Metal toxicity is a major stress affecting crop production. This includes metals that are essential for plants (copper, iron, zinc, manganese), and non-essential metals (cadmium, aluminum, cobalt, mercury). A primary common effect of high concentrations of metal such as cadmium, copper, cadmium, or mercury is root growth inhibition. Metal toxicity triggers the accumulation of reactive oxygen species leading to damage of lipids, proteins, and DNA. The plants response to metal toxicity involves several biochemical processes that require fine and precise regulation at transcriptional and post-transcriptional levels. MicroRNAs (miRNAs) are 21 nucleotide non-coding RNAs that regulate gene expression at the post-transcriptional level. A miRNA, incorporated into a RNA-induced silencing complex, promotes cleavage of its target mRNA that is recognized by an almost perfect base complementarity. In plants, miRNA regulation is involved in development and also in biotic and abiotic stress responses. We review novel advances in identifying miRNAs related to metal toxicity responses and their potential role according to their targets. Most of the targets for plant metal-responsive miRNAs are transcription factors. Information about metal-responsive miRNAs in different plants points to important regulatory roles of miR319, miR390, miR393, and miR398. The target of miR319 is the TCP transcription factor, implicated in growth control. miR390 exerts its action through the biogenesis of trans-acting small interference RNAs that, in turn, regulate auxin responsive factors. miR393 targets the auxin receptors TIR1/AFBs and a bHLH transcription factor. Increasing evidence points to the crucial role of miR398 and its targets Cu/Zn superoxide dismutases in the control of the auxin receptors TIR1/AFBs and a bHLH transcription factor. Increasing evidence points to the crucial role of miR398 and its targets Cu/Zn superoxide dismutases in the control of...
cytochrome c genes from the green alga *Chlamydomonas reinhardtii* (Quinn et al., 2000). Two promoter regions of the *PsSR2* gene from *Phaseolus vulgaris* contain heavy metal-responsive elements (HMREs; Qi et al., 2007).

Small and/or large non-protein coding RNAs (npcRNAs) may be involved in the regulation/signaling of metal toxicity response (Jones-Rhoades et al., 2006; Hobert, 2008; Ben Amor et al., 2009). One of the most studied classes of npcRNAs is the micro RNAs (miRNAs). miRNAs are 21 nucleotide npcRNAs that regulate gene expression at the post-transcriptional level in plants. A precursor miRNA (pre-miRNA) with imperfect hairpin structure is processed into a mature miRNA and this hairpin structure is processed into a mature miRNA and this

In regard to miRNAs that respond to Cd-toxicity the conserved miRNAs: miR160, miR164, and miR167 and the novel of Osa-miR602 and Osa-miR604 were identified in a library of small RNAs from rice seedlings exposed to Cd (Huang et al., 2009). Osa-miR602 is up-regulated in rice roots exposed for 2 h to high Cd; its predicted target is a xylemogenic endodermal cell. Osa-miR604, which was up-regulated in leaves treated with toxic levels of Cd for 6 h, down-regulated a lipid transfer protein (LPT; Huang et al., 2009). This type of protein is responsive to environmental stresses and to abscisic acid, salicylic acid, ethylene, and methyl jasmonate that has been proposed to participate in cutin and wax assembly and in defense of plant against pathogens (Arondel et al., 2000; Kim et al., 2008). Rice microarray data showed that miR528 is up-regulated, while miR162, miR166, miR171, miR390, miR168, and miR156 families were down-regulated under Cd stress (Ding et al., 2011). The search of possible metal-responsive cis-acting elements revealed that a MRE-like sequence (5′-TGCGCNC-3′) is present in promoter regions of most of the Cd-responsive miRNA genes (Ding et al., 2011). Other cis-acting elements related to different abiotic stresses such as ARE (anaerobic-responsiveness element); ABRE (ABA-responsive element); ERE (ethylene-responsive element); HSE (heat-stress responsive element); and DFA (low temperature-responsive element) were also identified in these miRNA genes promoters, thus implying that these miRNAs could be responsive to other stress signals besides metal toxicity (Ding et al., 2011). In roots of *Brassica napus* miR393, miR171, miR156, and miR396 are down-regulated after Cd exposure (6 h; Xie et al., 2007).

**Table 1 | Metal toxicity-responsive miRNAs.**

<table>
<thead>
<tr>
<th>Related metal</th>
<th>miRNA</th>
<th>Targets</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd, Hg, Al, Mn</td>
<td>miR319</td>
<td>TCP transcription factors</td>
<td>Zhou et al. (2008), Valdés-López et al. (2010), Chen et al. (2012)</td>
</tr>
<tr>
<td>Cd, Hg, Al</td>
<td>miR171</td>
<td>SCL transcription factors</td>
<td>Xie et al. (2007), Zhou et al. (2008, 2012)</td>
</tr>
<tr>
<td>Cd, Hg, Al</td>
<td>miR368</td>
<td>TASS</td>
<td>Ding et al. (2011), Chen et al. (2012), Zhou et al. (2012)</td>
</tr>
<tr>
<td>Cd, Hg, Al</td>
<td>miR393</td>
<td>TIR1/IAF1s (F-box auxin receptors) and bHLH transcription factors</td>
<td>Xie et al. (2007, Zhou et al. (2008)</td>
</tr>
<tr>
<td>Cd, Hg, Al</td>
<td>miR366</td>
<td>GPF transcription factors</td>
<td>Xie et al. (2007), Chen et al. (2012), Zhou et al. (2012)</td>
</tr>
<tr>
<td>Cd, Hg, Mn</td>
<td>miR167</td>
<td>Auxin responsive factors (ARFs)</td>
<td>Huang et al. (2009), Valdés-López et al. (2010), Zhou et al. (2012)</td>
</tr>
<tr>
<td>Cd, Hg</td>
<td>miR164</td>
<td>NAC, CUP transcription factors</td>
<td>Huang et al. (2009, Zhou et al. (2012)</td>
</tr>
<tr>
<td>Cd, Al</td>
<td>miR160</td>
<td>Auxin responsive factors (ARFs)</td>
<td>Huang et al. (2009, Chen et al. (2012)</td>
</tr>
<tr>
<td>Cd</td>
<td>miR156</td>
<td>SBP transcription factors</td>
<td>Xie et al. (2007), Ding et al. (2011)</td>
</tr>
<tr>
<td>Cu, Fe, Mn</td>
<td>miR398</td>
<td>CSD, COX5b.1, CCS</td>
<td>Sunkar et al. (2006), Zhou et al. (2008), Valdés-López et al. (2010)</td>
</tr>
<tr>
<td>Hg, Mn</td>
<td>miR172</td>
<td>AP2 transcription factors</td>
<td>Valdés-López et al. (2010, Zhou et al. (2012)</td>
</tr>
<tr>
<td>Mn</td>
<td>miR397</td>
<td>Laccases</td>
<td>Valdés-López et al. (2010)</td>
</tr>
</tbody>
</table>
In leaves of the model legume Medicago truncatula, miR393, miR171, miR319, and miR329 are up-regulated, while miR166 and miR398 are down-regulated after Cd, Hg, and Al exposure (Zhou et al., 2008). A high-throughput small RNA-sequencing approach revealed that miR159, miR160, miR319, miR396, and miR390 were down-regulated in response to Al (Chen et al., 2012). More recently, a study using a similar approach identified Hg-toxicity responsive miRNAs such as the miR167, miR172, miR169, miR164, miR395 families that are up-regulated, whereas the miR196, miR390, and miR171 are down-regulated in this legume. In addition, new M. truncatula Hg-responsive miRNAs were identified such as miR2681 targets the transcripts coding TIR-NBS-LRR disease resistance proteins (Zhou et al., 2012).

Our group has reported the miRNA expression profile in common bean (P. vulgaris), the most important legume for human consumption. Using a miRNA-macroarray hybridization approach we identified miRNAs that respond to nutrient deficiencies and to Mn-toxicity in different plant organs. In common bean plants exposed to high Mn miR397 is down-regulated in leaves, miR319 and miR398 are up-regulated in roots and nodules, miR172 is up-regulated in nodules and miR167 is up-regulated in roots (Valdés-López et al., 2010). Recently, the identification and characterization of miRNAs in P. vulgaris by high-throughput sequencing has been completed (Peláez et al., 2012).

Current information about metal-responsive miRNAs in different plants indicates the common relevant role of miR319, miR390, miR393, and miR398.

**ROLES OF miR319, miR390, miR393, AND miR398**

**miR319**
Plant growth and senescence are processes affected by metal toxicity (Maksymiec, 2007). Common responses of shoots to Al- and Cu-toxicity include cellular and ultrastructural changes in leaves, decreased photosynthetic activity leading to chlorosis and necrosis of leaves, total decrease in leaf number and size, and decreased shoot biomass (Thornton et al., 1986; Lanaras et al., 1993; Maksymiec, 1997; Panou-Filotheou et al., 2003). In addition, Ca-toxicity leads to rapid senescence in leaves (Luna et al., 1994). Interestingly, miR319 and its target TCP (Teosinte Branched/Cycloidea/PCF) TF (Table 1), implicated in growth control, have shown differential expression in most of the studies of miRNAs responding to metal toxicity. Members of the TCP family bind to promoter elements which are essential for the expression of the proliferating cell nuclear antigen (PCNA) gene (Kosugi and Ohashi, 1997). Other TCPs are involved in the morphogenesis of shoot lateral organs (Li et al., 2005). Lately, it has been demonstrated that miR319 plays a role on leaf senescence through the regulation of TCPs that positively control leaf senescence via JA biosynthesis and important senescence positive regulators like WRKY35 (Schommer et al., 2008).

**Figure 1** depicts the mode of action of miR319 and TCP. In leaves, high Cd, Hg, and Al induce miR319 leading to the degradation of TCP thus affecting growth and senescence. In the roots, this miRNA is up-regulated in response to Al while it is down-regulated in Mn-toxicity (Valdés-López et al., 2010; Chen et al., 2012). The opposite regulation of miR319 could be due to the different plant species and/or the different time of exposure and metal concentration used. When both metals are abundant in the ground Al may exert an antagonistic effect on the uptake of Mn thus ameliorating Mn-toxicity (Blair and Taylor, 1997; Yang et al., 2009). There are no reports about the regulation of miR319 when plant roots are exposed to the combination of Al and Mn; we find difficult to speculate about this issue since specific effects in the plant would depend on several variables (concentration, time of exposure, environmental conditions).

**miR390**
miR390 and its target TAS3 (Table 1) are related to metal toxicity response in different plants. The miR390-induced cleavage of TAS3 transcript initiates ta-siRNAs (trans-acting small interference RNAs) biogenesis, leading to the degradation of ARFs (auxin response factors) that play critical roles in lateral root development (Marín et al., 2010). miR390 is repressed in roots of plants under Cd, Al, and Hg toxicities, which would lead the accumulation of intact TAS3 transcript and the decrease of tasiARFs resulting in...
the inhibition of lateral root growth (Chen et al., 2011; Zhou et al., 2012; Figure 1).

Arabidopsis plants exposed to high Cu show decreased primary root growth and increased short lateral root density. Also, changes in auxin and cytokinin accumulation and in mitotic activity within the primary and secondary root tips were observed in Cu-exposed plants (Lequeux et al., 2010). We may speculate that miR390 and its targets could also respond to Cu-toxicity, but no information about miR390 regulation in this stress is available.

miR393

miR393 is regulated by Cd, Hg, and Al toxicities (Xie et al., 2007; Zhou et al., 2008). These metals induce miR393 in leaves, which would lead to the repression of its targets the F-box auxin receptors TIR1/AFBs and bHLH transcription factors (Table 1; Jones-Rhoades and Bartel, 2004; Navarro et al., 2006). TIR1 positively regulates auxin signaling, its level would be low when the miR393 increases leading to an inhibition of auxin signaling. Studies have shown the importance of miR393 regulation of leaf development, root system architecture, and root growth (Vidal et al., 2010; Si-Ammour et al., 2011; Chen et al., 2011; Figure 1). This miRNA also responds to bacterial infection with Pseudomonas syringae and to salinity (Navarro et al., 2006; Gao et al., 2011).

The root is the main organ affected by high concentration of metals such as Cu, Cd, Cu, Hg, and Al in the soil, and the common phenotypic response is changes in architecture (Karataglis et al., 1997; Törres et al., 2006). Plants exposed to virulent strains of Pseudomonas syringae pv tomato, CSD1 was negatively correlated with miR398 levels. Avirulent strains induce a biphasic accumulation of ROS (oxidative burst) leading to the accumulation of ROS at the beginning of the hypersensitive response and at a second phase accompanied by local cell death (Lamb and Dixon, 1997; Wojtaszek, 1997; Torres et al., 2006). Plants exposed to virulent strains do not show drastic changes in the levels of miR398, which could be due to the absence of the oxidative burst or to the presence of just the initial accumulation of ROS. The generation of ROS is one of the common responses to metal toxicities as well as the synthesis of active antioxidative enzymes. Both responses vary among different metal exposures (Sharma and Dietz, 2009), the specific response of miR398 or other ROS-responsive miRNAs may vary according to the metal and to the time of exposure to the stress (Figure 1).

CONCLUDING REMARKS

The identification and analysis of miRNAs responsive to different metal toxicities has provided information about their possible relations in the networks involved in plant adaptation to these abiotic stresses. These studies are recent so we can predict the discovery of additional novel metal stress-responsive miRNAs.
Further research is needed to deeply understand the role of miRNAs and their targets, including miRNAs interacting with metallochaperones and functions in metal detoxification of plants.

This should take into account that plant species varying in growth habits and genotypic backgrounds may have differential responses. Further research is needed to deeply understand the role of miRNAs in the response to environmental changes. A better understanding of the role of miRNAs during metal stress will contribute to the better design of strategies aimed at improving stress tolerance of crop plants.

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