCL1 (Dicer like enzyme1) stabilises the conversion of pri-miRNAs to stem-loop pre-miRNAs in dicing-bodies (D-bodies). And this enzyme also helps in the processing for the formation of miRNA/miRNA* duplex. Till now the complete process is occur in the nucleus which simply referred as miRNA processing complex. Now the duplex is export in the cytoplasm by the intermediate HASTY transport protein. The process occur in the cytoplasm is post translation regulation in which the miRNA duplex is export for loading into agronaute protein (AGO1). From this step the target mRNA is distributing in the 2 steps all depends on destiny. Some are perfect complementary which forms mRNA cleavage while some are partial complementary which suppressed the translation.

## Functions of miRNA in crop

Already we studied that the miRNAs have 20-24 nucleotide and naturally occuring in plant and further can be studied by various methods like computational methods and experimental techniques which are widely used in plants to identify more than 1000 miRNAs in plants. As the miRNA biogenesis is a dynamic process, so that the functions of miRNA can also vary. We see in biogenesis that the binding of target mRNA and inhibiting gene expression is one of the common mechanisms of miRNA (nogoy2018) [25]. By regulating a large number of biological processes in plants or crops, miRNAs play a key role that involves maintenance of order intergrity, development, metabolism, and accommodative responses toward environmental stresses (biotic and abiotic stresses etc). Every different plants and crops have different miRNAs which are depending on their function or the traits that are showing by their miRNAs. According to Bartel 2004 [2], the target mRNA have only one single miRNA that have complementary site which cleave the target mRNA at that complement sites. There are several miRNAs for different traits in many crops; however, no studies are available on their use in plant breeding. Recently, in several plant species, researchers have developed unique miRNA-based molecular markers (miRNA-SSRs / SNP) for several quantitative features.

The function of miRNAs are very well understood as the various experimental studies were completed by now and still various plant breeders and scientists are involved in detail study of miRNAs that are not discovered yet. In the table 1. We draw an important miRNA of various different species of plant/crops with their target genes and traits along with their specific gene. Alike in aradiopsis which is a model plant, too many miRNAs are discovered which show a specific function. MiRNAs are used in disease resistance as master regulators of NBS-LRR protein and TFs (nucleotide-binding site-leucine-rich repeat proteins and transcription factors). There are only three 22-nt miRNA families (miR1507, miR2109, and miR2118) that cause the development of phased short-interfering RNAs from NB-LRR encoding genes, according to Zhai *et al.* 2011 [37].

**Table 1:** Important miRNA of different plant species along with their trait

| S.no. | Crop | miRNA | Target gene | Traits | Reference |
|---|---|---|---|---|---|
| 1. | Aradiopsis | ▪ miR397a<br>▪ miR398<br>▪ miR398c<br>▪ miR169<br>▪ gma-miR172c<br>▪ Gma-miR394a<br>▪ miR394a/b | ▪ CBF, COR<br>▪ CSD1,CSD2 and CCS<br>▪ CSD1,CSD2 and CCS<br>▪ CLV1 and CLV2<br>▪ AP2-like TFs<br>▪ F-box genes<br>▪ LCR | ▪ Chilling and freezing tolerance<br>▪ Heat tolerance<br>▪ Freezing tolerance<br>▪ Disease resistence<br>▪ Salt stress and water deficiet<br>▪ Drought tolerance<br>▪ Salt tolerance | ▪ Dong and Pei 2014<br>▪ Gaun *et al.*, 2013<br>▪ Chen *et al.*, 2013 [9]<br>▪ Hanemian *et al.*, 2016<br>▪ Li *et al.*, 2016<br>▪ Ni *et al.*, 2012<br>▪ Song *et al.*, 2016 |
| 2. | Alfalfa | ▪ miR156 | ▪ SPL TFs | ▪ Tolerance to salinity stress | ▪ Muhammad *et al.*, 2017 |
| 3. | Jatropha curcas | ▪ miR398 | ▪ HD-ZIP | ▪ Abiotic and biotic stress | ▪ Zeng *et al.*, 2010 |
| 4. | Tomato | ▪ miR482/2118<br>▪ miR156<br>▪ Sly-miR169c | ▪ NBS-LRR<br>▪ CNR<br>▪ SlNF-YA1/2/3 and SlMRP1 | ▪ Disease resistency<br>▪ Fruit ripening<br>▪ Drought tolerance | ▪ Shivaprasad *et al.*, 2012<br>▪ Molesini *et al.*, 2012<br>▪ Zhang *et al.*, 2011a |
| 5. | Switchgrass | ▪ miR156 | ▪ SPL TFs | ▪ Increased biomass, starch and digestibility | ▪ Chuck *et al.*, 2011 |
| 6. | Barley (Hordeum vulgare) | ▪ miR156d<br>▪ miR396d | ▪ Squamosa binding protein<br>▪ Growth factor | ▪ Development, drought stress<br>▪ Seed development, cell differentiation | ▪ Curaba *et al.*, 2012<br>▪ Shuzuo *et al.*, 2012 |
| 7. | wheat | ▪ Tae-miR408 | ▪ TaTOC-A1, B1, C1and D1 | ▪ Heading date and plant architecture | ▪ Zhao *et al.* (2016) |

Some important effects of miRNAs which showing their traits in the plant aradiopsis:-

**miR397:** Different studies on miR397 have indicated its function in reactions to abiotic stress. For example, the expression of miR397 was up-regulated as a response to cold, drought, and high salinity in *Aradiopsis thaliana* **(**Sunkar and Zhu 2004). The expression of cold-regulated CBF genes and downstream COR genes was over-expressed under a constitutive promoter that affected miR397a. As a result of over-expression, the chilling and freezing stress resistance was enhanced by the Arabidopsis plants (Dong and Pei 2014) [12].

**miR398:** Families of miRNAs that have miR398 connected as their target gene CSD1, CSD2 and CCS to the stressed regulatory network as part of the oxidative burst product. By cleavage and ARGONAUTE10 (AGO10)-mediated translational repression, MiR398 guides the post-transcriptional regulation of CCS1 mRNAs. It shows the Reduces expression was reported as heat tolerance (Jia *et al.* 2009) [15]. Elevated temperatures in Chinese cabbage *(Brassica rapa)* decreased the expression of miR398 (Yu *et al*. 2012). If once exposed to salt stress for 48 hours, the reduced expression was recorded jointly in the genus Arabidopsis (Jia *et al*. 2009) [15]. Earlier study determined that the transgenic plants expressing the standard coding sequence of CSD1, CSD2 or CCS. These were show the heat tolerance in *A. thaliana*. In this the concentration of miR398 was showing increase which results the transcripts of its target genes were decreased. MiR398c was identified as a negative tolerance regulator against cooling stress in *Arabdiopsis thaliana*. The miR398c mutants showed higher rates of survival than wild type plants (Chen *et al*.2013) [9].

**miR169:** According to printed information, in one of the most significant and preserved miRNA families, the miR169 family is one important and it also includes angiosperm, dicotyledon, and a few ancient gymnosperms. Members of the miR169 family target the family of transcription factor encoding genes known as Nuclear Transcription Factor Y (Wu *et al*.2009; Li *et al*. 2010; Zhou *et al*. 2010b) [39]. Again, this element or factor is split into three subunits: NF-YA, NF-YB and NF-YC (Mantovani 1999). The miR169/NF-YA module is associated with drought stress (Li *et al*. 2008), nitrogen stress (Zhao *et al*. 2011; Liang *et al*. 2012), and cell expansion and metabolism of carbohydrate (Leyva-Gonzalez *et al*. 2012) in *Aradiopsis*.

## Role of miRNA in molecular breeding

MiRNA's role is as complex as it is in biogenesis, and miRNA's specific functions can differ. The common mechanism of miRNAs is to bind to and suppress the gene expression of the target mRNA. The mis-expression of miRNAs in plants can lead to different defects. By directing messenger RNA degradation or change it's location or translational arrest, microRNAs repress gene phenomena. Because of associate increasing world population, additionally as insufficiency of productive lands and water, international climate changes, and therefore an adequate potential international supply of food from crop plants is of great concern to the demands for biofuels as substitutive energy sources (Brown, Funk, 2008) [5, 6]. New agricultural technologies will be needed to resolve these challenges in order to ensure global food supply and stability, without regard to water and land conservation efforts (Liu, Chen, 2010). Plant molecular breeding tends to be an efficient and cost-effective way to customise crops to boost their qualitative and quantitative trajectory. Artificial miRNA technology was first applied in year 2004 to the model plant *Arabidopsis*.

Recent studies have started to reveal powerful and unexpected roles for microRNAs (miRNAs) through native sequence silencing in dominant plant development. Different aspects of plant development have been recorded to modulate miRNAs, including leaf morphogenesis and polarity, root initiation and development, floral differentiation and development, vascular development and transition from vegetative growth to reproductive growth of plant growth (Jones-Rhoades, Bartel; Chuck, Hake 2009). Moreover, miRNAs are concerned in growth regulator signal transduction (Liu, Chen 2009) [15] and signal transduction in response to environmental factors and pathogen invasion.

Flower development: The origin of the flower is described perfectly or completely by the ABC model. The flower organs are regulated by the behaviour of three classes- A, B and C of the transcriptional factor, according to this ABC model. APETALA2 (AP2) is the gene of class A which plays a essential function in morphology and flowering time. One of the targets for miR1722 is AP2 (Aukerman and Sakai 2003; Chen 2004). The overexpression of this miR172 gene prevents the translation of the genes AP2 and genes like AP2 and as a result early flowering is occur and it also disrupt the specification of floral organ.

Shoot apical meristems and the root apical meristems can help to develop plant shoot and root. The NAC family transcription factors known to be involved in shoot apical meristem formation and cotyledon separation during embryogenesis in Arabidopsis are encoded by cuc genes. The miR164 family targets are members (e.g., CUC1, CUC2, and NAC1) of the NAC family (Laufs *et al*. 2004; Mallory *et al*. 2004; Guo *et al*. 2005; Schwab *et al*. 2005) [18]. Plants that overexpress miR164 exhibit a number of developmental defects similar to mutants of the naked gene family. In comparison, miR164 mutants with loss of function accumulate higher levels of nac mRNA, leading to more lateral root formation (Guo *et al*. 2005).

Genome editing is one in all the foremost fortunate and helpful ways in plant molecular breeding. Genome editing may be a technique that enables scientists to modify the deoxyribonucleic acid in plants, bacteria, and animals as well as many species. Deoxyribonucleic acid editing can allow changes in physical characteristics, such as texture, shape, size and colour. Current plant genome editing has been principally achieved by combining the 2 techniques: adding the deoxyribonucleic acid encryption the streptococci pyogenes Cas9 (SpCas9) super molecule and an built guide ribonucleic acid into plant cells by Agrobacterium-mediated transformation, then create AN altered plant through tissue culture. Targeted genome editing technologies mediate by numerous sequence-specific nucleases are emerged as powerful tools for plant cistron perform studies and crop improvement. Gene editing in plants has been created abundant easier with the recently developed CRISPR/Cas9 system as a result of its simplicity and flexibility.

Early studies mistreatment CRISPR - Cas9 for cistron redaction have targeted on crops vital for agriculture. It absolutely was realised too soon that the system might be employed in crops to boost traits, like yield, plant design, plant aesthetics, and sickness tolerance.

## Successful example of miRNA editing

A group of researchers conducted a promising study on rice miRNA editing. Here, CRISPRCas9 was used to attack Osa-miR408 and Osa-miR528. Notably, this research showed that modifying single miRNA could result in several other unrelated miRNAs being altered in expression and shows the functional validation as modified trait. Osa-miR528 was proposed as the positive regulator of salt stress in the study (Zhou *et al*. 2017).

## Conclusion and discussion

This review serves in showing the character of plant microRNAs, their terribly dynamic biogenesis, its functions in gene network regulation, and therefore the role of microRNA-based technology in crop breeding. The biogenesis of miRNAs is known to be a awfully dynamic advanced beginning with the MIR gene transcription, localization, and super molecule assembly that all works along throughout the method. In post-translational regulation of miRNA biogenesis, methylation and phosphorylation play necessary roles in production of mature miRNAs and interference between proteins have an effect on the silencing pathway. Complete understanding of the mechanism of miRNA regulation is critical before continuing to transgenic ways, as a result of it will facilitate minimize undesirable trade-off in transgenic product. miRNA databases were cojointly created out of identification experiments to predict in silico the gene targets of microRNA through varied algorithms. Analysis of the preserved miRNA families completely different in several plants conjointly discovered every miRNA family might target another different role in each plant. Unsurprisingly, several studies gave proof however bound miRNAs will facilitate speedy grain filling in corn, improves tolerance of rice to drought, resistance of wheat to mycosis and a lot of. As new plant-breeding techniques develop, these efforts, along side a deeper understanding of the structure and performance of whole genomes, can alter the event of future technologies in breeding new and necessary traits in plants. Data conferred throughout this review would possibly perform a benchmark in turning out with experiments which is able to facilitate improve crop productivity whereas keeping in mind all the realizable ways that in utilizing the abundant data concerning miRNAs.

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## References

1. Achkar NP, Cambiagno DA, Manavell PA. miRNA Biogenesis: A dynamic pathway. Trends Plant Sci. 2016; 21(12):1034-1044. https://doi.org/10.1016/j.tplants.2016.09.003
2. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004; 116:281-297. https://doi.org/10.1016/ s0092-8674(04)00045
3. Bej S, Basak J. MicroRNAs: the potential biomarkers in plant stress response. Am J Plant Sci. 2014; 05(05):748-759. https://doi.org/10.4236/ajps.2014.55089
4. Brant EJ, Budak H. Plant small non-coding RNAs and their roles in biotic stresses. Front. Plant Sci. 2018;

9:1038. https://doi:10.3389/fpls.2018.01038
5. Brown ME, Funk CC. Climate. Food security under climate change. Science. 2008; 319:580-581
6. Brown ME, Funk CC. Climate. Food security under climate change, science. 2008; 319:580-1.
7. Budak H, Kantar M, Bulut R, Akpinar BA. Stress responsive miRNAs and isomiRs in cereals. Plant Sci. 2015; 235:1-13. https://doi:10.1016/j.plantsci.2015.02.008
8. Budak H, Akpinar BA. Plant miRNAs: biogenesis, organization and origins. Funct Integr 558 Genomic. 2015; 15(5):523-531. https://doi:10.1007/s10142-015-0451-2
9. Chen Y, Jiang J, Song A, Chen S, Shan H, Luo H *et al*. Ambient temperature enhanced freezing tolerance of Chrysanthmum dichrum CdICE1 Arabidopsis via miR398. BMC Biology, 2013, 11, 121.
10. Chuck G, Candela H, Hake S. Big impacts by small RNAs in plant development. Curr Opin Plant Biol. 2009; 12:81-86.
11. Chuck GS, Tobias C, Sun L, Kraemer F, Li C, Dibble D *et al*. Overexpression of the maize corngrass1 microRNA prevents flowering, improves digestibility, and increases starch content of switchgrass. Proceedings of National Academy of Sciences U.S.A. 2011; 108:17550-17555.
12. Dong CH, Pei H. Over-expression of miR397 improves plant tolerance to cold stress in Arabidopsis thaliana. Journal of Plant Biology. 2014; 57:209-217.
13. Fang X, Cui Y, Li Y, Qi Y. Transcription and processing of primary microRNAs are coupled by Elongator complex in Arabidopsis. Nat Plants. 2015; 1:15075
14. Guan Q, Lu X, Zeng H, Zhang Y, Zhu J. Heat stress induction of miR398 triggers a regulatory loop that is critical for thermotolerance in Arabidopsis. Plant Journal. 2013; 74:840-851.
15. Jia X, Wang WX, Ren L, Chen QJ, Mendu V, Willcut B *et al*. Differential and dynamic regulation of miR398 in response to ABA and salt stress in Populus tremula and Arabidopsis thaliana. Plant Molecular Biology. 2009; 71:51-59.
16. Jones-Rhoades MW, Bartel DP, Bartel B. MicroRNAs and their regulatory roles in plants. Ann Rev Plant Biol. 2006; 57:19-53.
17. Kim VN. MicroRNA biogenesis: coordinated cropping and dicing. Nat Rev Mol Cell Biol. 2005; 6:376-385. https://doi.org/10.1038/ nrm1644
18. Laufs P, Peaucelle A, Morin H, Traas J. MicroRNA regulation of the CUC genes is required for boundary size control in *Arabidopsis* meristems. Development. 2004; 131:4311-4322.
19. Lee RC, Feinbaum RL, Ambros V. The C elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993; 75(5):843-854. https://doi.org/10.1016/00928674(93)90529 –Y
20. Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH *et al*. MicroRNA genes are transcribed by RNA polymerase II. Embo J. 2004; 23:4051-4060. https://doi.org/10.1038/ sj.emboj.7600385
21. Li SJ, Castillo-Gonzalez C, Yu B, Zhang XR. The functions of plant small RNAs in development and in stress responses. Plant J. 2017; 90:654-670. https://doi:10.1111/tpj.13444
22. Mack GS. MicroRNA gets down to business. Nat Biotechnol. 2007; 25:631-638. https://doi.org/10.1038/nbt0607-631
23. Millar AA, Waterhouse PM. Plant and animal microRNAs: similarities and differences. Funct Integr Genomic. 2005; 5(3):129-135. https://doi:10.1007/s10142-005-0145-2
24. Molesini B, Pii Y, Pandolfini T. Fruit improvement using intragenesis and artificial microRNA. Trends Biotechnol. 2012; 30:8088. https://doi.org/10.1016/j.tibtech.2011.07.005
25. Nogoy Franz Marielle Nogoy1 · Marjohn C. Niño1 · Jae Young Song1 · Yu Jin Jung2 · Kwon Kyoo Kang2 · Illsup Nou3 · Yong-Gu Cho1 Plant microRNAs in molecular breeding, 2018. https://doi.org/10.1007/s11816-018-0468-9
26. Nozawa M, Miura S, Nei M. Origins and Evolution of MicroRNA Genes in Plant Species. Genome Biology and Evolution. 2012; 4:230-239.
27. Park MY, Wu G, Gonzalez-Sulser A, Vaucheret H, Poethig RS. Nuclear processing and export of microRNAs in Arabidopsis. Proc Natl Acad Sci USA. 2005; 102(10):3691-3696. https://doi.org/10.1073/pnas.0405570102
28. Reinhart BJ, Weinstein EG, Rhoades MW, Bartel B, Bartel DP. MicroRNAs in plants. Genes and Development, 2002; 16:1616-2166. https://doi.org/10.1101/gad.1004402
29. Rosegrant MW, Cline SA. Global food security: challenges and policies. Science. 2003; 302:1917-9.
30. Sandhya Tyagi, Sandeep Sharma, Showkat Ahmad Ganie, Mohd Tahir, Reyazul Rouf Mir, Renu Pandey *et al*. Plant microRNAs: biogenesis, gene silencing, web-based analysis tools and their use as molecular markers. 2019; 9:413. https://doi.org/10.1007/s13205-019
31. Shivaprasad PV, Chen HM, Patel K, Bond DM, Santos BA, Baulcombe DC. A microRNA super family regulates nucleotide binding site leucine rich repeats and other mRNAs. Plant Cell. 2012; 24:859-874. https://doi.org/10.1105/tpc.111.095380
32. Shriram V, Kumar V, Devarumath RM, Khare TS, Wani SH. MicroRNAs As potential targets for abiotic stress tolerance in plants. Front. Plant Sci. 2016; 7:817. https://doi:10.3389/fpls.2016.00817
33. Shuzuo LV, Nie X, Le W, Du X, Biradar SS, Jia X *et al*. Identification and characterization of microRNAs from barley (*Hordeum vulgare* L.) by high-throughput sequencing. Int J Biol Sci. 2012; 13:2973-2984. 10.3390/ijms13032973
34. Voinnet O. Origin, biogenesis, and activity of plant microRNAs. Cell. 2009; 136:669-687. https://doi.org/10.1016/j.cell.2009.01.046
35. Wang S, Quan L, Li S, You C, Zhang Y, Gao L *et al*. The protein phosphatase 4 complex promotes transcription and processing of primary microRNAs in Arabidopsis. Plant Cell, 2019. https://doi.org/10.1105/tpc.18.00556
36. Zeng C, Wang W, Zheng Y, Chen X, Bo W, Song S *et al*. Conservation and divergence of microRNAs and their functions in Euphorbiaceous plants. Nucleic Acids Res. 2010; 38:981-995. https://doi.org/10.1093/nar/gkp1035

37. Zhai J, Jeong DH, De Paoli E, Park S, Rosen BD, Li Y *et al*. MicroRNAs as master regulators of the plant NB-LRR defense gene family via the production of phased, trans-acting siRNAs. Genes Dev. 2011; 25:2540-2553. https://doi.org/10.1101/ gad.177527.111.
38. Zhang X, Zou Z, Gong P, Zhang J, Ziaf K, Li H *et al*. Over-expression of microRNA169 confers enhanced drought tolerance to tomato. Biotechnology Letters. 2011a; 33:403-409.
39. Zhou L, Liu Y, Liu Z, Kong D, Duan M, Luo L *et al*. Genome wide identification and analysis of drought-responsive microRNAs in Oryza sativa. Journal of Experimental Botany. 2010b; 61:4157-4168.
40. Zihan Gao, Jingtao Nie, Huasen Wang. MicroRNA biogenesis in plant. Plant Growth Regulation, 2020. https://doi.org/10.1007/s10725-020-00654-9.