Comparative response of SOD in different plants against cadmium and drought stress at the molecular level

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Abstract: Abiotic stress like drought and heavy metal imposes a negative impact on exposed plants’ growth and development, commences over production of reactive oxygen species (ROS) inside plant cells resulting in oxidative stress at the cellular level. After that, plants activate multiple defense mechanisms, within which the superoxide dismutase (SOD) family acts as the first line of defense to eliminate ROS. From the literature, it is evident that fewer studies have been carried out in combination with molecular evolution and phylogenetics, and expression profile of the SOD genes amidst dicot and the monocot at subcellular level against drought stress and cadmium (Cd) metal exposure. In the present study, SOD isogenes are identified in purposely elected two dicot plants i.e. Arabidopsis thaliana (9 genes), Solanum lycopersicum (8 genes) and two monocot plants namely Triticum aestivum (11 genes), and Oryza sativa (7 genes), respectively. Based on the amino acids sequence similarities, the identified proteins are classified into three subfamilies in accordance to their phylogenetic relationships, namely Cu/ZnSOD, FeSOD, and MnSOD. High variability observed between Cu/ZnSOD with other two groups i.e. FeSOD and MnSOD which showed lesser variation within them by using secondary structure predication. Subcellular localization suggested that genes encoding FeSOD, MnSOD and Cu/ZnSOD are predominant in chloroplasts, mitochondria, and cytoplasm, respectively in studied plants. The expression profiling through microarray analysis showed varied strategies of SOD isogenes against drought stress and Cd exposure individually. From the perspective of evolution, this study would expand our knowledge for vividly understanding the role of distinctive SOD isogenes in detoxifying ROS in different plants under various abiotic stresses.

Keywords: plants, superoxide dismutase, ROS, abiotic stress, bioinformatic analysis

1 Introduction

Plants in their habitat commital to deal with the ever-changing environmental condition and pollutants that are often stressful for their growth and development (Feng et al., 2019). The adverse circumstances are a consequence of the individual or cumulative effect of biotic stress (e.g. pathogen infection and herbivore attack) and abiotic stress (e.g. excess of toxic metal and drought, salt, heat, cold, nutrient deficiency), wherein drought and toxic metal stress are dominant factors affecting the plant productivity and threaten food security in agriculture (Zhu, 2016). At a cellular level, such abiotic factor creates non-equality in the level of reactive oxygen species (ROS), one of the initial biochemical effects caused by abiotic stress. Superoxide dismutase (SOD, EC 1.15.1.1) is a crucial enzyme functioning as the first line of defense against ROS in plant cells by virtue of its ability to convert superoxide radicals (O$_2^-$) into hydrogen peroxide (H$_2$O$_2$) (Xie et al., 2014; Lightfoot et al., 2017), thereby maintaining cell membrane stability and minimizing O$_2^-$ attack. SOD isoenzymes are usually induced by their metal co-factors such as Cu/Zn, Fe, and Mn, which endure in all subcellular organelles (Figure 1). Previous studies marked FeSOD (MW: 3.87 × 10$^4$) localization in the chloroplast, MnSOD (MW: 4.0 × 10$^4$) in the mitochondria and peroxisomes, while Cu/ZnSOD (MW: 3.2 × 10$^4$) is predominately located in the chloroplast and the cytosol (Xia et al., 2015). The cytosolic form of Cu/ZnSOD was also detected in the...
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Figure 1. Contributions of SOD isoenzymes in subcellular organelles in different types of plants

In the present research, transcriptional changes of each isogenes from the SOD genome in *A. thaliana*, *S. lycopersicum*, *T. aestivum*, *O. sativa* was obtained from the National Centre for Biotechnology Information (NCBI)/Gene Expression Omnibus Database (GEO). Based on the above background, the objectives of present research was 1) to identify the SOD gene family and its evolutionary pattern through phylogenetic tree construct; (2) to predicate the secondary structure of selected SOD isogenes from selected monocot and dicot plants; (3) to calculate the contribution of SOD isogenes at different subcellular organelles of plants; (4) to map the expression profiles of SOD isogenes against drought stress and Cd exposure.

2 Materials and Methods

2.1 Identification of SOD isogenes from dicot and monocot selected plants

In order to compare responses of SOD against Cd or drought stress at molecular levels, the dicot (*A. thaliana* and *S. lycopersicum*) and the monocot (*T. aestivum* and *O. sativa*) were selected. The protein sequences of SOD (At1g08830, At1g12520, At2g28190, At3g10920, At3g56350, At4g25100, At5g18100, At5g23310, and At5g51100) were obtained from the Arabidopsis database TAIR (http://www.arabidopsis.org/) and used for identification of their respective proteins in *S. lycopersicum*, *T. aestivum* and *O. sativa* by using Sol Genomics Network (https://www.sgn.cornell.edu/organism/Solanum_lycopersicum/organism), GrainGenes (https://wheat.pw.usda.gov/GG3/), and RAP-DB (http://rapdb.dna.affrc.go.jp/), respectively. After eliminating redundant hits, 7 (Solyc01g067740, Solyc02g021140, Solyc03g095180, Solyc06g048410, Solyc06g049080, Solyc08g079930, and Solyc11g066390), 11 (Traescs2a02g537100, Traescs4a02g390300, Traescs4a-
Table 1. Data sources of expression profiles of SOD isogenes in different species of plants

| NO. | Title                                                                 | Accession numbers from NCBI/GEO | References                               |
|-----|-----------------------------------------------------------------------|---------------------------------|------------------------------------------|
| 1   | Transcriptomic analysis of soil-grown Arabidopsis thaliana roots and shoots in response to a drought Stress. | GSE76827                       | Sultana et al., 2016                     |
| 2   | Action of multiple intra-QTL genes concerted around a co-localized transcription factor underpins a large effect QTL. | GSE78504                       | Dixit et al., 2015                       |
| 3   | Posttranscriptional control of photosynthetic mRNA decay under stress conditions requires 3’ and 5’ untranslated regions and correlates with differential polysome association in rice. | GSE32065                       | Park et al., 2012                        |
| 4   | Integression of novel traits from a wild wheat relative improves drought adaptation in wheat. | GSE42214                       | Placido et al., 2013                     |
| 5   | Transcriptomic and proteomic analyses of a pale-green durum wheat mutant shows variations in photosystem components and metabolic deficiencies under drought stress. | GSE47090                       | Peremarti et al., 2014                   |
| 6   | Molecular insights into the involvement of a never ripe receptor in the interaction between two beneficial soil bacteria and tomato plants under well-watered and drought conditions. | GSE106317                      | Ibort et al., 2018                       |
| 7   | Transcriptomic analysis of sweet potato under dehydration stress identifies candidate genes for drought tolerance. | GSE8161                        | Lau et al., 2018                          |
| 8   | The Arabidopsis nitrate transporter NRT1.8 functions in nitrate removal from the xylem sap and mediates cadmium tolerance. | GSE22114                       | Li et al., 2016                           |
| 9   | Comparative characterization of aluminum responsive transcriptome in Arabidopsis roots: comparison with other rhizotoxic ions at different stress intensities. | GSE108751                      | Kusunoki et al., 2018                    |
| 10  | Natural variation in Arabidopsis thaliana Cd responses and the detection of quantitative trait loci affecting Cd tolerance. | GSE94314                       | Fischer et al., 2017                     |
| 11  | Transcriptomic and metabolomic shifts in rice roots in response to Cr (VI) stress. | GSE25206                       | Dubey et al., 2016                       |
| 12  | Ammonium N influences the uptakes, translocations, subcellular distributions and chemical forms of Cd and Zn to mediate the Cd/Zn interactions in dwarf polish wheat (triticum polonicum l.) seedlings. | GSE132104                      | Cheng et al., 2018                       |
| 13  | Microarray analysis and real-time PCR assay developed to find biomarkers for mercury-contaminated soil. | GSE63024                       | Cui et al., 2016                          |

2.2 Phylogenetic analysis of SOD isogenes from studied plant species

The valid SOD isogenes from different species of plants were used for phylogenetic analysis by MEGA7.0.18 program using neighborhood-joining methods with 1000 bootstrap replicates (Kumar et al., 2016).

2.3 Subcellular localization of SOD isogenes

The information about locale of SOD isogenes at the subcellular level in different selected plant species was gained from the database of UniProtKB-SubCell (https://www.uniprot.org).

2.4 Secondary structure predication

One isogene against each subfamily of SOD genome from individual studied plant species was selected for secondary structure predication. Elements of the secondary structure were conducted using the database of PredictProtein (https://www.predictprotein.org).

2.5 Expression profiles of SOD isogenes of chosen plants

Expression profiles of SOD genes related to Cd and drought stress individually in A. thaliana, S. lycopersicum, T. aestivum and O. sativa were investigated using the genome microarray data from the NCBI and GEO database. Generally, hydroponic or soil culture were carried out in these experiments, and the exposure concentrations and exposure duration of these experiments were described in the literatures (Sultana et al., 2016; Dixit et al., 2015; Park et al., 2012; Peremarti et al., 2014; Ibort et al., 2018; Lau et al., 2018; Li et al., 2016; Kusunoki et al., 2018; Fischer et al., 2017; Dubey et al., 2016; Cheng et al., 2018; Cui et al., 2016). The accession numbers identified in the GEO database included drought stress (GSE76827, GSE78504, GSE32065, GSE42214, GSE47090, GSE106317, GSE8161), Cd stress (GSE22114, GSE108751, GSE94314, GSE25206, GSE63024, GSE132104) (Table 1). The RMA (Robust Multi-array Average) algorithm was used to normalize the consequent unprocessed data and log2 transformation. Heat-map analysis was conducted by the program MeV (MultiExperiment Viewer) v.4.9.0.

3 Results and Discussion

3.1 Identification of SOD gene families and phylogenetic tree construction and analysis

The phylogenetic analysis is the reliable method to explore the insight of molecular evolution pattern of SOD through comparing the sequences of different types of SOD genes in...
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different species or the same species considering the positions and numbers of introns and exons, and the sequences of SOD amino acid residues (Xie et al., 2014). In the present study, a homology search for SOD genes sequences in A. thaliana, S. lycopersicum, T. aestivum, and O. sativa was performed to identify the SOD isogenes by using T-BLAST search. The number of SOD isogenes noticed in A. thaliana, S. lycopersicum, T. aestivum, and O. sativa were 9, 8, 11, and 7, respectively, suggesting a quantitative and distributive variation between dicot and monocot. To examine the evolutionary relationships of the proteins of SOD isogenes, a phylogenetic tree of A. thaliana, S. lycopersicum, T. aestivum, and O. sativa was constructed (Figure 2). Based on the sequence similarities data of SOD families from different plants, the identified proteins were classified into 3 sub-families and 8 groups in accordance with their phylogenetic relationships. The 3 subfamilies are termed as FeSOD, MnSOD, and Cu/ZnSOD of which FeSOD and Cu/ZnSOD comprise 3 groups while MnSOD contains only 2 groups. In the case of FeSOD sub-family, the SOD sequences of T. aestivum had similarities with O. sativa (group 1), and the A. thaliana SOD sequences showed analogy to that of S. lycopersicum (group 2). Interestingly, similar results were observed in MnSOD sub-family (group 4 & 5), suggesting an evolutionary resemblance of FeSOD and MnSOD between dicot and monocot. In addition, we ascertained no significant evolutionary difference in Cu/ZnSOD sub-family between dicot and monocot (group 6-8).

Around 2.4 billion years ago, the transition happens in the environment from reducing to oxidizing state owing to the occurrence of oxygenic photosynthesis (Blankenship, 2010). Thus, the SOD can promote a living organism’s survival in the oxidizing condition on the earth. Back then, there are two types of SOD in prokaryotes i.e. Fe/MnSOD and Cu/ZnSOD. With the evolution of SOD genes, Fe/MnSOD gradually evolved into FeSOD and MnSOD, and the amino acid sequence and protein 3-D structure of the FeSOD and MnSOD present similarity (Smith and Doolittle, 1992). However, Cu/ZnSOD was developed separately during evolution, and it is very different from FeSOD and MnSOD in the crystal structure and catalytic mechanism (Smith and Doolittle, 1992; Tyagi et al., 2019). The higher availability of Fe and Mn ion in the reducing environment and the presence of FeSODs and MnSODs in aerobic/anaerobic bacteria suggested them as the most ancient form of SOD. Since these cambialistic SOD are present in primitive anaerobic organisms, they have evolved into the FeSODs and MnSODs found in different higher plants including dicots and monocots (Miller, 2012). Previous studies indicated that there are mainly three types of Cu/ZnSOD in plants including cytoplasmic Cu/ZnSOD, chloroplast Cu/ZnSOD, extracellular Cu/ZnSOD. Cytoplasmic Cu/Zn-SOD may be the original form of Cu/Zn-SOD, and its origin may be traced under the presence of oxygen in the atmosphere; chloroplast Cu/ZnSOD may originate from eukaryotes; extracellular Cu/ZnSOD originates from the arthropod phylum evolved independently by adding a signal peptide to the cytoplasmic Cu/Zn-SOD (Schmidt et al., 2009). Overall, the clustering of three types of SOD in dif-

Figure 2. Phylogenetic tree construction and analysis of SOD isogenes in different types of plants
different phylogenetic tree and occurrence in different types of plants provides an insight into the divergence in three forms during evolution. Due to the difference in evolution, the responses of SOD at genetic levels to the changing external environment are also different.

3.2 2-D structure analysis of SOD in different plants

Cu/ZnSOD is a homodimer that is shown in blue-green, with the relative molecular mass of 15-17 kD in each subunit. These subunits are linked through the hydrophobic interaction of non-covalent bonds, and each subunit contains one Cu$^{2+}$ and one Zn$^{2+}$, respectively (Perry et al., 2010). In the primary structure, each subunit of Cu/ZnSOD contains 150-160 amino acid residues, wherein 4 histidine residues are coordinated with Cu$^{2+}$ and 3 histidine and 1 aspartic acid residues coordinated with Zn$^{2+}$ is highly homologous among different species (Xia et al., 2015). Presently, five isogenes i.e. Os07g0665200, Os04g0573200, At1g28190, Traescs7d02g290700, and Solyco6g049070 were selected for mapping the protein secondary structure based on the result of phylogenetic tree analysis (Figure 3a). The secondary structure of Cu/ZnSOD anticipated from studied four plant species comprises β sheets in all of them. While, only T. aestivum, O. sativa, and A. thaliana contain the α helix. In addition, we found that α helix in T. aestivum (Traescs7d02g290700) and O. sativa (Os04g0573200) are localized in the same region. The number of β sheets in T. aestivum (Traescs7d02g290700) and O. sativa (Os07g0665200) is equivalent, while one of the isoform of Cu/ZnSOD in O. sativa (Os04g0573200) contains more α helices and β sheets than other (Os07g0665200). These results implied that the structural variability of Cu/ZnSOD in monocot is less than that of a dicot.

MnSOD in prokaryotic cells is composed of 2 subunits, and each containing one Mn$^{2+}$; while in a eukaryotic cell, especially mitochondrial MnSOD composed of 4 subunits, and similar to prokaryotes all subunit anchored with one Mn$^{2+}$. FeSOD also consists of 2 subunits, and each subunit containing 1 iron (Fe) element (Perry et al., 2010). Mn-SOD and Fe-SOD are highly similar in amino acid sequence and spatial structure, indicating that MnSOD and FeSOD are evolutionarily homologous. In the primary structure, each subunit consists of 200-220 amino acid residues, with a relative molecular mass of 18-20 kD, and the metal ligand of MnSOD and FeSOD consist of 3 histidines and 1 aspartic acid. The current research targeted five isogenes i.e. Os06g0115400, At5g23310, At4g25100, Traescs7d02g086400, and Solyco6g048410 of FeSOD for analyzing the protein secondary structure (Figure 3b). Interestingly, the secondary structure of FeSOD from considered plant species prominently contains α helix in all alike Cu/ZnSOD structures. In monocots, O. sativa has more α helix and β sheets than T. aestivum, and there is no α helix in O. sativa in the first 100 amino acid residues; in dicots, A. thaliana and S. lycopersicum have similar numbers and regions of β sheets, while the number of α helices in A. thaliana is significantly higher than that of S. lycopersicum. As for MnSOD, six isogenes i.e. Os05g0323900, At3g10920, At3g56350, Traescs2d02g538300, Traescs2a02g537100, and Solyco6g049080 were selected for mapping the protein secondary structure (Figure 3c). Generally, the secondary structure of MnSOD of these four species are relatively similar, all of which contain α helices. However, the number of α helices in monocots is more than 10, in reverse to this the number of α helices in dicots is less than 10. In monocots, more β sheets are found in O. sativa rather than in T. aestivum; in dicots, S. lycopersicum and A. thaliana contain 9 α helices. These results indicated that although the MnSOD and FeSOD are highly homologous in many cases, they still have certain specificity. For example, MnSOD and FeSOD have some specific amino acid residues in the primary structure. The former is Gly77, Gly78, Phe85, Gln145 and Asp146, and the latter are Ala77, Gln78, Tyr85, Ala145 and Gly146 (Parker and Blake, 1988; Xia et al., 2015).

Under the views of modern system theory, structure determines function, structural variations of SOD isoforms might be an important mechanism to generate functional differences, which could also ascertain to some extent the endurance of a high number of SOD isoforms in different subcellular fractions and tissues in response to different stress conditions (Pelloux et al., 2007). As expected, high variability observed between Cu/ZnSOD with other two group i.e. FeSOD and MnSOD which showed lesser variation within them (Figure 3), suggesting the different mechanisms involving in ROS metabolism through SOD isoenzymes.

3.3 Contributions of SOD isogenes in subcellular organelles

A previous study indicated that the responses of these three types of SODs isogenes to various stress conditions are quite different such as drought, heavy metal, salt, light, and temperature. In order to prevent the toxicity induced by oxygen, the plants deployed the SODs in the form of isoenzymes in various subcellular fractions to confirm that ROS can be effectively scavenging under various stress conditions (Tsang et al., 1991). In the present study, we found that the number of SODs isogenes varies greatly in subcellular organelles from different types of plants (Figure 1), suggesting that the strategies of subcellular organelles in coping with ROS burst differs from plant to plant. In A. thaliana, the number of FeSOD isogenes in the chloroplast is the largest (25%), followed by the Cu/ZnSOD isogenes in the cytoplasm (16.67%), chloroplast (16.67%), mitochondrion (16.67%), nucleus (8.33%) and peroxisome (8.33%), and the number of MnSOD and FeSOD isogenes in mitochondrion and plasma membrane account for 16.67% and 8.33%, respectively. In case of S. lycopersicum, the number of FeSOD isogenes in the chloroplast is the largest (42.86%), followed by Cu/Zn SOD isogenes in chloroplast (14.29%), cytoplasm (14.29%), and mitochondrion (14.29%), and the MnSOD isogenes in mitochondrion (14.29%). As for T. aestivum,
Figure 3. 2-D structure analysis of SOD in different plants. (a) Cu/ZnSOD, (b) FeSOD, (c) MnSOD
the number of FeSOD isogenes in chloroplast shows the largest (54.55%), followed by MnSOD in the mitochondrion (27.27%) and Cu/ZnSOD in extracellular region/extracellular space (18.18%). As for *O. sativa*, the number of Cu/ZnSOD isogenes in cytoplasm is the largest (44.44%), followed by the Cu/ZnSOD isogenes in chloroplast (22.22%) and nucleus (11.11%), and the FeSOD and MnSOD isogenes in chloroplast nucleus and mitochondrion account for same i.e. 11.11%. These results also indicated a special variation of SOD isogenes expression within four types of plants. Indeed, the increase of ROS content in the cytoplasm could induce the expression of Cu/Zn genes. Similarly, the increase of ROS content in chloroplast and mitochondria could trigger the expression of FeSOD and MnSOD genes, implying that the specific expression of SOD genes may be related to the subcellular localization of the encoded SODs (Bowler et al., 1992).

### 3.4 Expression profiles and subcellular distribution of SODs genes in different plants

Faced with a scarcity of water resources, drought stress is becoming the most critical threat to global food safety. Moreover, the upsurge in anthropogenic activities (e.g. mining and industrialization) has cropped up the issue of heavy metal contamination in environmental matrices which affect the comfort of plants. Recently, with the development of the high-throughput sequencing technique and bioinformatics, much progress has been made in observations of specific gene responses in different plants, and this technique is also helpful to unravel the adaptive mechanisms of plants to the external stimuli. Herein, we have a great interest in comparing the expression profiles of SOD isogenes at subcellular fractions in dicot and monocot under drought stress and Cd exposure.

#### 3.4.1 Drought stress

As shown in Figure 4a (*A. thaliana*), up-regulated SOD isogenes in shoots were mainly *AtCSD1* (*At1g08830*), *AtCSD3* (*At5g18100*), *AtMSD1* (*At3g10920*), and *AtMn/FeSOD* (*At3g56350*) in response to drought stress, which were located in cytosol/nucleus, peroxisome, and mitochondrion. Similarly, up-regulated SOD isogenes in roots were mainly *AtCSD1* (*At1g08830*), *AtFS3D* (*At5g23310*), *AtFSD1* (*At4g25100*), and *AtMn/FeSOD* (*At3g56350*) under drought stress, which were localized in cytosol/nucleus, plastid (chloroplast), and plasma membrane. Apparently, the fold changes of up-regulated SOD isogenes in roots were higher than that of shoots, but the down-regulation of SOD isogenes were more prominent in shoots to that of roots, suggesting that roots SOD isogenes play an important role in resisting the drought stress in *A. thaliana*.

As shown in Figure 4b (*S. lycopersicum*), *SIFS3D* (*Solyc02g021140*) and *SISOCC1* (*Solyc01g067740*) had greater expression in shoots in response to drought stress, which were located in chloroplast and cytoplasm, while *SICS* (*Solyc08g079830*) and *SIFSOD* (*Solyc06g048410*) had greater expression in roots under drought stress, which were situated in mitochondrion and plastid (chloroplast). Evidently, the fold changes of up- and down-regulated SOD isogenes in shoots were higher than those of in roots.

As shown in Figure 4c, we found that the SOD isogenes were generally up-regulated in both roots and shoots of *O. sativa* in response to drought stress. Specifically, *OsSOD1* (*Os03g0351500*), *OsSOD2* (*Os03g0219200*), *OsSOD4* (*Os08g0561700*), *OsCu/ZnSOD* (*Os04g0573200*), *OsCSD1* (*Os07g0665200*), and *OsMSD* (*Os05g023900*) had greater expression in shoots, which can be observed in all subcellular organelles, as well as *OsSOD2*, *OsSOD4*, *OsCu/ZnSOD*, *OsCSD1*, *OsMSD*, and *OsFSD1.1* (*Os06g0115400*) over expressed in roots, which can be cited clearly in all subcellular organelles. These results indicated that almost all the SOD isogenes play active roles in responding to drought stress.

Results from expression profiles of SOD isogenes indicated that *TaSOD3.1* (*TraesCS2D02G538300*) and *TaMnSOD* (*TraesCS2A02G537100*) were up-regulated genes in leaves of *T. aestivum* in response to drought stress, which were distributed in the mitochondrion (Figure 4d), *TaSOD1.1* (*TraesCS2D02G123300*), *TaSOD1.2* (*TraesCS7D02G290700*), *TaSOD3.1*, *TaMnSOD* and *TaFeSOD* (*TraesCS7D02G086400*) were up-regulated genes in roots of *T. aestivum* under drought stress, which were distributed in extracellular, mitochondrion, and plastid (chloroplast). These results indicated that roots SOD isogenes play an important role in resisting the drought stress in *T. aestivum*.

Noticeable the response of SOD isogenes in different plant tissues under drought condition is quite different. The up-regulated *AtCSD1*, *AtCSD3*, and *AtMn/FeSOD* are the common genes found in both roots and shoots of *A. thaliana* under drought stress. Similarly, the up-regulated *OsSOD2*, *OsSOD4*, *OsCu/ZnSOD*, *OsCSD1*, and *OsMSD* are the prevailing genes found in both roots and shoots of *O. sativa* against drought stress. These up-regulated genes may be the master regulator genes, which play a crucial role in ROS scavenging in response to drought stress (Fan et al., 2020). However, no common up- and down-regulated genes were observed in both roots and leaves of *T. aestivum* and *S. lycopersicum*, indicating that the expression of SOD isogenes among different types of plants under drought stress is tissue-specific. Based on the above findings, it can be asserted that the strategies of SOD isogenes in *T. aestivum* and *S. lycopersicum* in coping with drought stress may be different from *A. thaliana* and *O. sativa*.

#### 3.4.2 Cd stress

We also found that the responses of SOD isogenes in different types of plants under Cd stress varied greatly from those of drought stress. As shown in Figure 4a, almost all of SOD isogenes in roots of *A. thaliana* were generally down-regulated, except for *AtMnSOD* and *AtFeSOD* in mitochondrion, implying that the influence of Cd stress on the expression of SOD isogenes was greater than that of drought stress. Figure 4b depicts that almost all of SOD isogenes in roots of *S. lycoper-
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### Arabidopsis thaliana

|         | Shoots |         |         |         |
|---------|--------|---------|---------|---------|
| A1g08830| 1.00   | 0.95    | 0.96    | 0.91    | 1.20    |
| A1g12520| 1.00   | 0.90    | 0.91    | 0.83    | 1.05    |
| A1g18100| 1.00   | 1.12    | 0.86    | 0.87    | 1.02    |
| A1g21900| 1.00   | 0.98    | 0.83    | 0.78    | 0.61    |
| A1g25100| 1.00   | 1.00    | 0.90    | 0.91    | 1.33    |
| A1g51100| 1.00   | 0.95    | 0.64    | 0.91    | 0.89    |
| A1g51100| 1.00   | 1.00    | 1.55    | 1.66    | 1.67    |
| A1g56350| 1.00   | 0.93    | 1.02    | 0.29    | 1.26    |

**Drought stress**

**Cd stress**

### Solanum lycopersicum

|         | Shoots |         |         |         |         |         |
|---------|--------|---------|---------|---------|---------|---------|
| Solyc06g048410 | 1.00  | 1.17    | 0.86    | 0.85    | 0.69    | 0.55    |
| Solyc03g095180 | 1.00  | 1.20    | 0.89    | 0.88    | 0.99    | 0.66    |
| Solyc06g049080 | 1.00  | 0.94    | 0.81    | 0.79    | 0.71    | 0.61    |
| Solyc02g021140 | 1.00  | 1.27    | 1.42    | 1.18    | 1.08    | 1.27    |
| Solyc01g067740 | 1.00  | 1.08    | 1.45    | 1.99    | 1.76    | 2.10    |

**Drought stress**

**Cd stress**

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Figure 4. Expression profiles of SOD isogenes in different types of plant under drought stress and Cd exposure
sium were generally up-regulated, except for SIFeSOD in the plastid (chloroplast), implying that the influence of drought on the expression of SOD isogenes was greater than that of Cd stress. In O. sativa, as observed from Figure 4c, the SOD isogenes were generally down-regulated in roots, except for OsCu/ZnSOD in plastid (chloroplast)/nucleus/cytoplasm. Similarly, we also found that the SOD isogenes were generally down-regulated in roots of T. aestivum, except for TaSOD3.2 and TaMnSOD in mitochondrion (Figure 4d). These results also implied that the effect of Cd stress on the expression of SOD isogenes was greater than that of drought stress.

Overall, the mechanisms of ROS production triggered by drought stress are different from those of heavy metal stress. The drought stress mainly targets various physiological processes e.g. reduction in growth, photosynthetic rate, CO₂ fixation owing to stomata closure to avoid transpiration (Gill et al., 2010), and leads to the burst of ROS in plant cells. In chloroplast, oxygen (O₂) is forced to act as electron acceptors to form O₂⁻ on the reducing side of the PSI of the inner capsule membrane. The generated O₂⁻ can enter the extracellular matrix of the thylakoid membrane to generate H₂O₂ through enzymatic or non-enzymatic action, and it can also be used by PSII light-harvesting chlorophyll complex protein converts O₂⁻ from PSI into H₂O₂ (Pospisil, 2012).

In mitochondria, the activity of enzymes related to respiration is inhibited or the coupling of plant mitochondrial electron transport chain (ETC) and ATP is disrupted, resulting in the leakage of some electrons in the electron transfer of the respiratory chain, thereby generating ROS (Steffens, 2014). Compared with drought stress, accumulation of Cd in plants mainly cause morphological and physiological disorders and impact growth, photosynthesis, metabolic pathway, and enzymatic activity (Nazir et al., 2020). Cadmium ions were unable to directly generate ROS through Fenton and/or Haber Weiss reactions in biological systems under physiological conditions. However, the production of ROS after Cd exposure has been reported in multiple studies (Pathak and Khandelwal, 2006; Zhou et al., 2009). Cd indirectly produces cellular ROS by increasing the free Fe-concentration, possibly via replacement in various proteins (Dorta et al., 2003). Free redox-active metals directly enhance the production of ·OH (hydroxyl) radicals through the Fenton reaction. The reduction of the oxidized metal ion can be achieved by the Haber-Weiss reaction with O₂⁻ as a substrate. Therefore, the expressions of SOD isogenes in plant tissues under drought stress are quite different from those of Cd stress.

The relationship between the resistance of plants to environmental stress and the activities of SOD has been widely investigated in recent years. Various stress tolerant crop plants have been developed through the modification of SOD isogenes by using transgenic methods (Lee et al., 2020). For example, the drought resistance in O. sativa significantly increased when the MnSOD gene was transferred from Pisum sativum to O. sativa (Wang et al., 2005). Similarly, the tolerance against osmotic stress was attained when the MnSOD gene was transferred from Natrinema altunense to O. sativa. The enhanced expression of MnSOD gene, the activity of SOD, and photosynthesis in transformed O. sativa indicated the efficient ROS scavenging in plant cells (Chen et al., 2013). Multiple studies indicate the roles of SOD in providing tolerance to the plants for defending against various environmental stresses. However, in some cases, the extra genetic SOD failed to provide tolerance to the transgenic plants (Tyagi et al., 2019). It might be attributed to the differences in the response of SOD isoenzymes, their subcellular locations and the complexity of ROS scavenging system in different types of plants. Therefore, its urgent to depict the differential expression of SOD isogenes in most of the plants underlying abiotic stress from the perspective of molecular evolution.

4 Conclusions

In this study, the phylogenetic relationships, 2-D structure, subcellular localization, and expression profiles of SOD isogenes were investigated to reveal the roles of SOD in the interactions between plants and abiotic stresses. There is an evolutionary resemblance of FeSOD and MnSOD between dicot and monocot, while Cu/ZnSOD has no significant evolutionary difference between dicot and monocot, implying that Cu/ZnSOD is developed separately during evolution, and it is very different from FeSOD and MnSOD in the perspective of 2-D structure and function between dicot and monocot. Subcellular localization suggested that the number of SOD isogenes varies greatly in subcellular organelles between dicot and monocot. The differences in phylogenetic relationships, 2-D structure, and subcellular localization of SOD isogenes eventually result in the differential expression of SOD isogenes under drought stress and Cd exposure between dicot and monocot. Overall, understanding the stress signalling responses is helpful to increase the ability that improves the stress resistance in crop plants.

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Declaration of Competing Interest

The authors declare that there is no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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