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Role of microRNAs in Arsenic Stress Tolerance of Plants

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Abstract

Arsenic is a highly toxic carcinogenic element whose contamination in groundwater and soil has emerged as a problem of unprecedented scale in last few years. The arsenic exposure to humans occurs through drinking water and food and threatens to increase incidences of cancer and other ailments drastically in future. To safeguard people from arsenic through food, research has focused on understanding in depth mechanisms of arsenic stress responses in plants. These included laboratory-based and field studies and encompassed morphological, physiological, biochemical and molecular assays and also whole genome transcriptome and proteome analyses. These studies led to information about changes at biochemical and molecular levels which are reflected in morphological and growth changes. Further, the involvement of various signalling and regulatory elements has been revealed. Among these, microRNAs (miRNAs), which are 20-25 base pairs long RNAs, have emerged as important players in mediated arsenic stress responses in plants. miRNAs have been found to regulate signalling elements, transcription factors, hormones biosynthesis and responses, oxidative stress responses, sulphur metabolism etc. This review presents a discussion on miRNAs involvement in arsenic stress responses in plants and also sheds light on future perspectives.

The arsenic contamination of the environment is a problem concerning people throughout the world. The major affected areas include Southeast Asian countries like Bangladesh, India, China, Pakistan and Vietnam (Chakraborti *et al*., 2009; Ravenscroft *et al*., 2009; Podgorski *et al*., 2017). Both biogeochemical and anthropogenic activities are attributed to have led to arsenic contamination. Arsenic has been used as insecticides, pesticides and herbicides viz., sodium arsenite, calcium arsenite, copper acetoarsenite-Paris Green etc. Methylarsenic acid and dimethylarsenic acid have been used as herbicides (Bencko and Foong, 2016). Even today, arsenic finds usage in pharmaceutical and glass industries, in some herbicides (dimethylarsinic acid), and as feed additives for poultry and swine (roxarsone, arsanilic acid and its derivatives) (Mangalgiri *et al*., 2015; Bencko and Foong, 2016). Only a few microbes are able to use arsenic in their respiratory metabolism to gain energy. However, arsenic is toxic to other organisms. The toxicity of arsenic is dependent on its species. Arsenic exists in a number of inorganic and organic forms and the list of these compounds is increasing with the development of sophisticated analytical instruments. The major inorganic species include arsenite [As(III)] arsenate [As(V)] (Arslan *et al*., 2016). The As contaminated groundwater is used for drinking and irrigation purposes and so arsenic enters into the food chain (Tripathi *et al*., 2007). Rice is of specific importance in this scenario since it is a major crop cultivated in arsenic affected Asian countries and the growing conditions of rice favour greater arsenic accumulation as compared to other crops (Srivastava *et al*., 2012; Awasthi *et al*., 2017).

To tackle the issue of arsenic infiltration in rice, a lot of research has been conducted to delineate the details of arsenic uptake and transport in plants, the mechanisms of arsenic stress on plants and the response processes of plants to tackle the stress (Srivastava *et al*., 2011, 2012; Awasthi *et al*., 2017). If the mechanistic details are understood, agronomic or biotechnological mitigation strategies may be devised and implemented. The uptake and transport of arsenic species have been investigated and a lot of details are available presently. It is known that As(V) is taken up through phosphate transporters while As(III) enters through aquaglyceroporins (Li *et al*., 2016). Arsenic affects a number of physiological and biochemical processes in plants. In particular, Arsenic stress affects both the germination rate and frequency and early growth of seedlings after germination due to effects on ATP and NAD metabolisms (Abedin and Meharg, 2002; Srivastava *et al*., 2013a). Arsenic stress is also known to markedly influence the photosynthetic efficiency of plants, cause a decline in chlorophyll levels, affect the enzymes of chlorophyll synthesis and degradation and induce changes in chloroplast ultrastructure (Jain and Gadre, 2004; Li *et al*., 2006; Srivastava *et al*., 2013b). The effects of arsenic on carbon, nitrogen and sulphur metabolisms are demonstrated and it is also known that these metabolic

changes lead to perturbed amino acid biosynthesis (Pathare *et al*., 2013). Several transcriptomics and proteomics studies have also been performed to obtain greater details of arsenic-induced changes in plants (Requejo and Tena, 2006; Norton *et al*., 2008; Yu *et al*., 2012; Srivastava *et al*., 2013c; 2015; Castrillo *et al*., 2013). These analyses demonstrate wide changes in signalling elements (mitogen activated protein kinases, hormones, microRNAs, transcription factors), and metabolic pathways (carbon, nitrogen, sulphur, lipid, amino acid), photosynthesis and respiratory processes, transposable elements etc. MicroRNAs have emerged as important regulators of arsenic stress responses in the last few years and have been demonstrated to regulate a number of processes. This review discusses the importance of microRNAs in plant responses to arsenic stress.

2. The Biogenesis of microRNAs in Plants

Regulatory endogenous small RNAs are ubiquitous components of endogenous plant transcriptomes and has been categorized into several groups based on differences in biogenesis and function (Axtell, 2013). Some are derived from ss-RNA precursors and contain a hairpin structure (hairpin RNAs; hpRNAs) while others originate from dsRNA precursors (small interfering RNAs; siRNAs). hpRNAs are further divided into miRNAs and non-miRNAs in plants (Axtell, 2013). MicroRNAs are a group of highly conserved small RNAs, which do not encode proteins and are of short (20–24 nucleotide) size (Xie *et al*., 2015; Stepien *et al*., 2017). miRNAs modulate various processes through interaction with specific messenger RNAs (mRNAs) at post-transcriptional levels via homology alignment. miRNAs regulate various developmental processes, phase transition as well as stress responses. miRNA production occurs in a tissue-specific and time-dependent manner and varies in response to various stresses (Srivastava *et al*., 2013c; Achkar *et al*., 2016). miRNAs are encoded by distinct genomic loci and their biogenesis related processes occur in the subnuclear dicing bodies (D-bodies). The transcription of plant miRNA genes (MIRs) occurs by RNA polymerase II (RNA Pol II) producing primary transcripts (pri-miRNAs), which contain two specific features; a cap structure at the 5'end and a poly(A) tail at the 3'end (Stepien *et al*., 2017). The production of miRNAs occurs from these pri-miRNA by a Dicer-Like 1 (DCL1) RNase and other proteins, Serrate (SE; a zinc-finger-domain-containing protein) and Hyponastic Leaves 1 (HYL1; a dsRNA-binding-domain-containing protein) (Achkar *et al*., 2016). pri-miRNAs release pre-miRNAs after the action of RNase III (DCL1) that contain stem loop structure and then these pre-miRNAs are processed into a duplex. For stabilizing the duplex during transport from nucleus to cytoplasm via the exportin Hasty (HST) pathway, small RNA methyltransferase (Hua Enhancer1; HEN1) adds a methyl group to the 3' end of the duplex. One strand of the duplex, miRNA (known as guide strand) binds with argonaute (AGO) proteins to form RNA-induced silencing complex (RISC) to regulate gene expression either via cleavage or by translational inhibition of target RNAs (Brodersen *et al*., 2008), while the other strand, miRNA* (known as passenger strand) is degraded (Xie *et al*., 2015). RISC assembly is facilitated by HSP90 (Heat Shock Protein 90) and CYP40 (Cyclophilin 40) inducing conformational changes in AGO protein to facilitate the entry of miRNA-miRNA* duplxes (Stepien *et al*., 2017). Figure 1 presents a brief picture of miRNA biogenesis in plants.

3. Responses of microRNAs to Arsenic Exposure

A number of studies to date have focused on identifying the roles of miRNAs in metal(loid) (Al, As, Cd, Cu, Hg etc.) stress responses of various plants. The adopted approaches included both computational and experimental and have led to the discovery of several conserved and novel miRNAs responsive to metal(loid)s from various plants. A list of briefly summarized miRNA responses is given in Table 1. Various studies point to a few specific features of miRNA responses in plants. The miRNA responses vary (a) from plant to plant or one species of a plant to another species (b) with respect to metal(loid) treatment, (c) in tissue-specific manner in one plant (d) in time-dependent manner in a tissue and/or in a plant, and (e) in a dose-dependent manner in a tissue and/or in a plant. Further, the number of metal(loid) responsive miRNAs was also found to vary in different studies even when conducted in same tissue, plant and with same metal(loid).

Tuli *et al*. (2010) quoted putative As responsive miRNAs from rice. These included miRNAs like miR160f, miR168a/b, miR169q, miR319a, miR394, miR397a, miR414, miR416, miR444a, miR531, miR820a/b/c, miR1427, miR1430, miR1431, and miR1437. Sharma *et al*. (2015) analyzed miRNA profile in response to As(III) and As(V) and in high As accumulating genotype (HARG) and low As accumulating genotype (LARG). It was found that some miRNAs were down-regulated or up-regulated in both genotypes and in response to both As species (viz., miR164, miR171, miR395, miR529, miR820, miR1432, were down-regulated while miR408, miR1861, miR2102, miR2907 were up-regulated). However, some miRNAs showed a differential response, such as miR396 was up-regulated in response to As(V) while down-regulated in response to As(III). The reverse response was found for miR528. In case of As(III), miR399 was down-regulated in HARG and up-regulated in LARG while miR1846 showed opposite response. Even the miRNA species of a family can show different response to a stress viz., miR2907a and

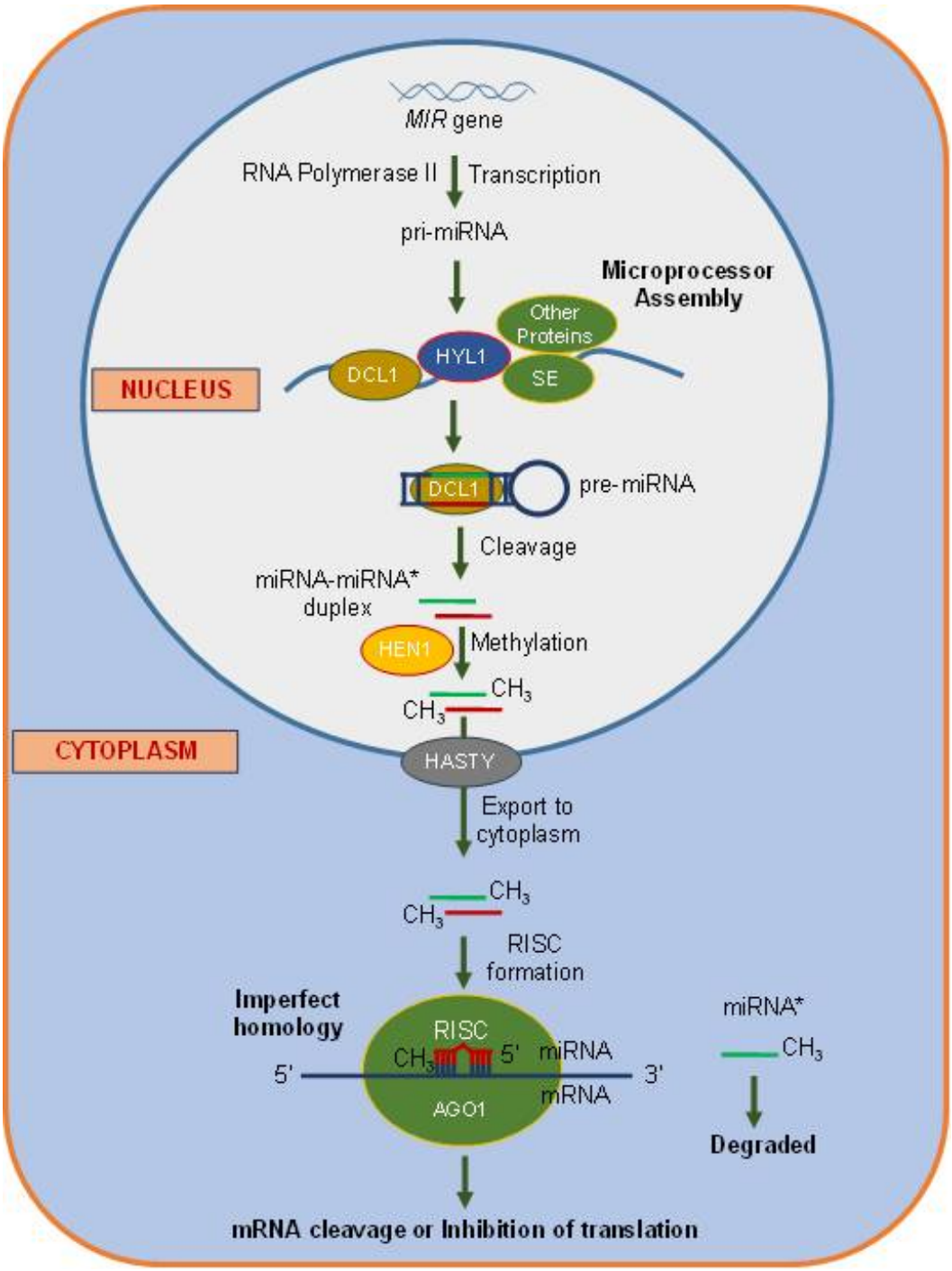

Fig. 1: Schematic diagram showing miRNA biogenesis in plants. Primary-miRNA (pri-miRNA) transcripts are formed after transcription of *MIR* genes by RNA polymerase II. Then, pre-miRNAs are produced by DCL1 endonuclease in a microprocessor assembly, which is supported by proteins like HYL1, SE and other proteins. DCL1 further acts on pre-miRNAs to form miRNA-miRNA* duplexes. Then, duplexes are methylated by HEN1 and then the duplex is exported from nucleus to cytoplasm via HASTY. In cytoplasm, miRNA binds to AGO1 to form RNA-Induced Silencing Complex (RISC) where target mRNA is either cleaved or its translation is inhibited due to imperfect homology. The other strand, miRNA* is mostly degraded

Table 1: List of arsenic-responsive miRNAs found in various plants

S.N.	miRNA	Name of Plant (s)	Reference
1	miR156	*Brassica juncea, Oryza sativa*	Yu *et al.* (2012), Srivastava *et al.* (2013c)
2	miR159	*B.juncea*	Srivastava *et al.* (2013c)
3	miR164	*B. juncea, O. sativa*	Srivastava *et al.* (2013c), Sharma *et al.* (2015)
4	miR166	*O. sativa*	Yu *et al.* (2012)
5	miR167	*B. juncea*	Srivastava *et al.* (2013c)
6	miR168	*O. sativa*	Yu *et al.* (2012)
7	miR169	*B. juncea*	Srivastava *et al.* (2013c)
8	miR171	*O. sativa*	Yu *et al.* (2012), Sharma *et al.* (2015)
9	miR172	*B. juncea*	Srivastava *et al.* (2013c)
10	miR319	*B. juncea*	Srivastava *et al.* (2013c)
11	miR390	*O. sativa*	Liu and Zhang (2012)
12	miR395	*O. sativa, B. juncea*	Srivastava *et al.* (2013c), Sharma *et al.* (2015)
13	miR396	*O. sativa*	Yu *et al.* (2012), Sharma *et al.* (2015)
14	miR397	*O. sativa*	Liu and Zhang (2012)
15	miR399	*O. sativa*	Sharma *et al.* (2015)
16	miR408	*O. sativa*	Liu and Zhang (2012), Sharma *et al.* (2015)
17	miR528	*O. sativa*	Liu and Zhang (2012), Sharma *et al.* (2015)
18	miR529	*O. sativa*	Sharma *et al.* (2015)
19	miR820	*O. sativa*	Sharma *et al.* (2015)
20	miR838	*B. juncea*	Srivastava *et al.* (2013c)
21	miR854	*B. juncea*	Srivastava *et al.* (2013c)
22	miR1318	*O. sativa*	Liu and Zhang (2012)
23	miR1432	*O. sativa*	Sharma *et al.* (2015)
24	miR1846	*O. sativa*	Sharma *et al.* (2015)
25	miR1861	*O. sativa*	Sharma *et al.* (2015)
26	miR2102	*O. sativa*	Sharma *et al.* (2015)
27	miR2907	*O. sativa*	Sharma *et al.* (2015)

miR2907d showed up-regulation and miR2907b and miR2907c showed down-regulation in rice low As accumulating genotype in response to As(III) (Sharma *et al.*, 2015). Similarly, in response to As(V), various species of miR156, miR159, miR169 and miR319 showed a variable response in *B. juncea* (Srivastava *et al.*, 2013c). In As-treated *O. sativa* and *B. juncea* (Liu and Zhang, 2012; Srivastava *et al.*, 2013c), miR164 and miR172 were depressed in *O. sativa*, while induced in *B. juncea*. Hence, the response of miRNAs was dependent on the metal(loid) or species of the metalloid and also on the plant and its genotype.

Time-dependent expression of miRNAs was demonstrated by Srivastava *et al*. (2013c) in *B. juncea* where various miRNAs showed change in expression when As(V) exposure was increased from 1 h to 4 h e.g., miR426, miR472, miR390 were up-regulated at 1 h but showed down-regulation at 4 h, while miR395 showed down-regulation at 1 h but up-regulation at 4 h. This was followed by further time dependent analysis of selected miRNAs at 6 h, 24 h and 72 h. It was found that exposure duration did affect the expression profile of miRNAs. Tissue-specific miRNA expression is another important feature of miRNAs. In case of *B. juncea* exposed to As(V), miR167

and miR838 were down-regulated in roots but up-regulated in shoots. Yu *et al*. (2012) performed a transcriptomic analysis of rice plants and identified 36 As(III) responsive miRNAs showing variation in expression in low or high As(III) treatments. 25 miRNAs of 22 families were observed in roots while 30 miRNAs of 23 families were found in shoots.

Even the number of As(III)-responsive miRNAs in different studies has been found to be different. A total of 69 miRNAs belonging to 18 plant miRNA families had significantly altered expression. The As-responsive miRNAs also exhibited a time- and organ-dependent change in their expression. A total of 67 arsenite stress-responsive miRNAs have also been identified in rice (Liu and Zhang, 2012). Yu *et al*. (2012) also assessed the response of mIRNAs in rice and found 25 As(III)-responsive miRNAs in roots and 30 miRNAs in shoots. Pandey *et al*. (2015) analyzed miRNA expression in rice seedlings and observed a total of 46 miRNAs to be responsive to As. Sharma *et al*. (2015) assessed miRNA profile in contrasting low and high As accumulating rice genotypes. They found variable number of responsive miRNAs upon exposure to As(III) and As(V). Further, there have been differences observed with respect to number of pri- and mature-miRNAs.

4. Regulatory Functions of miRNAs

miRNAs have been suggested to regulate metal stress through several ways from changes in the metabolic and biochemical profile of plants to hormonal biosynthesis, and from transport to oxidative stress regulation. Metal(loid) detoxification in plants requires complexation with S-containing ligands, glutathione (GSH) and phytochelatins (PCs) for minimizing the concentration of free metal(loid) ions and regulation of oxidative stress induced by metal(loid) through increased activity of antioxidant enzymes (Srivastava *et al*., 2007). This involves coordinated regulation of C, N and S metabolism (Pathare *et al*., 2013) through the action of several phytohormones and signaling networks. miRNAs act in concerted action with various signaling components to tackle metalloid load (Curaba *et al*., 2014). A study by Srivastava *et al*. (2009) proposed stress exerted on sulphur metabolism upon arsenic exposure to act as a signal to plants for mediating various responses through the action of hormones like jasmonates, ethylene, auxins, and cytokinins. In this connection, Srivastava *et al*. (2013c) observed miR395, miR838, and miR854 to be responsive to As exposure in *B. juncea* within 4 h of exposure. miR395 regulates S uptake and allocation through its targets sulphur transporter (SULTR2;1) and ATP sulphurylase (APS) (Liang *et al*., 2010). miR838 and miR854 were predicted to attenuate the translation of SULTR2;1 and SULTR2;2, and serine acetyl transferase (SAT), which are involved in sulphate uptake and synthesis of O-acetylserine, respectively. Thus, miRNAs act at a very early time point to regulate sulphur metabolism. In oxidative stress tolerance, miR398 plays crucial role through regulation of Cu/Zn superoxide dismutase (Bouche, 2010). A role of miR395 and miR398 in ameliorative effects of Se on As stressed rice plants was demonstrated by Pandey *et al*. (2015).

A role of microRNAs in the regulation of hormones like auxin (miR160, miR167, miR528, miR393) and jasmonates (miR319) is demonstrated (Wang *et al*., 2005; Schommer *et al*., 2008). miR319 was found to be an As-responsive miRNAs in *B. juncea* (Srivastava *et al*., 2013c). This miRNA affects jasmonate biosynthesis through interaction with TCP transcription factors (Schommer *et al*., 2008). miR319 was also found to be responsive to As in rice (Tuli *et al*., 2010; Liu and Zhang, 2012) Further, Srivastava *et al*. (2013c) also found miRNA838 to target a lipase in *B. juncea* that can regulate oxylipin biosynthesis and thus, jasmonate biosynthesis. Tuli *et al*. (2010) also suggested miR168b and miR169q in rice to target lipoxygenase 1 and 12-oxophytodienoate reductase 2, respectively. These genes can affect jasmonate biosynthesis. Srivastava *et al*. (2013c) also demonstrated interconnections of jasmonates and miRNAs in response to arsenic stress through external jasmonic acid supply that resulted in the altered expression pattern of miRNA319. The involvement of auxins and associated regulatory miRNAs was also demonstrated by Srivastava *et al*. (2013c). miRNA167 (Meng *et al*., 2010), miRNA164 (Guo *et al*., 2005), and miR390 (Yoon *et al*., 2010), which regulate auxin signalling and lateral root growth and density, were found among the arsenic responsive miRNAs and the levels of auxins were also found to vary (Srivastava *et al*., 2013c). The role of miRNAs in the regulation of other hormones like gibberelins, cytokinins and ABA etc. is also reported. Further, miRNAs also functions in the cross-talk of several other phytohormones viz., miRNA (Curaba *et al*., 2014). A number of *cis*-acting elements have been identified in promoters of miRNAs which include that of phytohormones like salicylic acid, ethylene, gibberellins, methyl jasmonate, ABA etc. (Sharma *et al*., 2015) that suggest interactions among miRNAs and various signalling networks.

5. Future Directions

The studies, though still preliminary, do suggest strong involvement of miRNAs in the regulation of arsenic stress perception and signalling and response of plants. Future research needs to be directed towards the development of transgenic lines particularly for miRNAs involved in regulation of sulphur metabolism to get deeper knowledge of miRNA functions. In addition, research is needed to conclusively demonstrate miRNA involvement

in regulation of various processes during arsenic stress through in depth physiological, and biochemical analyses.

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