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MICRORNA: A MYRIAD ROLES IN PLANT TOLERANCE TO VARIOUS ABIOTIC STRESSES

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ABSTRACT

Global population scale is daily increasing violently and this population needs enough food production and supply. For which, crop productivity needs to be improved by using strategies which should be free of bio-safety and ethical issues and crop harvest index is hampering by both the biotic and abiotic insults. These ailments can be surpassed by using novel miRNA based gene editing strategy and these miRNAs are the small non-coding RNAs over expressed under harsh environmental conditions, these miRNAs nullify the stress susceptible gene expression by translation inhibition/ RNA DICER formation. MiRNA research has been given an opportunity to develop the crop plants by using non-transgenesis approach. This review will be helpful in the development various crop plants tolerant to various abiotic stresses by using miRNA approach and also given an idea for the digging of miRNAs in maize after imposing various stresses like drought, salt and heat stress. The recent advancements in miRNA research has been given space to identification and characterization of miRNAs under different stress imposed plants and these miRNAs are most useful for the drought and heat stress prone vulnerable crops such as maize, rice and other cereals.

KEYWORDS

MiRNA, Drought and Heat stress, Translation, DICER, ROI and Maize.

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INTRODUCTON

Plants grow in compound and harsh environments which include both the biotic and abiotic stresses and these stresses mainly disturb the yield index of the crop. An intensity of the stresses depends on various geographical regions and these stresses showed the negative impacts on both crop reproductive as well as vegetative stages of the crop plant. Finally, endogenous plant changes will be occurred during the stress which includes molecular, physiological and biochemical manifestations which is having various quenching

pathways for the sequestration of Reactive Oxygen Intermediates (ROI) which were generated under various environmental insults. By adopting various methodologies includes both the conventional and biotechnological interventions can improves the genotypes with tolerance for various abiotic stresses. But, biotechnological improvement of the crop plants has several bio-safety and ethical issues towards consumption of humans as well as animals. Henceforth, there is an urgent need to develop crop plants with classical breeding and techniques. In this scenario, primarily need to identify the tolerant genotypes by applying various range of abiotic stresses and then the identified genotypes will be used to isolate the various stress responsive genes and their metabolites for the development of tolerant varieties. Under abiotic stress, various pathways and their controlling genes and its proteins, enzymes will be elevated and up regulated to neutralize the ionic homeostasis by underwent series of reactions in plants at different locations and cells wherever it needs. During the stress, plants will generate various genes which include microRNA and these were played a vital role in the scavenging of ROI generated under stress.

Plants play a key role in the maintenance of ecosystem and these plants will be used human beings from the beginning of the life as shelter, medicine and food. The major constraints to plant productivity are human activities, abiotic stresses like drought, soil toxicity, climate change, and biotic threats like insects, herbivores, microbial pathogens, etc. the increased population needs lot of food sources from plants and these plants underwent various threats raised by harsh environment. Various intervention strategies developed in molecular biology especially in the field of microRNA research and these molecules were play a vital role various metabolic processes at the post-transcriptional level (Sanan-Mishra *et al.*, 2009¹, Bej and Basak, 2014², Djami-Tchatchou and Dubery, 2015)³ and these RNA mediated as riboregulators are the key molecules in improving the plant productivity index and this review gives a brief idea of miRNA genesis, miRNAs are play a crucial role in regulating gene expression in cereals,

legumes, tubers, fruits, biofuel sources, beverages, and fiber crops and also described the improvement economically important plants are also described.

MicroRNAs

MicroRNAs are the genes accumulated under various stresses and these are an endogenous small molecules contain 20-24 nucleotides (Jones-Rhoades *et al.*, 2006)⁴. These RNAs are the regulatory genes for the induction of various cycles which squeeze the ROI and these are small non-coding RNAs and play a key role in the improving plant tolerance to various stresses caused by various sources. MiRNAs are the powerful ribo-molecules in the regulation of gene expression, mainly through cleavage and/or translation inhibition of the target mRNAs during or after transcription.

Origin and cherishing of miRNA in plants for various stresses

The miRNAs are naturally observed in non-coding regions of the genome with both the sense and anti-sense orientation and these miRNAs are transcribed into polycistronic RNAs in a group manner. They are originated from nucleus and these were transcribed by gene specific RNA pol-II (Bartel, 2004⁵, Jones-Rhoades *et al.*, 2006)⁴ and these stretches are capped with 5' and ends with 3' which is having many poly A tails (Pantaleo *et al.*, 2010)⁶ and these were cleaved by RNaseIII-like enzymes called DICER-LIKE (DCL1) with the association of various suitable proteins (1 (HYL1) and serrate (SE) into miRNA precursors (pre-miRNAs) and finally formed dimer of miRNA:miRNA in the cytoplasm (Papp *et al.*, 2003⁷, Bartel, 2004⁵, Peláez *et al.*, 2012)⁸ and these dimers are methylated at their 3' end by the conserved S-adenosyl-L-methionine-dependent RNA methyl-transferase and HUA enhancer 1 (HEN1; Sun *et al.*, 2012)⁹ and then exported to the cytoplasm with the help of EXPORTIN-5, HASTY as plant homolog (Park *et al.*, 2005¹⁰, Peláez *et al.*, 2012)⁸. Then the duplexes are loaded into the RNA-induced silencing complex (RISC), containing ARGONAUTE (AGO) proteins and then miRNA:miRNA duplexes are separated by the AGO1 protein (Arribas-Hernández *et al.*, 2016¹¹, Iki, 2017)¹², during this process one strand of the duplex miRNA is directed to the exosome for

degradation by a small-RNA degrading nuclease while the mature miRNA is inserted into the RISC which contains AGO proteins (Sun *et al.*, 2012)⁹. The mature miRNA directs the RISC to complementary target which is having high homology to the target mRNA lead to site-specific cleavage of the mRNA and finally target mRNA showed translational repression (Sun *et al.*, 2012)⁹.

MiRNA-mediated gene regulation and expression

These miRNAs are the basic powerful molecules for the expression of various genes for the production of compatible proteins and the silencing the targeted genes expression at post transcriptional level and these are the primary molecules for the induction of various genes expression to quench the ROI (Sanan-Mishra and Mukherjee, 2007)¹³, Lelandais-Brière *et al.*, 2010¹⁴, Pantaleo *et al.*, 2010⁷, Sun, 2012⁹, Djami-Tchatchou and Dubery, 2015)³. In this scenario, the expressed miRNA is bound to the target mRNA which results in the degradation of the target mRNA and leads to imperfect protein expression (Sun, 2012)⁹. In this process, poly (A) tail removed by miRNA on the targeted mRNA which results mRNA collapse (Guleria *et al.*, 2011)¹⁵. These miRNA molecules are able to silence the target gene expression by following post transcriptional modifications (Ebert and Sharp, 2012)¹⁶.

Bioinformatic tools acquired for miRNA research

Various methodologies were adopted for the isolation and characterization of miRNAs which were up-regulated under various stresses are discussing very elaborative. Schommer *et al.*, 2012¹⁷ used the bioinformatic tools for discovery and analysis of miRNA for the genetic screening and in *Arabidopsis thaliana* (Llave *et al.*, 2002¹⁸, Park *et al.*, 2002¹⁹, Reinhart *et al.*, 2002)²⁰ the first plant miRNAs such as miR156, miR159, miR164, miR171, etc., were described. MiRNAs shows maximum complementarity to their target mRNAs and these were identified by BLAST (Basic Local Alignment Search Tool, www.ncbi.nlm.nih.gov/blast/) analysis of the known miRNA sequences against expressed sequence tags

(ESTs), and genome sequences of cotton (Zhang *et al.*, 2007)²¹, switchgrass (Matts *et al.*, 2010)²², wheat (Han *et al.*, 2013)²³, potato (Xie *et al.*, 2011)²⁴, and others plant species. Next generation sequencing (NGS) method is the potential method of miRNA discovery and target identification in plants is (Pantaleo *et al.*, 2010⁷, Kulcheski *et al.*, 2011²⁵, Peláez *et al.*, 2012⁸, Djami-Tchatchou and Dubery, 2015)³. Various databases were used for the identification and validation of miRNAs in the plants are miRBase (<http://www.mirbase.org>) (Zhang and Wang, 2015)²⁶, Plant MicroRNA Database (PMRD; <http://mirnablog.com/plant-micronadatabase-goes-online/>) contains a large amount of information for plant miRNAs, such as the miRNA and their target(s), secondary structure, expression profiling and genome browser (Zhang *et al.*, 2010)²⁷ and also, NGS analysis was also supported by complex computational programmes such as Next-Gen sequence databases (<https://mpss.udel.edu/index.php>), AthaMap (<http://www.athamap.de/>) (Steffens *et al.*, 2004)²⁸ and the CLC Genomic workbench 6 software (CLC Bio, Cambridge, MA, USA). Including above tools, several other tools were also used for the miRNA screening like: psRNATarget (<http://plantgrn.noble.org/psRNATarget/>) (Zhang and Wang, 2015)²⁶, TAPIR (<http://bioinformatics.psb.ugent.be/webtools/tapir/>) (Zhang and Wang, 2015)²⁶, miRTour (<http://mirnablog.com/mirtour-plantmirna-and-target-prediction-tool/>; Zhang and Wang, 2015)²⁶ and miRTarBase, the experimentally validated microRNA-target interactions database (<http://mirtarbase.mbc.nctu.edu.tw/>) (Hsu *et al.*, 2011)²⁹, Shriram *et al.*, 2016)³⁰.

Functional roles of miRNA in plants tolerance to abiotic stress

Explication of previous reports and studies in the miRNA research suggested that the miRNAs are involved in many biological processes where they play crucial role in development and growth, maintenance of genome integrity, signal transduction, hormone signaling pathways, hormone homeostasis, innate immunity, and response to different environmental abiotic and biotic stresses

(Navarro *et al.*, 2006³¹, Sun, 2012⁹, Xie *et al.*, 2014³², Zhang and Wang, 2015)²⁶. MiRNAs played a key role in the regulation of various stress responses during the development of the plants towards various stresses (Mallory and Vaucheret, 2006)³³. Microbe-associated molecular pattern (MAMP) molecules play a vital role in the resistance in the plants under pathogen attack by underwent differential transcriptional regulation for the altered cellular metabolism for plant resistance to pathogen infestations (Djami-Tchatchou and Dubery, 2015)³.

Role of microRNAs in maize development towards various stresses

Maize (*Zea mays* L.) is the substitute crop for after rice and wheat and also Maize is the second most important food crop worldwide, used for food, feed, and forage and as a source of ethanol for fuel production. Despite of maize is an agriculturally and economically important crop and also been used in research as a model plant (Bennetzen and Hake, 2009)³⁴. Yield index of maize will be mainly affected by various environmental challenges which include drought, salt, cold, heat, and nutrient deficiency. Recent research on developing stress tolerant maize has been taken up top priority by analyzing various miRNAs under various stresses discussed here very briefly. Among the various miRNAs screened for drought tolerance, miR156, miR160, miR166, miR167, miR169 are being involved in maize development, growth, and responses to biotic stress were firstly described and characterized with their potential target genes; squamosa promoter-binding protein, auxin response factor, HD-ZIP TF, CCAAT-binding factor, and HAP-2-like proteins, respectively (Mica *et al.*, 2006)³⁵. In addition to this, 321 miRNAs of maize were analyzed by using miRbase version 21 and these identified miRNAs are play a critical functions in growth, development and plant responses to biotic and abiotic stresses and miR164 negatively regulates ZmNAC1 (NAM, ATAF, and CUC), a plant-specific TF family involved in development and stress regulation (Li *et al.*, 2012)³⁶. Several miRNAs of maize will be having the prominent roles in the drought vanishing

process which includes 95 conserved miRNAs such as miR156a, miR160a, miR164e, miR164a, miR167d, miR168, miR169a, miR393a, miR397b, miR408b, miR528a, etc., belonging to 20 families, including 11 novel miRNA families, were identified and characterized to play regulatory roles during maize endosperm development (Gu *et al.*, 2013)³⁷. In another study showed that three maize miRNAs, miR528a (the regulator of a putative laccase, a Ring-H2 zinc finger protein and a MADS box-like protein), miR167a and miR160b (regulators of auxin response factor and a member of the B3 TF family), are important for ear germination, development and physiology of the plant (Ding *et al.*, 2013)³⁸. Research findings in maize on miRNA based abiotic stress tolerance, 124 conserved maize miRNAs and 68 novel maize miRNAs were identified to be associated with drought stress resistance where they play a crucial role in the regulation of genes involved in the drought stress response (Sheng *et al.*, 2015).

Development crops using miRNA for enhanced stress tolerance

MiRNAs are the very potent gene regulators under severe stress by expressing the functional genes to nullifying the impact of stress. Several studies have been reported that the miRNAs are the most important ribo-regulators which control the plant growth and development, finally leads to positive response to abiotic and biotic stress tolerance in plants. Genetic modification technology is one of the most promising solutions by using miRNA as a key gene which can contribute to agricultural productivity in order to produce superior crop cultivars. Transgenic technology an offers the expression of foreign genes in the crop plants under suitable promoters, as like this miRNA based control on gene expression include the over expression of miRNA-resistants and artificial gene targets of desired trait (Gupta, 2015)³⁹ and these artificial miRNA crop improvement resulted into negative effect as developed plants would be susceptible to various stresses (Franco-Zorrilla *et al.*, 2007)⁴⁰. By using the artificial miRNA based gene silencing successfully launched in efficiently in rice and several plant species (Khraiwesh *et al.*,

2008⁴¹, Sharma *et al.*, 2015b)⁴² and cucumber mosaic virus suppressor gene was used in tobacco for resistance for tobacco mosaic virus (Qu *et al.*, 2007)⁴³ and these miRNA based genetically modified crops may enhance the stress tolerance to abiotic and biotic stresses and for increasing yield.

Improvement of crops for abiotic and biotic stresses by using miRNA

Abiotic stresses are the main yield limiting factors in the crop plants. Under stress, plants will be taken up various methodologies to quench the ROI by up-regulating stress responsive genes and various plant molecular responses were observed towards to mitigating the abiotic stresses. Under this situation, plants undergo development and regulation of genes responsible to stresses as miRNA regulation (Bej and Basak, 2014)⁴⁴. Several studies reported that the over-expression of miRNAs leads to stress tolerance are being discussed here very apparently i.e., over-expression of a drought responsive miR169 enhanced drought tolerance by lowering stomatal opening, which decreased transpiration rates and reduced leaf water loss (Zhang *et al.*, 2011) and in rice it was demonstrated that the over-expression of osa-MIR396c led to a susceptible plant by reducing salt and alkali stress tolerance (Gao *et al.*, 2010)⁴⁵, transgenic rice lines which over-expression of miR398 in transgenic rice showed resistant to high salinity and water stress than non-transgenic rice by over accumulation cu/zn-sod levels (Lu *et al.*, 2011)⁴⁶. Ectopically over-expression of miR319 impacted on leaf morphogenesis and led to enhanced cold tolerance after chilling acclimation in rice (Yang *et al.*, 2013)⁴⁷. Hence, the retrospection of contemporary and previous studies proved that miRNAs play key roles during plant response to stress and it is anticipated to that regulation of specific genes for specific traits in crop plants to combat various abiotic stresses.

CONCLUSION

As epitomize the various studies was under taken for the development of tolerant crops toward various stresses by using miRNA based gene silencing methodologies, miRNAs are currently regarded among the most important gene regulators

and also a significant progress has been made in recent years on analysis and characterization of plant miRNAs with an increasing number of research reports on the crucial function of miRNAs in crop plants and this review summarizes the role of miRNAs in agronomically/economically important plants tolerance to abiotic stresses. Explication of above literature discussed in this review, these small nucleic acids are involved in vital aspects which include plant growth and development, vegetative to reproductive phase change, hormone signaling, and signal transduction pathways and homeostasis responses to different environmental abiotic and biotic stresses (Sun *et al.*, 2012)⁹ and also contemporary studies also revealed that plant miRNAs are involved as molecular regulators of plant immune and defense responses (Li *et al.*, 2010⁴⁸, Balmer and Mauch-Mani, 2013⁴⁹, Djami-Tchatchou and Dubery, 2015)³. By following these evidences, the miRNAs are the crucial gene regulators for the crop plants towards stress tolerance and also a miRNAs are the emerging as a next generation targets for genetic engineering for improvement of the agronomic properties of crops. Development of tolerant crop plants with miRNAs would be the effective cutting edge crop improvement technology for both the biotic and abiotic stresses. In this scenario, the manipulation of miRNA expression levels would represent an effective strategy for improving the responses of crop plants to environmental stress and pathogen resistant (Zhang and Wang, 2015)²⁶. Different transgenic approaches, focused on miRNAs of importance and the corresponding identified target genes, can be used to constitutive over-expression of miRNAs, stress-induced, or tissue-specific expression of miRNAs and the expression of miRNA resistant target genes, artificial target mimics and artificial miRNAs (Zhou and Luo, 2013⁵⁰, Gupta, 2015)³⁹. An over-expression of the target gene or selection of miRNA-resistant target genes or artificial target mimics in crop plants showed improved and enhanced tolerance to various abiotic stresses (Gupta, 2015)³⁹. Even though, successful development of crops with suitable miRNAs for various stress tolerance, still the stress

tolerance is multi gene trait, henceforth, the plants needs to develop with mutigenes for multiple/or single trait. Various hinders are there in miRNA research towards development of crops with suitable improved traits, miRNA over-expression and knockout of major target genes normally produce very similar phenotypes, and this is generally contrary to what is observed in plants with reduced activity of the miRNA (Palatnik *et al.*, 2007)⁵¹. Expression analysis in whole plant and the detrimental effect of miRNA need to study and also approach should be carefully and specifically designed for better results and these artificial miRNAs designed to suppress the target gene expression of a protein-coding mRNA and this strategy is very essential for the understand the mechanisms of miRNA regulation of plant growth and development or plant responses to various abiotic and biotic stresses and this will facilitate the design of suitable strategies to improvising the plants to various abiotic stress tolerance without using any marker genes as well as any alien genes. By using this above eco-friendly miRNA strategy can develop the heat and drought tolerant maize varieties without any ethical and bio-safety issues.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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