Research Journal of Agriculture and Biological Sciences, 5(5): 803-814, 2009

Review of Plant miRNAs in Environmental Stressed Conditions

LIN Hai-Jian, ZHANG Zhi-Ming, Shen Ya-OU, Gao Shi-Bin, PAN Guang-Tang

(Institute of Maize Research, Sichuan Agricultural University, Sichuan Ya'an, 625014, China)
LIN Hai-Jian, Institute of Maize Research, Sichuan Agricultural University, Sichuan Ya'an 625014,

Abstract: Stress conditions including biotic and abiotic stress influence the normal growth of plant and cause great loss to crop yield. In the long course of evolution, plants have developed intricate mechanism to resist stressed conditions. Responsive induced expression genes are the representative examples. Under various stressed conditions, not only the protein-coding genes, the non-protein-coding genes will be induced expression also. More and more researches show that the transcripts of these non-protein-coding genes play a big role in regulation of gene expression. miRNAs are one of the group of these no-coding regulatory small RNAs. Recent research findings show that in order to resist the biotic and abiotic stresses, miRNA (microRNA) gene will be induced expression and the transcripts (miRNAs) can regulate gene expression by guiding target mRNA cleavage or translational inhibition. This paper focuses on the advances of plant miRNAs research in some stressed conditions, especially the induced expression of miRNA and target genes regulation and its role on the adaptation to stressed conditions.

Key words: microRNA biotic/abiotic stress target gene gene regulation

INTRODUCTION

Stressed conditions including aboitic and biotic stress is one of the important limited factors for crop growth and yield, and much researches have been made in revealing this complicated biological mechanism. Under various stressed conditions of plants, many genes, including the protein-coding and non-protein-coding genes will be induced expression. miRNAs are a class of small, non-coding, with ~21 nucleotide length RNA as a newly regulator in gene expression both in animals and plants[1-2]. miRNA was first discovered in *Caenorhabditis elegans*[3-4], and then discovered in plants such as Arabidopsis[5], rice[6], maize[7], wheat[8] and moss[9] through clone and computational methods. An increasing number of miRNAs have been identified and deposited in major miRNA databases (http://www.sanger.ac.uk/ Software/Rfam/ mirna/index.shtml). The discover of miRNA in plants has broadened our perspectives on the mechanism of gene regulation which mediating many plant biological processes such as development, protein degradation, cell proliferation and differentiation, keeping genome complete and signal transduction[10]. More and more researches have demonstrated that many plant miRNAs will be induced by abiotic/biotic stress and may play a big role in the process of adaptation by plant itself in order to resist the stressed environments[11-13]. With the deeply researches, miRNAs may provide us the newly thinking to understand the tolerant/resistant mechanism of plants and can be used as a tool to enhance plant tolerance to stressed conditions. This paper mainly takes some model plants (Arabidopsis, *poulus,* rice et al) as the examples and focuses on the roles of miRNAs in plant abiotic/biotic stress such as nutrition depletion, virus/bacterial infection, UV-B radiation, mechanic stress, oxidative stress and other stressed conditions which are the two types of mechanic stress; miRNA408 is up-regulated expression in both tension and compression stress; miRNA159, 476 and 479, exhibit preferentially up-regulated expression only in compression tissues; miRNA160 and 172 aree down-regulated expression only in compression tissues; miRNA168 is up-regulated expression only in tension stress.

The Plant miRNAs Biogenesis and Target Genes Recognition: Plant miRNAs are products of transcription of MIRNA genes which distribute in the whole genomic of plants, and plants miRNAs are transcribed from their own transcriptional units which constrast with animals miRNAs which sometimes appear to be processed from introns of protein-coding genes [14]. First, the MIR genes are transcribed into one or more long length primary transcripts (pri-miRNAs) with a specific stem-loop structure mediated by RNA polymerase [15] . Northern, EST, and mapping evidence

Corresponding Author: PAN Guang-Tang, Institute of Maize Research, Sichuan Agricultural University, Sichuan Ya'an 625014,China.
E-mail: pangt1956@yahoo.com. China. E-mail: linhj521@yahoo.com.cn

Table 1: Plant stress/defense-related miRNAs and its validated or putative target genes

MicroRNA	Stress Conditions	Response	Validated (V) or Putative (P) Target Genes	Reference
miRNA399	Low phosphorus supply in the media	Up-regulated	Ubiqiutin conjugating enzyme-E2 (V)	[12][23][31]
miRNA395	Low sulfate supply in the media	Up-regulated	Sulfate transporter,AST68 (V) ATP sulfurylase1, APS1 (V) ATP sulfurylase2, APS2 (V) ATP sulfurylase3, APS3 (V)	[13]
miRNA159	Fungi infected	Unknown	MYB transcription factor (V) TCP transcription factor (V)	[47][74] [24]
miRNA160-3	Unknown	Unknown	Pathogenesis-related protein (P)	[57]
miRNA1-39	Unknown	Unknown	A mucin-like protein (P)	[57]
miRNA171e	Unknown	Unknown	RGA1(P)	[53]
miRNA393	Leaves infected with bacterial flagellin 22 Leaves infiltrated with P*seudomonas syringae pv. Tomato* Cold, dehydration, salt and ABA stress	Up-regulated Up-regulated Up-regulate	F-box protein, AFB2 (V) Auxin receptor, TIR1 (V) F-box protein, AFB1 (V) F-box protein, AFB3 (V)	[74] [11]
miRNAs*[1]	UV-B radiation	Up-regulated	Auxin response factors, ARF (P)	[61]
miRNAs*[2]	Mechanic stress (tension and compression stress)	Up or down- Regulated*[3]	Development and stress/defense-related genes, especially the biosynthesis of cell wall metabolites (P)	[63]
miRNA398	Oxidative stress	Down-regulated	Superoxide dismutase 1, CSD1 (V) Superoxide dismutase 1, CSD1 (V) Cytochrome c oxidase subunit V (V)	[25]
miRNA319c	Cold	Up-regulate	MYB transcription factor (V) TCP transcription factor (V)	[11][15]
miRNA389	Cold, dehydration, salt and ABA stress	Down-regulated	Unknown proteins	[11]

Note:
*[1] Four miRNAs, miRNA160, 165/166, 167 and 393 may be involved in.
*[2], A major of the 21 miRNAs involve in response to the mechanic stress of *poulus trichocarpa.*
*[3], miRNA156, 162, 164, 475,480 and 481 are down-regulated by tension and compression

indicate that plant pri-miRNAs are longer than needed to encompass the miRNA stem-loops[13,16,17]. At least some of these pri-miRNA transcripts appear to be spliced, polyadenylated, and capped [16,17,18]. A key step of miRNA processing is excising the mature miRNA from pri-miRNA by RNase -type endonucleases such as *Dice-like* [19] and another protein, *HYL1*[20], which probably collaborates with *Dicer-like* during the process of cut sites recognition and subsequent functions[18]. Be different from animals, the maturation process of plant miRNAs involves an additional step, methylation[21]. Methylation of miRNA requires protein *HEN1* and studies indicate that mutations of *HEN1* result in 3'-end uridylation of miRNAs, which apparently leads to reduced miRNA accumulation and function[22,23]. Following *Dicer-like* and *HYL1*-meidated cleavage and *HEN1*-mediated methylation, most miRNAs exit the nucleus and enter the cytoplasm facilitated by a member of the importinβfamily of nucleocytoplasimic transporter, *HST*[24], which is another important protein in the process of miRNA maturation. The final step of miRNA maturation is one of the strand of the miRNA/miRNA duplex collaborates with the protein *AGO* and incorporates into a induced silencing complex (RISC) [25] [Figure 1].

MiRNA can direct the RISC to regulate the target genes expression by following two posttranscriptional mechanisms: mRNA cleavage or translation repression. The choice of the target genes recognition is not determined by the type of the small RNA (siRNA or miRNA) but instead is determined by the complementary degree of the target gene and miRNA sequence, partially or absolutely. Once incorporated into a cytoplasmic RISC, the miRNA will specify cleavage if the target mRNA has sufficient complementarity to the miRNA, or it will repress productive translation if the target mRNA has partially complementarity to the miRNA[26][Fig. 2]. Most of miRNAs in animals are not sufficiently complementary to the target genes and they bind the 3'UTR region of mRNA and repress the translation. Different with animal miRNAs, most of the plant miRNAs are sufficiently complementary and chose the first mechanism which leads the target mRNA cleavage.

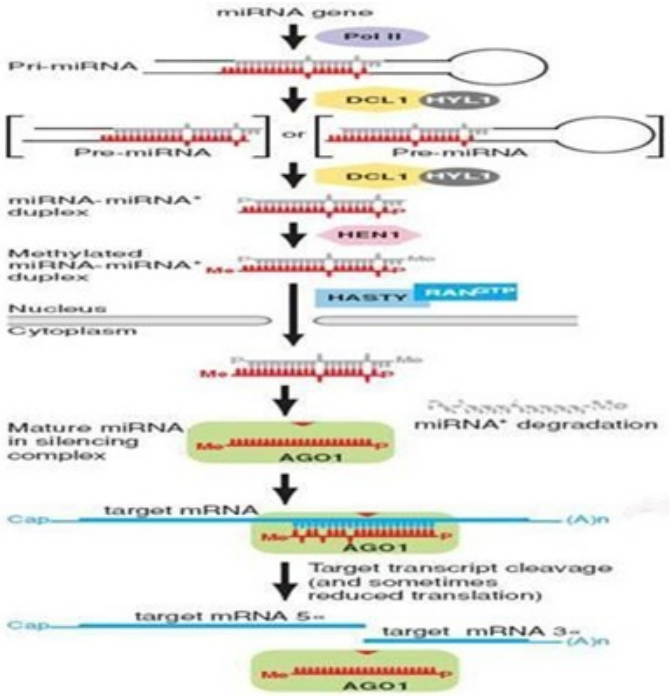

Fig. 1: The process of miRNA biogenesis and RISC assembling [18]

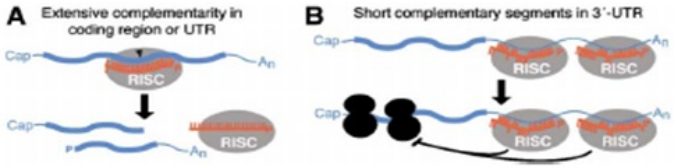

Fig. 2: Two modes of target gene recognition by miRNA [26]
(A) Messenger RNA cleavage specified by a miRNA. Black arrowhead indicates site of cleavage.
(B) Translational repression specified by miRNAs

Plant miRNAs and Stress Conditions: Many studies have demonstrated that stress conditions can induce related genes expression of plants. These stress-responsive genes expression result in the accumulation of some metabolites and alterations of some biochemical or physiological pathways which are vital for adaptations in order to resist the stressed conditions by plant itself[27-29]. Many genes are regulated by stressed conditions such as drought, salt, cold, nutrition depletion, virus infection and so on. These regulation levels include transcriptional, posttranscriptional and translation level and the posttranscriptional regulation is the most common in plants under stressed conditions, especially the regulation mode of transcription factor directly acts on the conversed *cis*-acting promoter elements[28]. Although the clear mechanism of posttranscriptional regulation is not well understand, the miRNA regulation mode under stressed conditions is likely to be this one[13]. The biogenesis, expression level and target gene's recognition of plant miRNAs under stressed conditions will provide the newly thinking about the stress response of plants. Functional analysis indicated that many plant miRNAs play a big role in plant tolerance to stress conditions including abiotic and biotic stress[12,30]. Well understanding the regulatory networks guided by plant miRNAs under stressed conditions can well elucidates the mechanism of plant tolerance and provides the new tools to improve the ability of crop resistance.

***microRNA399* and low-phosphorus Stress:** Phosphorus is one of the important mineral elements for plant growth and the least available essential nutrients in the soil, which become an important limited factor for the crop yield in some regions[31]. In response to persistently low level of available phosphorus in the environment, plants have developed highly specialized physiological and biochemical

adaptation changes to absorb and utilize Pi from the environment [29][32]. These adaptation changes depend on the correct gene expression at right times and places. To date, many researches have been done in unraveling low-phosphorus tolerant mechanism of plants, however, little is known about the overall pathway and regulatory system. But recent findings of *miRNA399* involved in phosphorus starvation response in Arabidopsis stride a step forward in well understanding the mechanism. *miRNA399* targets two genes belonging to two different gene family: the one is phosphate transporter [13] and the other is ubiquitin conjugating enzyme(*UBC24*) which is involved in protein degradation pathway [12].

The Arabidopsis genome encodes six *MIR399* genes, which strongly induced by the phosphorus starvation and can be reversed by the supply of phosphorus [33]. The *miRNA399* level is fluctuation according to the phosphorus supply level. Studies indicate that there is an inverse correlation between *miRNA399* and *UBC24* mRNA level under normal and low-phosphorus conditions[12,34-35]. The down-regulation of UBC under low-Pi stress can promote the elongation of primary root and induce the expression of high-affinity Pi transporter such as AtPT1, which are important for the Pi homeostasis in Arabidopsis[12][35]. Under low-*Pi* stress, the expression of *miRNA399* is induced and *UBC24* expression is suppressed. However, under normal conditions, the expression of *miRNA399* is suppressed and the *UBC24* is expressed. Studies indicate that under low-Pi stress condition, the induction of *miRNA399* and repression of UBC24 result in alleviation of *Pi* transporter expression and alteration of root architecture, which are very important for maintaining of Pi level in plants. Under normal phosphorus supply condition, *miRNA399* expression is suppressed and *UBC24* expressed and presumably participates in a ubiquitin or proteosome pathway that negatively regulates the expression of Pi transporters and controls hormonal signaling for root growth regulation to prevent the over-absorption of Pi from media which is very toxic for plants [35]. So, *miRNA399* is very important for the maintaining of phosphorus level both in normal and low-phosphorus supply in plants. *miRNA399* exists and functions in other plant species also. Studies in rapeseed and pumpkin indicate that *miRNA399* expression is strongly and specifically increased in phloem sap during Pi deprivation. By performing micro-grafting experiments using Arabidopsis, the study further show that chimeric plants constitutively over-expressing *miR399* in the shoot accumulate mature *miR399* species to very high levels in their wild-type roots, while corresponding primary transcripts are virtually absent in roots, demonstrating that there is a long distance shoot-to-root transport process [36].

miRNA395 and Sulfate Homeostasis: The relationship of miRNA and sulfate nutrition comes from the study of *miRNA395* in Arabidopsis. Sulfate is the fourth important inorganic element ranking in need next to N, P and K, which is indispensable for plant growth. Sulfate is taken up by plant roots mainly as the form of inorganic [37]. Sulfate starvation can induce many responsive genes expression which result in the changes of physiological and biological such as the alterations in the expression levels of sulfate assimilation enzymes so that sulfate acquisition can be sustained to some extent and sulfate assimilation is suspended [35]. In addition of protein –coding genes expression, low sulfate stress can induce miRNA expression also. The expression of *miRNA395* was induced under low sulfate condition, but this was not detected under sulfate sufficient media [13]. ATP sulfurylases (*ASP1*, *ASP3* and *ASP4*) that involved in the sulfate assimilation pathway was targeted by *miRNA395*. Computational and northern blot analysis indicates that the *miRNA395* is complementary to mRNA of *ASP1* and *ASP1*. And the ASP expression decreased while miRNA395 increased under low sulfate condition [38][13]. Another gene, *AST68*, is also targeted by *miRNA395*. *AST68* is a low-affinity sulfate transporter, which is implicated in the internal translocation of sulfate from roots to shoots [35][39]. Both ASP and *AST68* are very important for the sulfate homeostasis of Arabidopsis under sulfate starvation. Although the overall regulatory network involved in miRNAs under low sulfate stress is unknown, the available evidence implies an important role of *miRNA395* in regulating sulfate homeostasis [13]. Isam et al have cloned some miRNAs in *Physcomitrella* and one of which target two mRNAs that encode proteins involved in sulfur metabolism [40]. It presumably that *Physcomitrella* uses more diverse routes of sulfate assimilation than angiosperms [41], thus a need for their specific regulation through miRNAs is likely.

miRNA398 and Copper Homeostasis: Copper (Cu)is required for catalytic and structural properties of many proteins and are therefore essential for growth and development of all organisms[42]. The plastocyanin, copper/zinc superoxide dismutase, and cytochrome *c* are the major copper proteins in the cytoplasm of plant cells. Copper/Zinc superoxide dismutase (SOD) is involved in plant defense system against ROS and catalyzes the dismutation of superoxide radicals to molecular oxygen and hydrogen peroxide[43]. And the role of SOD in defense system will be discussed later. Copper/zinc SOD (Cu/Zn SOD), manganese SOD (Mn SOD), and iron SOD (Fe SOD) are three types of SODs reported in plants so far. Under copper limited conditions, expression of copper/zinc superoxide dismutase is down-regulated and the protein is replaced

by iron superoxide dismutase in chloroplasts. It was validated that this process is the adaptation change of arabidopsis when copper starvation which mediated by *miRNA398*[44]. The *miRNA398* directs the degradation of mRNA of *Cu/Zn SOD* when copper limited. Sequence analysis indicated that the transcripts encoding cytosolic Cu/Zn SOD and *COX5b-1*, a subunit of the mitochondrial cytochrome *c* oxidase, are also targeted by *miRNA398*. This regulation via *miRNA398* takes place in response to changes in a low range of copper levels, indicating that *miRNA398* is involved in a response to copper limitation. But another major copper protein, plastocyanin, which is important in higher plants, is not targeted by *miRNA398*. The study show that *miRNA398* is a key factor in copper homeostasis in plants and play big role in regulating stability of mRNAs of mRNAs of major copper proteins under copper-limited conditions[44].

miRNAs and Plant Virus Infection: Viruses can influence plant growth and development, causing great loss to crop production. In the long course of evolution, plants have developed some complicated defense mechanisms. One of the mechanisms is gene silencing which widely adopted in plant immunity [45-47]. Gene silencing is a specific and efficient degradation mechanism for exogenous factors such as transposons and virus. There are three types of gene silencing in different species, post-transcriptional gene silencing (PTGS) co-suppression in plants, RNA interference (RNAi) in animals and quelling in fungi [45,48-49]. The PTGS was first found in transgenic petunia plant [50], and then later studies have found that plant virus can also induce this co-suppression phenomenon and presume the putative biological mechanism which PTGS involved in defending virus infection [51]. With the deeply research, researchers have found that these phenomenon are correlated and the essence is to break down the exogenous factors in order to keep the genome integrity [52-53]. Virus and fungi can induce plant small RNA expression in the process of infection and some of these small RNAs are validated to be miRNAs [54-55]. Recent researches show that miRNAs are involved in modulating plant viral diseases [56-57]. Virus are known to exploit the host plant nucleic acids as a part of their infection strategy and miRNAs-mediated gene silencing are granted one general defense mechanism against plant virus [58]. Interestingly, virus can also generate miRNA and employ them to interfere host gene in order to circumvent the plant defense system [57,59].

Under virus infection process, plant can generate miRNAs, which involved in the regulation of the virus defense process in plants or targeting some key genes of virus development to suppress its increase. Many miRNAs have been predicted or validated to be involved in plant virus defense[30,58,60]. For example, *miRNA393* was found to be induced in response to flagellin that can enhance the resistance of Arabidopsis to *Pseudomonas syringae*. *miRNA393* can negatively regulate messenger RNAs for the *F-box* auxin receptors *TIR1*, *TIR2*, and *TIR3*. Repression of auxin signaling can restrict the growth of *Pseudomonas syringae* and represent resistance [30]. 9 miRNAs are involved in defense pathway in *physcomitrella*[41], *miRNA1-39* targets a gene coding for a mucin-like protein carrying a dense sugar coating against proteolysis, which is a key step in pathogen invasion. *miRNA160-3* targets on intracellular pathogenesis-related protein and *miRNA408* provides defense though interaction with the genes coding for a copper ion binding protein, and with electron transporter or Phytocyanin homolog[30,41]. The available nucleotide databases of genome survey sequences (GSS), high-throughput genomics sequences (HTGS), expressed sequenced tags (ESTs) and nonredundant (NR) nucleotides have been used for identification of plant miRNAs[13][61-63]. 36 in 476 EST contigs, which are predicted to contain miRNA, are involved in pathogen infection [61]. It indicated that *miRNA171e* in *V. vinifera* can target the *RGA1* protein which represent resistance to blight, a serious disease caused by *botrytis cinerea* in grapevine. Osmotin-like protein was induced by *phytophthora infestans* infection and the *miRNA166a* may be involved in targeting of its precursor [30]. In addition, researches show that the artificial miRNAs can also be applied and function in virus resistance of plants [64-65].

In the course of co-evolution with host plants, viruses have also developed counter defense strategy to escape the defensive systems of host plants [66]. One of the actions is gene silencing suppressor which can help viruses infection at the early stage. For example, *Tobacco rattle virus* (TRV) encodes a 16-kDa (16K) protein that allows TRV to transiently invade the meristem[67]. The biogenesis of miRNAs also be affected by *tombus virus p19* and the possible mechanism is the disruption of miRNA maturation process by *p19*[68]. It is a general feature contributing to pathogenicity of many viruses that interference with miRNA-directed processes. A normally labile intermediate in the miRNA biogenesis/RNA-induced silencing complex (RISC) assembly pathway, miRNA*, accumulated specifically in the presence of suppressors (P1/HC-Pro, p21, or p19) that inhibited miRNA-guided cleavage of target mRNAs [59]. *AC4* protein, as a gene silencing suppressor encoded by *African cassava mosaic* virus can bind with *miRNA159* and prevent the cleavage of the target mRNA, which can interfere the PTGS process and influence the arabidopsis development [69]. Virus also can directly influence the

host plant miRNA level, which is independent of viral posttranscriptional gene silencing suppressor activity. A correlation was observed between symptom severity and alteration in levels of *miRNA156*, *160*, *164*, *166*, *169*, and *171* in *Nicotiana tabacum* infected by plant viruses representative of the *Tobamoviridae*, *Potyviridae*, and *Potexviridae* families [55]. It shows that virus infection and viral proteins influence miRNA balance without affecting posttranscriptional gene silencing and contributes to the hypothesis that viruses exploit miRNA pathways during pathogenesis[55]. Virus infection and plant defense can be described as the co-evolution process. It is likely that miRNAs have existed since the very beginning and cooperated with each other to optimize the effect [58]. However, the particular mechanism of plant defense and virus counter-defense involved in miRNAs is not well understood up to now.

miRNAs and UV-B Radiation: Light is one of the key factors that govern a multitude of developmental process during entire plant life cycle [70-71]. Ultraviolet in sunlight can be divided into three classes: UV-A, UV-C, and UV-B. UV-A, as the photomorphogenic signal, is less harmful and very important for plant growth [72]. High energetic UV-C can be strongly absorbed by oxygen and ozone in the stratosphere such that none of this sterilizing radiation is present in terrestrial sunlight [73]. 96% UV-B is absorbed by atmospheric ozone, but approximately 4% of terrestrial radiation is caused by it. High fluence rates of UV-B produce reactive oxygen species and may cause damage to DNA, proteins, membranes, and lipids [74-76], which have been raised by depletion of stratospheric ozone catalyzed by chlorofluorocarbons and other pollutants [77]. Plants must resist the deleterious effects of UV light because they are dependent on sunlight for photosynthesis and cannot avoid UV light exposure. In the course of evolution, plants have developed protective adaptations including accumulation of UV-absorbing pigments, and damage repair. For example, the utilization of UV-A photons to reverse some types of UV induced DNA lesions[73,78-79]. UV-B radiation also causes the accumulation of ROS species and induces the oxidative stress defense system [80-81], which involves many responsive genes induction expression [73,82]. But the overall mechanism is little known.

The relationship between miRNA and UV-B radiation comes from the research in *Arabidopsis* [83]. Zhou et al have utilized Arabidopsis miRNA gene chip to screen miRNAs response to UV-B radiation and put forward an improved computational approach to identify miRNAs genes induced by UV-B radiation and characterize the functions in regulating gene expression. It was validated that 21 miRNAs belonging to 11 miRNA gene families were up regulated under UV-B stress condition and 11 putative miRNAs involved in UV-B stimuli. Remarkably, 8 of the 11 putative light-inducible miRNAs (except *miRNA393*, *miRNA398*, *miRNA401*) have targets that encode transcription factors. Four miRNAs, *miRNA160*, *miRNA165/166*, *miRNA 167* and *miRNA393* may be involved in the Auxin signaling pathway [83], which is very important for plant development and stress resistance/tolerance [84-85]. This is the first work that demonstrates the relationship between plant miRNA and UV-B radiation so far. And the novel computational approach adopted in their work also can be directly applied to miRNAs responding to other abiotic and biotic stresses and extended to other plants.

miRNAs and Plant Mechanical Stress: Mechanical stress is also intituled dynamical stress in trees, which is described the branch or stem bend generated by outside force such as wind and gravity[86-87]. The deposition of secondary xylem, or wood, provides strength to support the tremendous crown structure. Under outside force stress, the stem or branch will be inclined and can be compensated by reaction wood, a specialized woody tissue [88]. The reaction wood can be considered as the defense system and corrective adaptation growth resulted by coordinately enhanced development of strength-contributing cells and wall metabolites [86-87,[89], but the comprehension of overall defense or adaptation regulatory network require further studying.

It validated that miRNAs were induced by mechanical streses and may play a big role in the defensive adaptations in *Populus trichocarpa* [89]. Lu et al cloned 22 miRNAs from 4 -days-stressed *Populus trichocarpa* by tension and compression stresses, two constant mechanical loads in trees. These induced miRNAs belong to 21 gene families and represent 98 loci in the *Populus trichocarpa* genome. The expression of induced miRNAs have specific of tissue and mechanical stress types. *miRNA156*, *162, 164, 475, 480*, and *481* are down-regulated by tension and compression stresses. Contrast, miRNA408 is up-regulated in both tension and compression stresses. *miRNA159, 476*, and *479*, exhibit preferentially up-regulated expression in compression tissues. The expression of *miRNA160* and *miRNA172* is suppressed only in compression tissues and *miRNA168* is induced in tension stress. The prediction of target gene indicated that some of these miRNAs are possibly associated with the formation of specialized wood. The results show that plant miRNAs can be induced by mechanical stress and may function in one of the important defense mechanism for structure and mechanical fitness [89].

miRNAs and Oxidative Stress: Reactive oxidative species (ROS), derivated or activated from oxygen, are harmful and toxic for plant growth and development and can lead to cell death by causing damage to proteins, lipids, carbohydrates, and DNA [90]. Plant cell can generate reactive oxidative species (ROS) both in normal and stress conditions. There is a delicate balance between the generation and scavenging of ROS under normal conditions. But under stress conditions such as drought stress, salty stress, virus infection stress, nutrition stress and heavy metal stress can induce more ROS production which can't be scavenged immediately by plant itself [91-93]. In the course of evolution, plants have developed complicated and efficient antioxidant mechanism to scavenge the overabundant ROS [90,94]. One example is Cu-Zn superoxide dismutase and super radicals, O_2^- that can be inverted to more toxic $^{\cdot}OH$ [95]. Cu-Zn superoxide diamutase is located in thylakoid of chloroplasts where is the site of superoxide generation and, thus, plays an important role in immediate scavenging of super radicals [96]. It was demonstrated that oxidative stress can induce SOD gene expression to scavenge the excess superoxide radicals[97]. The work about miRNA in oxidative stress is also come from the research of Cu-Zn superoxide diamutase and O_2^- in Arabidopsis [98] and reviewed detailedly by Sunkar [35].

It was validated that miRNA398 target CSD1 and CSD2 genes [11,13,99] and the target its target sites on CSD1 and CSD2 mRNA are conserved in dicotyledonous and monocotyledonous plants [11,13,82,91,92,100]. It indicates that oxidative stress can't induce accumulation transcription products of two CSD genes in transcription level but the up-regulated regulation of two CSD genes is dependent on the *miRNA398* level [91]. When growth under normal conditions of plants, two CSD genes are transcribed but their transcripts don't accumulate because of the cleavage guided by *miRNA398*. Contrast, in response to oxidative stress, the transcripts of two CSD genes accumulate because *miRNA398* is transcriptional down-regulated to release its suppression on mRNA of two CSD genes and represents tolerance to stress [91] [FIGURE 3]. Additionally, This work show that transgenic Arabidopsis thaliana plants over-expressing a miR398-resistant form of CSD2 accumulate more CSD2 mRNA than plants over-expressing a regular CSD2 and are consequently much more tolerant to high light, heavy metals, and other oxidative stresses. Thus, relieving *miR398*-guided suppression of CSD2 in transgenic plants is an effective new approach to improving plant productivity under oxidative stress conditions [91]. Another gene, *Cytochrome c oxidase subunit V* is also targeted by miRNA398 [11,13,35,91], but the regulatory network is unknown and whether function in antioxidant system needs further studying.

miRNA and Other Stressed Conditions: Sunkar et al have constructed small RNA library of Arabidopsis seedling treated by dehydration, salty, cold and ABA stress [11]. Sequencing analysis found that some of these small RNA are miRNAs including 26 new miRNAs belonging to 15 new miRNA gene families. According to the complementary characteristic of miRNA and its target genes, 41target genes with different functions were predicted. Northern blot show that many miRNAs are regulated (up or down-regulated) by one or more stress conditions and represent tissue specific. For example, *miRNA393* is strongly up-regulated by cold, dehydration, salty and ABA stress and the induced expression is comparatively weak for *miRNA 402* and *miRNA397b*. But *miRNA319c* is just regulated under cold stress. And interestingly, only *miRNA389a* is down-regulated by four stress conditions. The results show that miRNAs may and take part in the responsive genes expression and regulation and function in adaptation of plant.

Concluding Remarks: The discovery of miRNAs is a landmark in plant molecular biology field and provide a useful tool for us to comprehend the gene expression and regulation under stressed conditions. There is approximately 1% miRNA-coding gene in whole genome, which means that many unknown miRNAs are waiting for researchers to discover. The researches of miRNAs in model plants such as Arabidopsis and rice have shown that these non-coding small RNAs are common regulatory RNA and represent diversity in many aspects such as sequence, structure, content, and function. In addition, the expression of miRNA is faster than that of protein-coding genes, because miRNAs can't be restricted or influenced by the process of translation and can efficiently regulate target gene. These findings not only sublime our comprehension to gene regulation, but also deepen our understanding of the gene complexity from another aspect. Generally speaking, the process of plant breeding is the course of resistance to stress conditions. So far, Stress conditions are main limited factors for crop yield in some regions and the understanding of overall resistant mechanism is the premise solve this problem. The important role of plant miRNAs involved in gene expression and regulation under stress conditions provides the newly thinking to understanding the complicated biological mechanisms. We can forecast that miRNAs can be used as a useful tool to enhance the crop resistance or tolerance to stress conditions in the future.

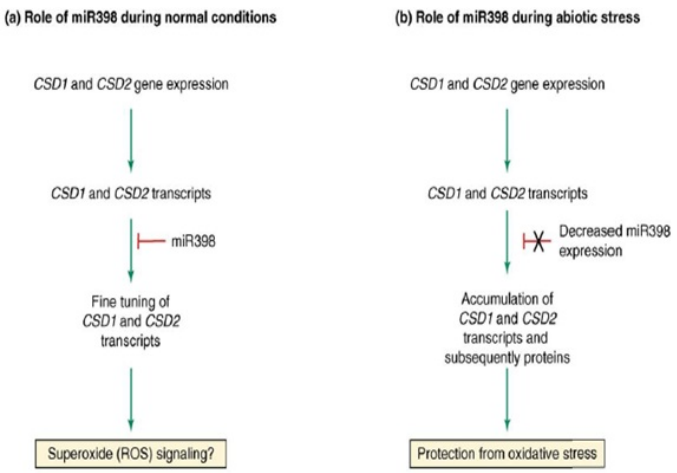

Fig.3: The regulation models of expression of CSD genes guided by miRNA398 under normal and oxidative stress conditions [35].

(a) Under normal conditions, the expression of miRNA399 leads to the cleavage of transcripts of two CSD genes, but whether is there any superoxide (ROS) signaling is unknown, so mark a question symbol.

(b) Under stress conditions, miRNA298 expression is suppressed and releases the cleavage to transcripts of two CSD genes. The accumulation of transcripts of CSD genes enhances the tolerant ability to oxidative stress.

REFERENCE

1. Carrington, J.C., V. Ambros, 2003. Role of microRNAs in plant and animal development. Science, 301: 336-338.
2. Hake, S., 2003. MicroRNAs: a role in plant development. Current Biology, 13: 851–852.
3. Lee, R.C., R.L. Feinbaum, V. Ambros, 1993. The C. elegans heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. Cell., 75: 843-854.
4. Wightman, B., I. Ha, G. Ruvkun, 1993. Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates temporal pattern formation in *C. elegans*. *Cell.,* 75: 855-862.
5. Sunkar, R., J.K. Zhu, 2001. Novel and stress-regulated micro-RNAs and other small RNAs from *Arabidopsis*. *Plant Cell.,* 16: 2001-2019.
6. Ramanjulu Sunkar, Thomas Girke, Pradeep Kumar Jain, Jian-Kang Zhu, 2005. Cloning and characterization of microRNAs from rice. *Plant Cell.,* 17: 1397–1411.
7. Erica Mica, 2006. Luca Gianfranceschi, Mario Enrico Pe. Characterization of five microRNA families in maize. Journal of Experimental Botany., 57(11): 2601-2612.
8. Yingyin Yao, Ganggang Guo, Zhongfu Ni, Ramanjulu Sunkar, Jinkun Du, Jian-Kang Zhu, 2007. Qixin Sun. Cloning and characterization of microRNAs from wheat (*Triticum aestivum* L.). Genome Biology, 8: R96.
9. Arazi, T., M. Talmor Neiman, R. Stav, M. Riese, P. Huijser, D.C. Baulcombe, 2005. Cloning and characterization of micro-RNAs from moss. Plant J., 43: 837-848.
10. Mallory, A.C., H. Vaucheret, 2006. Functions of microRNAs and related small RNAs in plants. Nat Genet., 38: S31-S36.
11. Sunkar, R., J.K. Zhu, 2004. Novel and stress-regulated microRNAs and other small RNAs from *Arabidopsis*. Plant Cell., 16: 2001-2019.
12. Fujii, H., T.J. Chiou, S.I. Lin, K. Aung, J.K. Zhu, 2005. A miRNA involved in phosphate-starvation response in *Arabidopsis*. Curr. Biol., 15: 2038-2043.
13. Jones-Rhoades M.W., D.P. Bartel, 2004. Computational identification of plant microRNAs and their targets, including a stress induced miRNA. Mol Cell., 14: 787-799.
14. Baskerville, S., D.P. Bartel, 2005. Microarray profiling of microRNA reveals frequent coexpression with neighboring miRNAs and host genes. *RNA*., 11: 241-247.
15. Lee, Y., M.1. Kim, J.1. Han, K.H. Yeom, S. Lee, S.H. Baek, V.N. Kim, 2004. MicroRNA genes are transcribed by RNA polymerase I. The EMBO Journal, 23: 4051-4060.
16. Aukerman, M.J., H. Sakai, 2003. Regulation of flowering time and flora organ identity by a miRNA and its APETALA2-like target genes. Plant Cell., 15: 2730-2741.

17. Xie, X., E. Allen, N. Fahlgren, A. Calamar, S.A. Givan, J.C. Carrington, 2005. Expression of Arabidopsis MIRNA genes. Plant Physio., 138: 2145-2154.
18. Jones-Rhoades M.W., D.P. Bartel, B. Bartel, MicroRNAs and their regulatory roles in plants. Annu. Rev. Plant Biol., 57: 19-53.
19. Bernstein, E., A.A. Caudy, S.M. Hammond, G.J. Hannon, 2001. Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature., 409: 363-366.
20. Lu, C., N.A. Fedoroff, 2000. mutation in the *Arabidopsis HYL1* gene encoding a dsRNA binding protein affects response to abscisic acid, auxin, and cytokinin. Plant Cell., 12: 2351-2366.
21. Yang, Z., Y. Ebright, B. Yu, X. Chen, 2006. *HEN1* recognizes 21~24 nt small RNA duplexes and deposits a methyl group onto the 2'OH of the 3' terminal nucleotide. Nucleic Acids Research, 34: 2667-2675.
22. Boutet, S., F. Vazquez, J. Liu, C. Beclin, M. Fagard, 2003. Arabidopsis HEN1: a genetic link between endogenous miRNA controlling development and siRNA controlling transgene silencing and virus resistance. Curr. Biol., 13: 843-848.
23. Li, J., Z. Yang, B. Yu, J. Liu, X. Chen, 2005. Methylation protects miRNAs and siRNAs from a 3'-end uridylation activity in Arabidopsis. Curr. Biol., 15: 1501-1507.
24. Bollman, K.M., M.J. Aukerman, M.Y. Park, C. Hunter, T.Z. Berardini, R.S. Poethig, 2003. *HASTY*, the Arabidopsis ortholog of exportin5/MSN5, regulates phase change and morphogenesis. Development, 130: 1493-1504.
25. Baumberger, N., D.C. Baulcombe, 2005. *Arabidopsis ARGONAUTE1* is a RNA slicer that selectively recruits microRNAs and short interfering RNAs. Proc. Natl. Acad. Sci. USA., 102: 11928-11933.
26. Bartel, D.P., 2004. MicroRNA: genomics, biogenesis, mechanism, and function. Cell., 116: 281-297.
27. Fowler, S., M.F. Thomashow, 2002. Arabidopsis transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. Plant Cell., 14: 1675-1690.
28. Zhu, J.K., 2002. Salt and drought stress signal transduction in plants. Annu. Rev. Plant Biol., 53 :247-273.
29. Raghothama, K.G., 1999. Phosphate acquisition. Annu. Rev. Plant Physiol. Plant Mol Biol., 50: 665-693.
30. Navarro, L., P. Dunoyer, F. Jay, B. Arnold, N. Dharmasiri, M. Estelle, O. Voinnet, D.G. Jonathan, 2006. Jones A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. Science., 312: 436-439.
31. Marschner, H., 1995. Mineral nutrition of higher plants(2^{nd} edn). Academic press.
32. Roghothama, K.G., A.S. Karthikeyan, 2005. Phosphate acqiucition. Plant and Soil., 274: 37-49.
33. Bari, R., B.D. Pant, M. Stitt, W.R. Scheible, 2006. *PHO2*, microRNA399, and *PHR1* define a phosphate-signaling pathway in plants. Plant Physiol., 141: 988-999.
34. Chiou, T.J., K Aung, S.I. Lin, C.C. Wu, S.F. Chiang, C.L. Su, 2006. Regulation of phosphate homeostasis by microRNA in *Arabidopsis*. Plant Cell., 18: 412-421.
35. Sunkar, R., V. Chinnusamy, J.H. Zhu, J.K. Zhu, 2007. Small *RNAs* as big players in plant abiotic stress responses and nutrient deprivation. Trends Plant Sci., 12: 301–309.
36. Pan, B.D., A. Buhtz, J. Kehr, W.R. Scheible, 2008. MicroRNA399 is a long-distance signal for the regulationof plant phosphate homeostasis. Plant Journal, 53: 731-738.
37. Nikiforova, V.J., M. Bielecka, B. Gakiere, S. Krueger, J. Rinder, S. Kempa, R. Morcuende, W.R. Scheble, H. Hesse, R. Hoefgen, 2006. Effect of sulfur availability on the integrity of amino acid biosynthesis in plants. Amino Acids, 30: 173-183.
38. Lappartient, A.G., J.J. Vidmar, T. Leustek, A.D. Glass, B. Touraine, 1999. Inter-organ signaling in plants:regulation of ATP sulfurylase and sulfate transporter genes expression in roots mediated by phloem-translocated compound. Plant J., 18: 89-95.
39. Takahashi, H., A. Watanabe-Takahashi, F.W. Smith, M. Blake-Kalff, M.J. Hawkesford, K. Saito, 2000. The roles of three functional sulphate transporters involved in uptake and translocation of sulphate in Arabidopsis thaliana. Plant J., 23: 171-182.
40. Isam, F., Voß Bjo¨rn, R. Ralf, R.H. Wolfgang, F. Wolfgang, 2007. Evidence for the rapid expansion of microRNA-mediated regulation in early land plant evolution. Plant Biol., 7: 13.
41. Koprivova, A., A.J. Meyer, G. Schween, C. Herschbach, R. Reski, S. Kopriva, 2002. Functional knockout of the adenosine 5'-phosphosulfate reductase gene in Physcomitrella patens revives an old route of sulfate assimilation. The Journal of biological chemistry, 277(35): 32195-32201.
42. Guo, W.J., M. Meetam, P.B. Goldsbrough, 2008. Examining the specific contributions of individual arabidopsis metallothioneins to copper distribution and metal tolerance. Plant Physio., 146: 1697-1706.

43. Chu, C.C., W.C. Lee, W.Y. Guo, S.M. Pan, L.J. Chen, H.M. Li and T.L. Jinn, 2005. A Copper Chaperone for Superoxide Dismutase That Confers Three Types of Copper/Zinc Superoxide Dismutase Activity in Arabidopsis. Plant Physio., 139: 425-436.
44. Yamisaki, H., S.E. Abdel-Ghany, C.M. Cohu, Y. Kobayashi, T. Shikanai and M. Pilon, 2007. Regulation of copper homeostasis by microRNA in Arabidopsis. Journal of Biological Chemstry, 282: 22.
45. Carr, J.P., L.E. Marsh, G.P. Lomonossoff, M.E. Sekiya, M. Zaitlin, 1992. Resistance to tobacco mosaic virus induced by the 54-kDa gene sequence requires expression of the 54-kDa protein. Mol Plant Microbe Interact., 5: 397–404.
46. Baulcombe, D., 2004. RNA silencing in plants. Nature., 431: 356–363.
47. Plasterk, R.H.A., 2003. RNA silencing. The genome's immune system. Science, 296: 1263-1265.
48. Kooter, J.M., M.A. Matzke, P. Meyer, 1999. Listening to silent gene: transgene silencing, gene regulation and pathogen control. Trends Plant Sci., 4: 340-347.
49. Hannon, G.J., 2002. RNA interference. Nature., 418: 244-251.
50. Napoli, C., C. Lemieux, R. Jorgensen, 1990. Introduction of a chimeric chalcone synthase gene into petunia results in reversible cosuppression of homologous gene in trans. Plant Cell., 2: 279-289.
51. Ruiz, M.T., O. Voinnet, D.C. Baulcombe, 1998. Initiation and maintenance of virus-induced gene silencing. Plant Cell., 10: 937-946.
52. Takeda, A., M. Tsukuda, H. Mizumoto, K. Okamoto, M. Kaido, K. Mise, T.A. Okuno, 2005. plant RNA virus suppresses RNA silencing through viral RNA replication. The EMBO Journal, 24: 3147-3157.
53. Lecellier, C.H., O. Voinnet, 2004. RNA silencing: no mercy for viruses? Immunol Rev., 198: 285-303.
54. Katiyar-Agarwal, S., S. Gao, A. Vivian-Smith, H.L. Jin, 2007. A novel class of bacteria-induced smallRNAs in Arabidopsis. Genes & Dev., 21: 3123-3134.
55. Bazzini, A.A., H.E. Hopp, R.N. Beachy, S. Asurmendi, 2007. Infection and coaccumulation of tobacco mosaic virus proteins alter microRNA levels, correlating with symptom and plant development. Proc Natl Acad Sci USA., 104(29): 12157-12162.
56. Dunoyer, P., C.H. Lecellier, E.A. Parizotto, C. Himber, O. Voinnet, 2004. Probing the microRNA and small interfering RNA pathways with virus-encoded suppressors of RNA silencing. Plant Cell., 16: 1235–1250.
57. Carmen, S.M., A.G. Juan, 2006. MicroRNA-guided processing impairs plum pox virus replication, but the virus readily evolves to escape this silencing mechanism. J Virol., 80: 2429–2436.
58. Lu, Y.D., Q.H. Gan, X.Y. Chi, S. Qin, 2008. Roles of miRNA in plant defense and virus offense interaction Plant Cell Rep., 27(10): 1571-1579.
59. Elisabeth, J., 2004. Chapman, Alexey I. Prokhnevsky, Kodetham Gopinath, Valerian V. Dolja and James C. Carrington. Viral RNA silencing suppressors inhibit the microRNA pathway at an intermediate step. Genes & Dev., 18: 1179-1186.
60. Navarro, L., P. Dunoyer, F. Jay, B. Arnold, N. Dharmasiri, M. Estelle, O. Voinnet, D.G. Jonathan, 2006. Jones. A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. Science, 312: 436-439.
61. Zhang, B.H., X.P. Pan, Q.L. Wang, G.P. Cobb, T.A. Anderson, 2005. Identification and characterization of plant microRNA using EST analysis. Cell Res., 15: 336–360.
62. Sunkar, R., G. Jagadeeswaran, 2008. In silico identification of conserved microRNAs in large number of diverse plant species. BMC Plant Biology, 8: 37.
63. Isam, F., Voß Bjo¨rn, R. Ralf, R.H. Wolfgang, 2007. Wolfgang F.Evidence for the rapid expansion of microRNA-mediated regulation in early land plant evolution. Plant Biol., 7: 13.
64. Qu, J., J. Ye, R.X. Fang, 2007. Artificial MicroRNA-Mediated Virus Resistance in Plants. JOURNAL OF VIROLOGY, 81(12): 6690-6699.
65. Niu, Q.W., S.S. Lin, J.L. Reyes, K.C. Chen, H.W. Wu, S.D. Yeh, N.H. Chua, 2007. Corrigendum: expression of artificial microRNAs in transgenic Arabidopsis thaliana confers virus resistance. Nat Biotechnol, 25: 254.
66. Simo´n-Mateo, C., J.A. Garcı´a, 2006. MicroRNA-guided processing impairs Plum pox virus replication, but the virus readily evolves to escape this silencing mechanism. J Virol., 80: 2429–2436.
67. Ana, M., Martı´n-Herna´ndez, C. David, 2008. Baulcombe. Tobacco Rattle Virus 16-Kilodalton Protein Encodes a Suppressor of RNA Silencing That Allows Transient Viral Entry in Meristems. JOURNAL OF VIROLOGY, 82(8): 4064-4071.
68. Papp, I., M.F. Mette, W. Aufsatz, L. Daxinger, S.E. Schauer, A. Ray, van der J. Winden, M. Matzke, A.J.M. Matzke, 2003. Evidence for nuclear processing of plant microRNA and short interfering RNA precursors. Plant Physiol, 132: 1382-139.

69. Padmanabhan, C., V. Ramachandran, M. Claude, 2005. Fauquet. MicroRNA-binding viral protein interferes with Arabidopsis development. Proc Natl Acad Sci USA., 102(29): 10381-10386.
70. Chory, J., M. Chatterjee, R. Cook, T. Elich, C. Fankhauser, J. Li, P. Nagpal, M. Neff, A. Pepper, D. Poole, J. Reed, V. Vitart, 1996. From seed germination to flowering, light controls plant development via the pigment phytochrome. Proc Natl Acad Sci USA., 93: 12066-12071.
71. Jiao, Y., L. Ma, E. Strickland, X. Deng, Conservation and divergence of light-regulated genome expression patterns during seedling development in rice and Arabidopsis. Plant Cell., 17: 3239-3256.
72. Bjorn, L.O., 1994. Introduction. In RE Kendrick, GHM Kronenberg, eds, Photomorphogenesis in Plants, Ed 2. Kluwer Academic Publishers, Boston., 3–25.
73. Paula Casati and Virginia Walbot. Gene Expression Profiling in Response to UltravioletRadiation in Maize Genotypes with Varying Flavonoid Content1. Plant Physiology, 132: 1739–1754.
74. A-H-Mackerness, S., C.F. John, B. Jordan, B. Thomas, 2000. Early signaling components in ultraviolet-B responses: distinct roles for different reactive oxygen species and nitric oxide. FEBS Lett., 489: 237–242.
75. Ulm, R., F. Nagy, 2005. Signaling and gene regulation in response to ultraviolet light. Curr Opin Plant Biol., 8: 477-482.
76. Jenkins, G.I., B.A. Brown, 2007. UV-B perception and signal transduction. In GC Whitelam, KJ Halliday, eds, Light and Plant Development, Vol 30. Blackwell Publishing, Oxford, 155–182.
77. Searles, P.S., S.D. Flint, M.M. Caldwell, 2001. A meta analysis of plant field studies simulating stratospheric ozone depletion. Oecologia, 127: 1-10.
78. Stapleton, A.E., V. Walbot, 1994. Flavonoids can protect maize DNA from the induction of ultraviolet-radiation damage. Plant Physiol., 105: 881-889.
79. Britt, A.B.,1996. DNA damage and repair in plants. Annu Rev Plant Physiol Plant Mol Biol., 4: 75-100.
80. Dai, Q., B. Yan, S. Huang, X. Liu, S. Peng, M.L.M. Miranda, A.Q. Chavez, B.S. Vegara, D. Olszyk, 1997. Response to oxidative stress defense systems in rice (*Oryza sativa*) leaves with supplemental UV-B radiation. Plant Physiol., 101: 301-308.
81. Jansen, M.A.K., V. Gaba, B.M. Greenberg, 1998. Higher plants and UV-B radiation: balancing damage, repair and acclimation. Trends Plant Sci., 3: 131-135.
82. Ulm, R., A. Baumann, A. Oravecz, Z. Ma´te´, E. A´da´m, J. Edward, 2003. Oakeley. Schafer E, Nagy F. Genome-wide analysis of gene expression reveals function of the bZIP transcription factor HY5 in the UV-B response of Arabidopsis. Proc Natl Acad Sci USA., 101(5): 1397-1402.
83. Zhou, X.F., G.D. Wang, W.X. Zhang, 2007. UV-B responsive microRNA genes in Arabidopsis thaliana. Mol Syst Biol., 3: 103.
84. Keithanne Mockaitis, 2008. Auxin Receptors and Plant Development: A New Signaling Paradigm. Annual Review of cell and developmental biology, 24.
85. Roseline Remans, Stijn Spaepen 2006. and Jos Vanderleyden. Auxin Signaling in Plant Defense. Science, 313(5784): 171.
86. Sinnott, E.W., 1952. Reaction wood and the regulation of tree form. Am. J. Bot., 39: 69-78.
87. Timell, T.E., 1986. Compression Wood in Gymnosperms. (Berlin:Springer Verlag).
88. Wardrop, A.B., G.W. Davies, 1964. The structure of reaction wood: The structure and differentiation of compression wood. Aust. J. Bot., 12: 24-38.
89. Lu, S., Y.H. Sun, R. Shi, C. Clark, L. Li, V.L. Chiang, 2005. Novel and mechanical stress-responsive microRNAs in *populus trichocarpa* that are absent from arabidopsis. Plant Cell., 17: 2186-2203.
90. Mittler, R., S. Vanderauwera, M. Gollery, F. Breusegem, 2004. Reactive oxygen gene network of plants. Trends Plant Sci., 9: 490-498.
91. Malan, C., M.M. Gregling, J. Gressel, 1990. Correlation between Cu-Zn superoxide dismutase and glutathione reductase and environmental and xenobiotic stress tolerance in maize inbreds. Plant Sci., 69: 157-166.
92. Desikan, R., S. A-H-Mackerness, J.T. Hancock, S.J. Neill, 2001. Regulation of the *Arabidopsis* transcriptome by oxidative stress. Plant Physiol., 127: 159-172.
93. Yan Xiong, Anthony L. Contento, Phan Quang Nguyen, 2007. and Diane C. Bassham. Degradation of oxidized proteins by autophagy during oxidative stress in Arabidopsis. Plant Physio., 143: 291-299.
94. Apel, K. and H. Hirt, 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol., 55: 373-399.
95. Fridovich, I., 1995. Superoxide radical and superoxide dismutases. Annu Rev Biochem., 64: 97-112.
96. Kliebenstein, D.J., R.A. Monde, R.L. Last, 1998. Superoxide dismutase in *Arabidopsis*: an eclectic enzyme family with disparate regulation and protein localization. Plant Physiol., 118: 637-650.
97. Bowler, C., L. Slooten, S. Vandenbranden, De R. Rycke, J. Botterman, C. Sybesma, M. Van

Montagu, D. Inzé, 1991. Manganese superoxide diamutase can reduce cellular damage mediated by oxygen radicals in transgenic plants. The EMBO J., 10: 1723-1732.

98. Sunkar, R., A. Kapoor, J.K. Zhu, 2006. Posttranscriptional induction of two Cu/Zn superoxide dismutase genes in *Arabidopsis* is mediated by down-regulation of miR398 and important for oxidative stress tolerance. Plant Cell., 18: 2051-2065.
99. Bonnet, E., J. Wuyts, P. Rouze, de Y.V. Peer Detection, 2004. of 91 potential conserved plant microRNAs in *Arabidopsis thaliana* and *Oryza sativa* identifies important target genes. Proc. Natl. Acad. Sci. USA., 101: 11511-11516.
100. Sunkar, R., T. Girke, P.K. Jain, J.K. Zhu Cloning, 2005. and characterization of microRNAs from rice. Plant Cell., 17: 1397-1411.