RESEARCH ARTICLE

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

Li Yang[1†], **Yu-Xi Feng**[1†] **and Xiao-Zhang Yu**[1*]
[1] College of Environmental Science & Engineering, Guilin University of Technology, Guilin 541004, China
[†] These authors contribute equally to this work

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

*Correspondence to: Xiao-Zhang Yu, College of Environmental Science & Engineering, Guilin University of Technology, Guilin 541004, China; E-mail: xzyu@glut.edu.cn

Received: August 29, 2020; **Accepted:** October 16, 2020; **Published Online:** October 27, 2020
Citation: Yang, L., Feng, Y.-X. and Yu, X.-Z., 2020. Comparative response of SOD in different plants against cadmium and drought stress at the molecular level. *Applied Environmental Biotechnology*, 5(2): 14-27. http://doi.org/10.26789/AEB.2020.01.003

1 Introduction

Plants in their habitat committal 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 responses in plants cells under stress conditions, persist by accretion of reactive radicals in cells (Wu et al., 2010; Ghosh et al., 2016; Fan et al., 2020). However, abundant ROS concentration could instigate oxidative damage at the molecular and biochemical levels, thus resulting in DNA breakage, lipid peroxidation, protein modification and enzyme inactivation (Choudhury and Panda, 2005). Mostly, ROS at a relatively low level can act as a signaling molecule to mediate signal transduction between different tissues for improving stress tolerance in plants (Smirnoff and Arnaud, 2019; Fan et al., 2020). An in-depth understanding of stress signalling responses will be helpful to improve stress resistance in crop plants to achieve agricultural sustainability and food security for a global population (Zhu, 2016).

Predominantly, plants initialize a complex and systematic defense mechanism to safeguard themselves from detrimental 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 ($O_2^{\cdot-}$) into hydrogen peroxide (H_2O_2) (Xie et al., 2014; Lightfoot et al., 2017), thereby maintaining cell membrane stability and minimizing $O_2^{\cdot-}$ 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

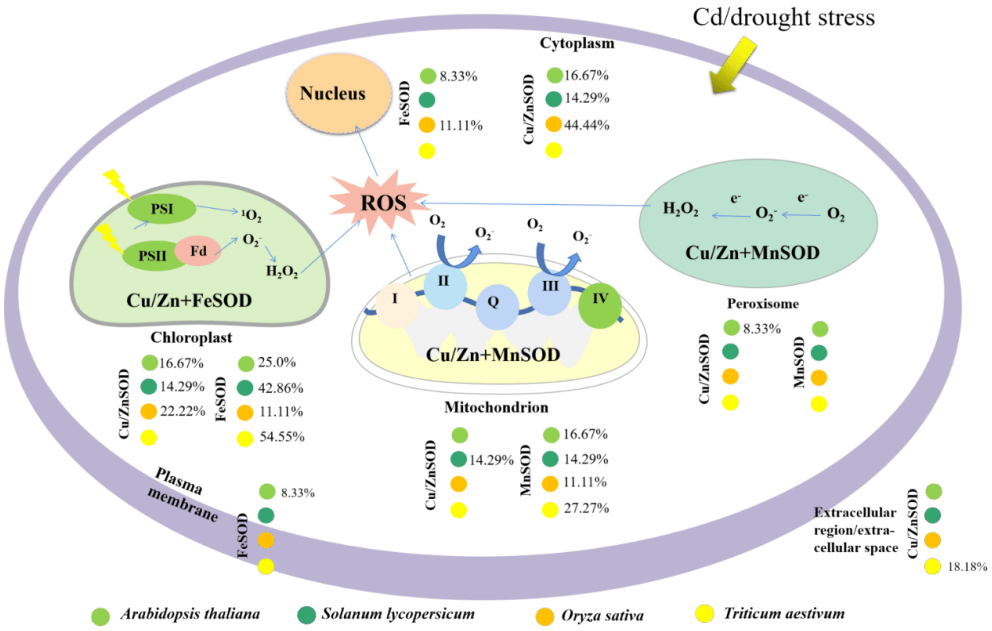


Figure 1. Contributions of SOD isogenes in subcellular organelles in different types of plants

located in the chloroplast and the cytosol (Xia et al., 2015). The cytosolic form of Cu/ZnSOD was also detected in the nucleus (Lightfoot et al., 2017; Xia et al., 2015). Comparative analysis using amino acid sequences of aforementioned three SOD isoenzymes indicated that MnSOD and FeSOD are often very old types of SOD, and most probably both isoenzymes have evolved from the same ancestral enzyme, whilst Cu/ZnSOD has no sequence similarity with MnSOD and FeSOD representing its diverged evolution pattern within eukaryotes (Grene et al., 2002).

Numerous studies have reported the roles of SOD isogenes in plant tolerance opposed to abiotic stresses. For example, over-expression of Cu/ZnSOD derived from *Avicennia marina* in *Indica* rice enhanced salt stress tolerance (Prashanth et al., 2008). Similarly, over-expression of MnSOD gene improved drought tolerance in transgenic *Arabidopsis* (Liu et al., 2013). Under Cd stress, the transcript levels of Cu/Zn-SOD were decreased in both roots and leaves of *Arabidopsis thaliana*; additionally, the transcript levels of FeSOD and MnSOD were increased in roots, while no significant differences were detected in leaves (Smeets et al., 2008). Overall, genetic expression associated with SOD in different plants responding to abiotic stresses varied greatly. Until now, few studies have been carried out to analyze the evolutionary pattern and expression profile of the SOD genome from the dicot (*Arabidopsis thaliana* and *Solanum lycopersicum*) and the monocot (*Triticum aestivum* and *Oryza sativa*) at subcellular organelles level against drought stress and Cd exposure. It is noticed that analyzing gene expression of various enzymes in *A. thaliana* has become a model experiment for researching stress-resistance mechanism (Locke et al., 2005). *S. lycopersicum* is one of the most popular fleshy fruit, and it is also an important vegetable in the globe (Dorais et al., 2008). *O. sativa* is one of the most important staple food crops worldwide, especially in eastern Asian countries (Gadal et al., 2019). *T. aestivum* is the most widely planted crop in the world, providing a fifth of mankind's calories (Petrenko et al., 2018).

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/genome), 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,

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	Introgression 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

Solyc06g049080, Solyc08g079830, and Solyc11g066390), 11 (Traescs2a02g537100, Traescs4a02g390300, Traescs4a-02g434000, Traescs7a02g048600, Traescs7a02g090400, Traescs2b02g567600, Traescs2d02g123300, Traescs2d02g-538300, Traescs7d02g043000, Traescs7d02g086400 and Traescs7d02g290700), and 9 (Os03g0351500, Os03g02192-00, Os08g0561700, Os04g0573200, Os07g0665200, Os05g-0323900 and Os06g0115400) recognized as SOD isogenes obtained from *S. lycopersicum*, *T. aestivum* and *O. sativa*.

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

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/Zn-SOD, 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-

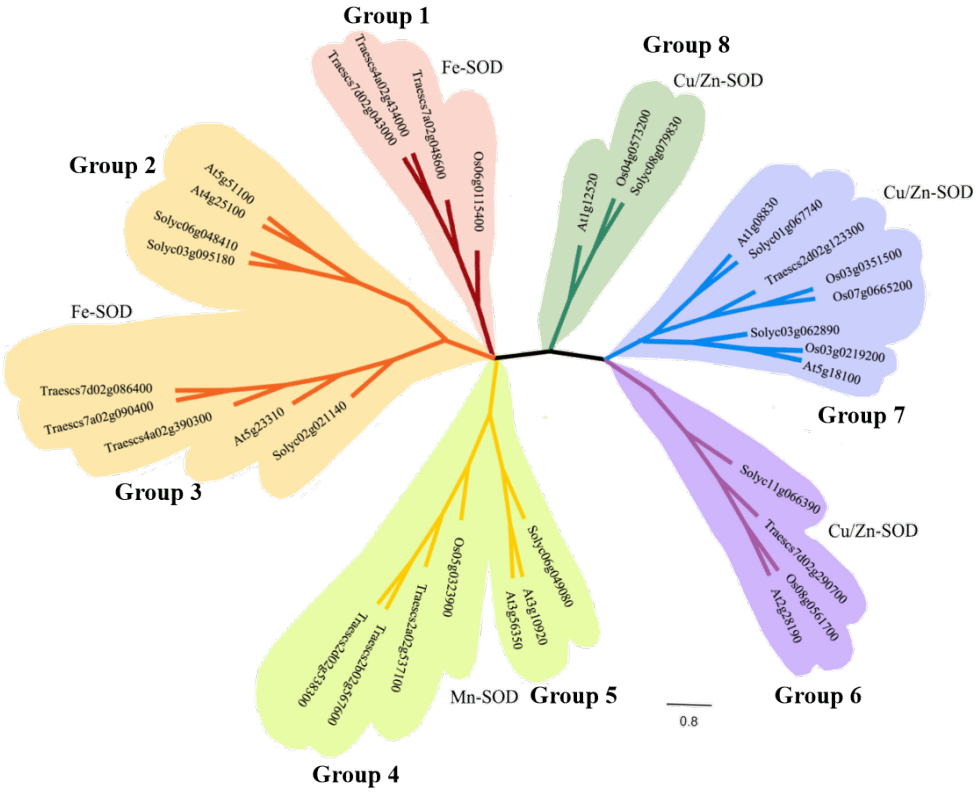


Figure 2. Phylogenetic tree construction and analysis of SOD isogenes in different types of plants

ferent 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

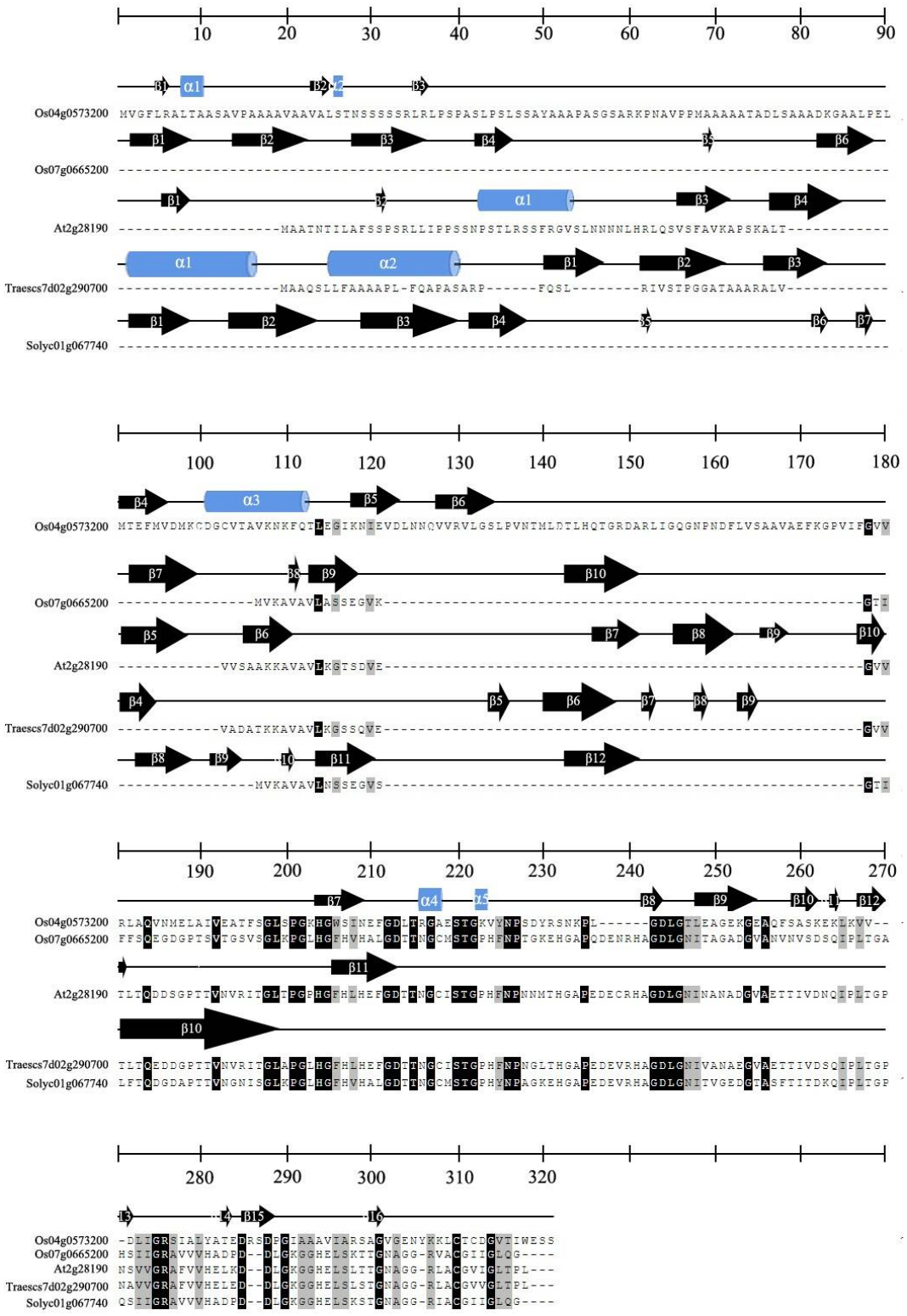
Cu/ZnSOD is a homodimer that is shown in blue-green, with the relative molecular mass of 15-17 kD in each subunit. These subunits are linked through the hydrophobic interaction of non-covalent bonds, and each subunit contains one Cu^{2+} and one Zn^{2+}, respectively (Perry et al., 2010). In the primary 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, At2g28190, Traescs7d02g290700, and Solyc01g067740 of Cu/Zn SOD were selected for predicting 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 Solyc06g048410 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 Solyc06g049080 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).

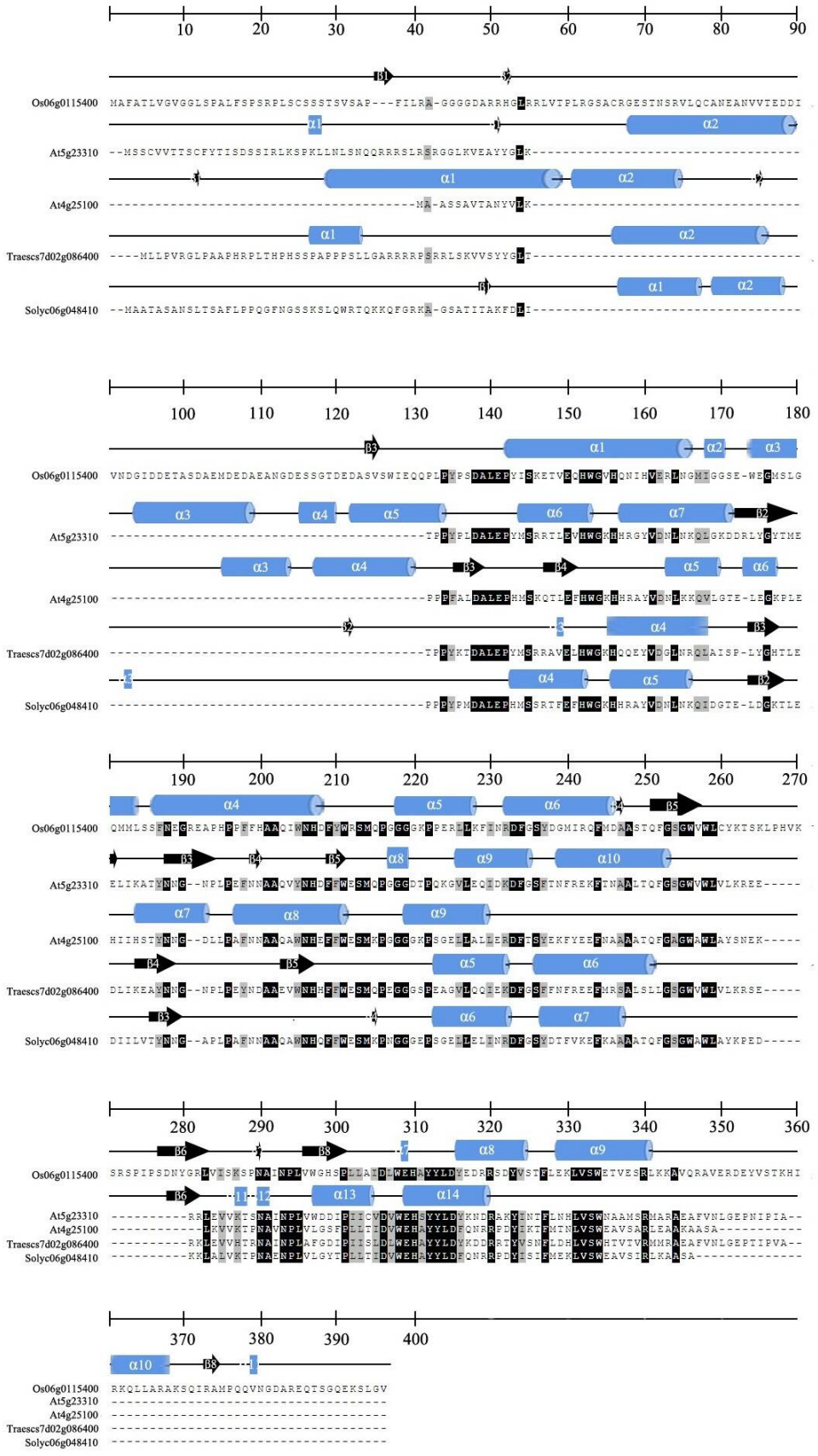
Under the views of modern system theory, structure determines function, structural variations of SOD isoforms might be an important mechanism to generate functional differences, which could also ascertain to some extent the endurance of a high number of SOD isoforms in different subcellular fractions 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

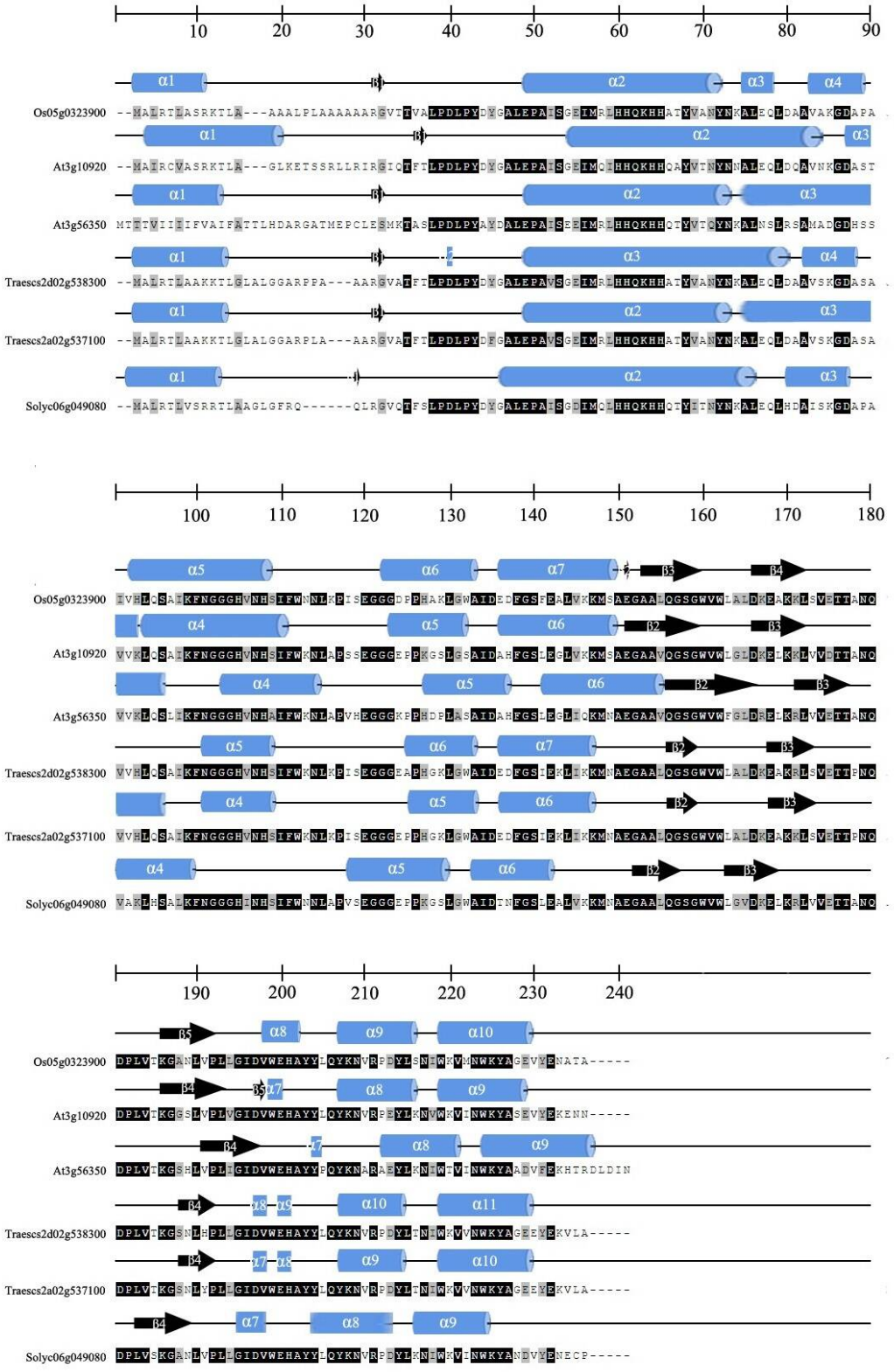
A previous study indicated that the responses of these three types of SODs isogenes to various stress conditions are quite different such as drought, heavy metal, salt, light, and temperature. In order to prevent the toxicity induced by oxygen, the plants deployed the SODs in the form of isoenzymes in 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 also presents 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*,


Os04g0573200
Os07g0665200
At2g28190
Traescs7d02g290700
Solyc01g067740

(a)


Os06g0115400
At5g23310
At4g25100
Traescs7d02g086400
Solyc06g048410

(b)



(c)

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/Zn-SOD 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 nucleoid 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 matrixes 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), *AtFSD3* (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*), *SlFSD3* (Solyc02g021140) and *SlSODCC1* (Solyc01g067740) had greater expression in shoots in response to drought stress, which were located in chloroplast and cytoplasm, while *SlCCS* (Solyc08g079830) and *SlFeSOD* (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.

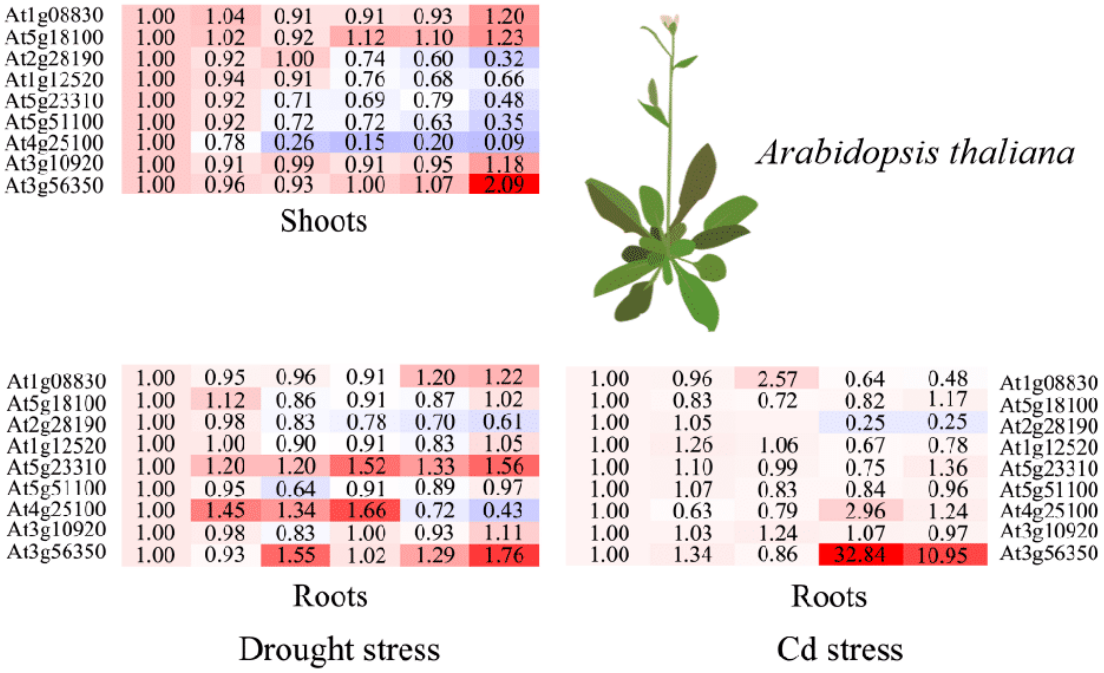
As shown in Figure 4c, we found that the SOD isogenes were generally up-regulated in both roots and shoots of *O. sativa* in response to drought stress. Specifically, *OsSOD1* (Os03g0351500), *OsSOD2* (Os03g0219200), *OsSOD4* (Os08g0561700), *OsCu/ZnSOD* (Os04g0573200), *OsCDS1* (Os07g0665200), and *OsMSD* (Os05g0323900) had greater expression in shoots, which can be observed in all subcellular organelles, as well as *OsSOD2*, *OsSOD4*, *OsCu/ZnSOD*, *OsCDS1*, *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*.

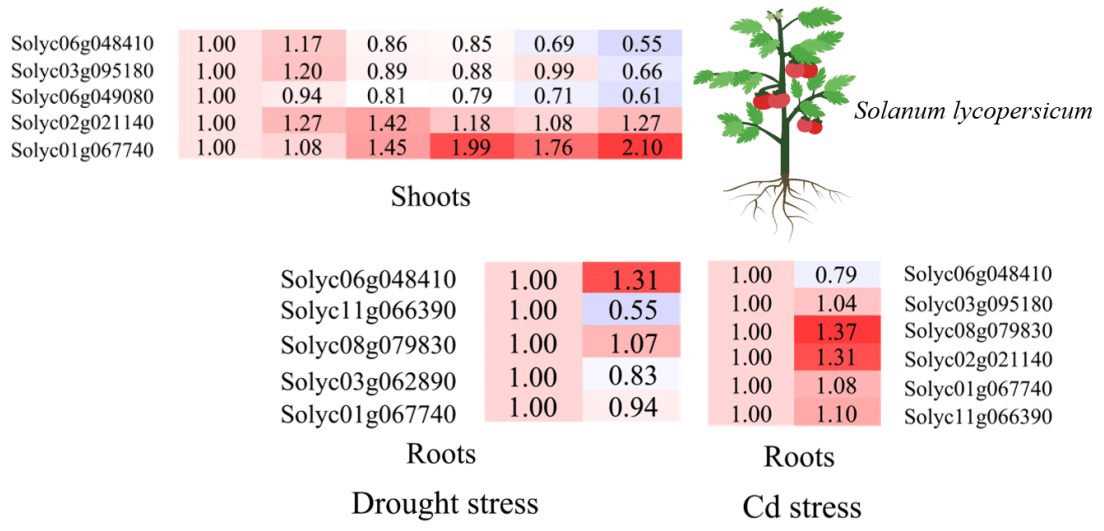
Noticeable the response of SOD isogenes in different plant tissues under drought condition is quite different. The up-regulated *AtCSD1*, *AtCSD3*, and *AtMn/FeSOD* are the common genes found in both roots and shoots of *A. thaliana* under drought stress. Similarly, the up-regulated *OsSOD2*, *OsSOD4*, *OsCu/ZnSOD*, *OsCDS1*, *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

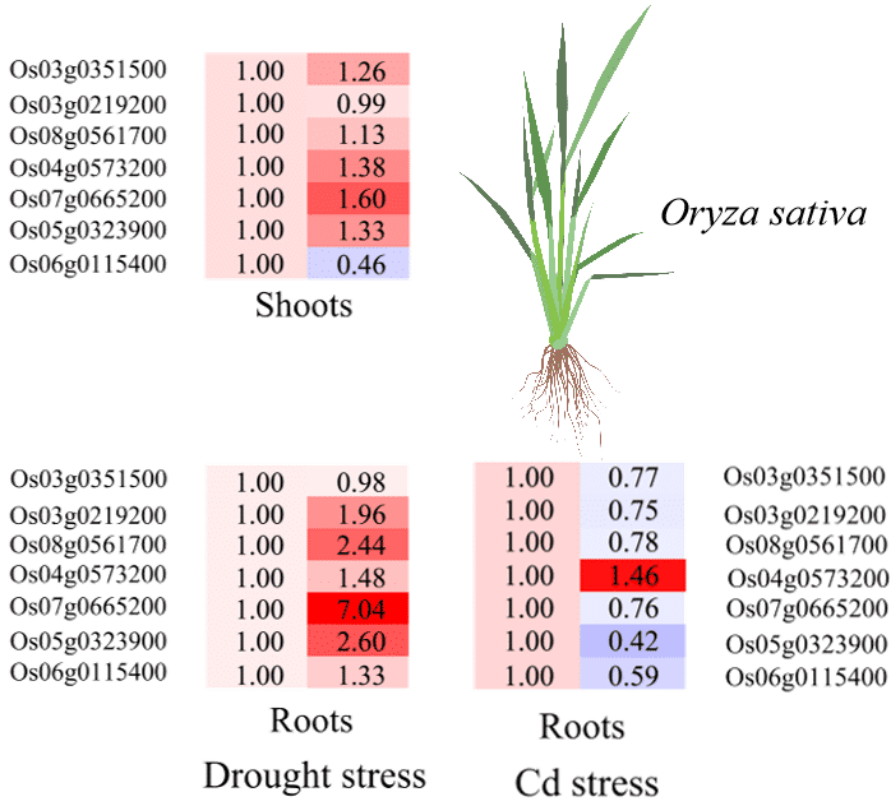
We also found that the responses of SOD isogenes in different types of plants under Cd stress varied greatly from those of drought stress. As shown in Figure 4a, almost all of SOD isogenes in roots of *A. thaliana* were generally down-regulated, except for *AtMnSOD* and *AtFeSOD* in mitochondrion, implying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


At1g08830 1.00 1.04 0.91 0.91 0.93 1.20
At5g18100 1.00 1.02 0.92 1.12 1.10 1.23
At2g28190 1.00 0.92 1.00 0.74 0.60 0.32
At1g12520 1.00 0.94 0.91 0.76 0.68 0.66
At5g23310 1.00 0.92 0.71 0.69 0.79 0.48
At5g51100 1.00 0.92 0.72 0.72 0.63 0.35
At4g25100 1.00 0.78 0.26 0.15 0.20 0.09
At3g10920 1.00 0.91 0.99 0.91 0.95 1.18
At3g56350 1.00 0.96 0.93 1.00 1.07 2.09
Shoots
Arabidopsis thaliana
At1g08830 1.00 0.95 0.96 0.91 1.20 1.22
At5g18100 1.00 1.12 0.86 0.91 0.87 1.02
At2g28190 1.00 0.98 0.83 0.78 0.70 0.61
At1g12520 1.00 1.00 0.90 0.91 0.83 1.05
At5g23310 1.00 1.20 1.20 1.52 1.33 1.56
At5g51100 1.00 0.95 0.64 0.91 0.89 0.97
At4g25100 1.00 1.45 1.34 1.66 0.72 0.43
At3g10920 1.00 0.98 0.83 1.00 0.93 1.11
At3g56350 1.00 0.93 1.55 1.02 1.29 1.76
Roots
Drought stress
1.00 0.96 2.57 0.64 0.48 At1g08830
1.00 0.83 0.72 0.82 1.17 At5g18100
1.00 1.05 0.25 0.25 At2g28190
1.00 1.26 1.06 0.67 0.78 At1g12520
1.00 1.10 0.99 0.75 1.36 At5g23310
1.00 1.07 0.83 0.84 0.96 At5g51100
1.00 0.63 0.79 2.96 1.24 At4g25100
1.00 1.03 1.24 1.07 0.97 At3g10920
1.00 1.34 0.86 32.84 10.95 At3g56350
Roots
Cd stress

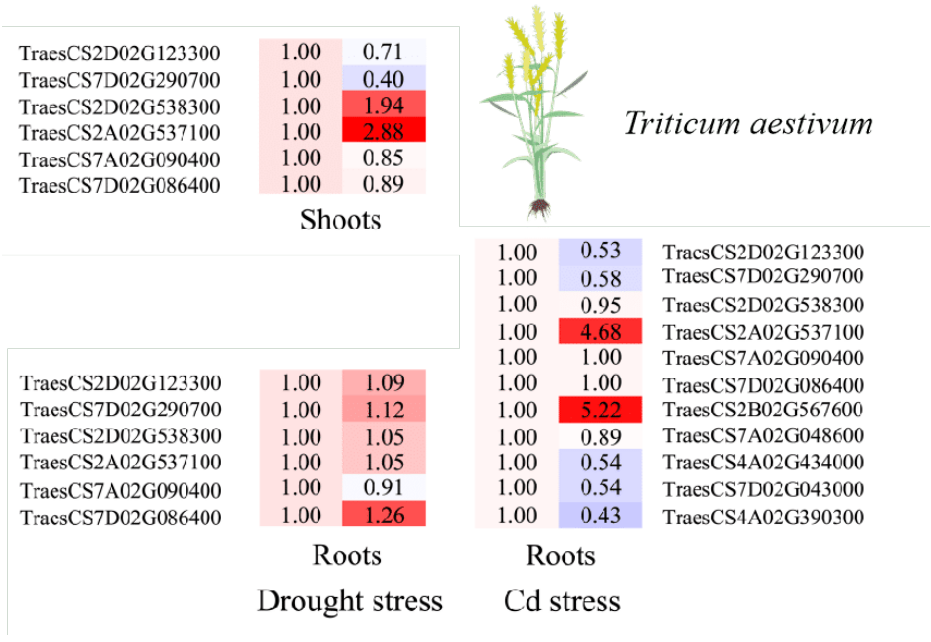
(a)


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
Shoots
Solanum lycopersicum
Solyc06g048410 1.00 1.31
Solyc11g066390 1.00 0.55
Solyc08g079830 1.00 1.07
Solyc03g062890 1.00 0.83
Solyc01g067740 1.00 0.94
Roots
Drought stress
1.00 0.79 Solyc06g048410
1.00 1.04 Solyc03g095180
1.00 1.37 Solyc08g079830
1.00 1.31 Solyc02g021140
1.00 1.08 Solyc01g067740
1.00 1.10 Solyc11g066390
Roots
Cd stress

(b)



(c)



(d)

Figure 4. Expression profiles of SOD isogenes in different types of plant under drought stress and Cd exposure

isogenes was greater than that of drought stress. Figure 4b depicts that almost all of SOD isogenes in roots of *S. lycopersicum* were generally up-regulated, except for *SlFeSOD* 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, CO_2 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 (O_2) is forced to act as electron acceptors to form $O_2^{\cdot-}$ on the reducing side of the PSI of the inner capsule membrane. The generated $O_2^{\cdot-}$ can enter the extracellular matrix of the thylakoid membrane to generate H_2O_2 through enzymatic or non-enzymatic action, and it can also be used by PSII light-harvesting chlorophyll complex protein converts $O_2^{\cdot-}$ from PSI into H_2O_2 (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 $O_2^{\cdot-}$ 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 Mn-SOD 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.

Acknowledgements

This work is financially supported by the Natural Science Foundation of Guangxi (No. 2018GXNSFDA281024). Thanks to Mr. Sheng Huang in preparation of Figure 4.

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.

References

Blankenship, R.E., 2010. Early evolution of photosynthesis. Plant Physiol, 154:43443. https://doi.org/10.1104/pp.110.161687

Bowler, C., Montagu, M.V. and Inze, D., 1992. Superoxide dismutase and stress tolerance. Annu Rev Plant Physiol Plant Mol Biol, 43:83116. https://doi.org/10.1146/annurev.pp.43.060192.000503

Chen, Z., Pan, Y.H., An, L.Y., Yang, W.J., Xu, L.G., Zhu, C., 2013. Heterologous expression of a halophilic archaeon manganese superoxide dismutase enhances salt tolerance in transgenic rice. Russ J Plant Physiol, 60:359366. https://doi.org/10.1134/S1021443713030059

Cheng, Y., Wang, C.C., Song, Y. et al., 2018. 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. Chemosphere Environmental Toxicology & Risk Assessment.

Choudhury, S. and Panda, S.K., 2005. Toxic effects, oxidative stress and ultrastructural changes in moss Taxithelium nepalense (Schwaegr.) Broth. under chromium and lead phytotoxicity. Water Air Soil Pollut, 167:7390. https://doi.org/10.1007/s11270-005-8682-9

Cui, B.S., Bai, J.H., Hou, J. et al., 2016. Microarray analysis and real-time pcr assay developed to find biomarkers for mercury-contaminated soil. Toxicology Research, 5(6), 1539-1547. https://doi.org/10.1039/c6tx00210b

Dixit, S., Kumar Biswal, A., Min, A., Henry, A., Oane, R.H., Raorane, M.L. et al., 2015. Action of multiple intra-qtl genes concerted around a co-localized transcription factor underpins a large effect qtl. Scientific Reports, 5, 15183. https://doi.org/10.1038/srep15183

Dorais, M., Ehret, D.L. and Papadopoulos, A.P., 2008. Tomato (*Solanum lycopersicum*) health components: from the seed to the consumer. Phytochem Rev, 7:231250. https://doi.org/10.1007/s11101-007-9085-x

Dorta, D.J., Leite, S. and DeMarco, K.C., 2003. A proposed sequence of events for cadmium-induced mitochondrial impairment. J Inorg Biochem, 97: 251257. https://doi.org/10.1016/S0162-0134(03)00314-3

Dubey, S., Prashant, M., Sanjay, D., Sandipan C. et al., 2016. Transcriptomic and metabolomic shifts in rice roots in response to Cr (VI) stress https://doi.org/10.1186/1471-2164-11-648

Fan, W.J., Feng, Y.X., Li, Y.H., Lin, Y.J., Yu, X.Z., 2020. Unraveling genes promoting ROS metabolism in subcellular organelles of *Oryza sativa* in response to trivalent and hexavalent chromium. Sci Total Environ, 744:140951. https://doi.org/10.1016/j.scitotenv.2020.140951

Feng, Y.X., Yu, X.Z., Mo. C.H., Lu, C.J., 2019. Regulation network of sucrose metabolism in response to trivalent and hexavalent chromium in *Oryza sativa*. J Agri Food Chem, 67:97389748. https://doi.org/10.1021/acs.jafc.9b01720

Fischer, S., Spielau, T. and Clemens, S., 2017. Natural variation in *Arabidopsis thaliana* cd responses and the detection of quantitative trait loci affecting cd tolerance. entific Reports, 7(1), 3693. https://doi.org/10.1038/s41598-017-03540-z

Gadal, N., Shrestha, J., Poudel, M.N., Pokharel, B., 2019. A review on production status and growing environments of rice in Nepal and in the world. Arch Agri Environ Sci, 4:8387. https://doi.org/10.26832/24566632.2019.0401013

Ghosh, B., Ali, M.N. and Saikat, G., 2016. Response of rice under salinity stress: a review update. J Res Rice, 4:167 https://doi.org/10.4172/2375-4338.1000167

Gill, S.S. and Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem, 48:909930. https://doi.org/10.1016/j.plaphy.2010.08.016

Grene, A.R., Neval, E. and Heath, L.S., 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J Exp Bot, 372:13311341. https://doi.org/10.1093/jexbot/53.372.1331

Ibort, Pablo, Molina, Sonia, Manuel, Ruiz-Lozano et al., 2018. 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. Molecular Plant Microbe Interactions.

Kumar, S., Stecher, G. and Tamura K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33: 18701874. https://doi.org/10.1093/molbev/msw054

Kusunoki, K., Kobayashi, Y., Kobayashi, Y. amd Koyama, H., 2018. Comparative characterization of aluminum responsive transcriptome in *Arabidopsis* roots: comparison with other rhizotoxic ions at different stress intensities. Soil Science & Plant Nutrition, 1-13. https://doi.org/10.1080/00380768.2018.1454253

Lau, K.H., del Rosario Herrera, María, Crisovan, E., Wu, S., Fei, Z., Khan, M.A. et al., 2018. Transcriptomic analysis of sweet potato under dehydration stress identifies candidate genes for drought tolerance. Plant Direct, 2(10). https://doi.org/10.1002/pld3.92

Lee, S.Y., Cheon, K.S., Kim, S.Y., Kim, J.H., Kim, W.H., 2020. Expression of sod2 enhances tolerance to drought stress in roses. Horticulture, Environment and Biotechnology. https://doi.org/10.1007/s13580-020-00239-5

Li, J.Y., Pike, S.M., Bao, J., Tian, W., Zhang, Y. et al., 2010. The *Arabidopsis* nitrate transporter nrt1.8 functions in nitrate removal from the xylem sap and mediates cadmium tolerance. THE PLANT CELL ONLINE. https://doi.org/10.1105/tpc.110.075242

Lightfoot, D.J., Mcgrann, G.R.D. and Able, A.J., 2017. The role of a cytosolic superoxide dismutase in barley-pathogen interactions. Mol Plant Pathol, 18:323335. https://doi.org/10.1111/mpp.12399

Liu, X.F., Sun, W.M., Li, Z.Q., Bai, R.X., Li, J.X., Shi, Z.H., Geng, H.W., Zhang, Y., Zhang, G.F., 2013. Over-expression of ScMnSOD, a SOD gene derived from Jojoba, improve drought tolerance in *Arabidopsis*. J Integra Agri, 12:17221730. CNKI:SUN:ZGNX.0.2013-10-004

Locke, J.C., Millar, A.J. and Turner, M.S., 2005. Modelling genetic networks with noisy and varied experimental data: the circadian clock in *Arabidopsis thaliana*. Journal of theoretical biology, 234: 383393. https://doi.org/10.1016/j.jtbi.2004.11.038

Miller, A.F., 2012. Superoxide dismutases: Ancient enzymes and new insights. FEBS Lett, 586:585595 https://doi.org/10.1016/j.febslet.2011.10.048

Nazir, F., Fariduddin, Q. and Khan, T.A., 2020. Hydrogen peroxide as a signalling molecule in plants and its crosstalk with other plant growth regulators under heavy metal stress. Chemosphere, 252:126486. https://doi.org/10.1016/j.chemosphere.2020.126486

Park, S.H., Chung, P.J., Juntawong, P., Bailey-Serres, J., Kim, Y.S., Jung, H. et al., 2012. Posttranscriptional control of photosynthetic mrna decay under stress conditions requires 3änd 5üntranslated regions and correlates with differential polysome association in rice. Plant Physiology, 159(3), 1111-1124. https://doi.org/10.2307/41549927

Parker, M.W. and Blake, C.C., 1988. Iron-and manganese-containing superoxide dismutases can be distinguished by analysis of their primary structures. FEBS Lett, 229:377388. https://doi.org/10.1016/0014-5793(88)81160-8

Pathak, N. and Khandelwal, S., 2006. Oxidative stress and apoptotic changes in murine splenocytes exposed to cadmium. Toxicology, 220:2636

Pelloux, J., Rusterucci, C. and Mellerowicz, E.J., 2007. New insights into pectin methylesterase structure and function. Trends Plant Sci, 12:267277. https://doi.org/10.1016/j.tplants.2007.04.001

Perry, J.J., Shin, D.S., Getzoff, E.D., Tainer, J.A., 2010. The structural biochemistry of the superoxide dismutases. Biochim Biophys Acta, 1804:245262. https://doi.org/10.1016/j.bbapap.2009.11.004

Peremarti, A., Marè, Caterina, Aprile, A., Roncaglia, E., Cattivelli, L., Villegas, D. et al., 2014. Transcriptomic and proteomic analyses of a pale-green durum wheat mutant shows variations in photosystem components and metabolic deficiencies under drought stress. Bmc Genomics, 15(1), 125-125. https://doi.org/10.1186/1471-2164-15-125

Petrenko, V., Spychaj, R., Prysiazhniuk, O., Sheiko, T., Khudolii, L., 2018. Evaluation of three wheat species (*Triticum aestivum* L, T. spelta L, T. dicoccum (Schrank) Schuebl) commonly used in organic cropping systems, considering selected parameters of technological quality. Romanian Agri Res, 35:255264.

Placido, D.F., Campbell, M.T., Folsom, J.J., Cui, X., Kruger, G.R. and Walia, B.H., 2013. Introgression of novel traits from a wild wheat relative improves drought adaptation in wheat. Plant Physiology, 161(4), 1806-1819. https://doi.org/10.1104/pp.113.214262

Pospisil, P., 2012. Molecular mechanisms of production and scavenging of reactive oxygen species by photosystem II. BioChim Biophys Acta (BBA)-Bioenergetics, 1817:218231. https://doi.org/10.1016/j.bbabio.2011.05.017

Prashanth, S.R., Sadhasivam, V. and Parida, A., 2008. Over expression of cytosolic copper/zinc superoxide dismutase from a mangrove plant *Avicennia marina* in *Indica* rice var Pusa Basmati-1 confers abiotic stress tolerance. Transgenic Research, 17:281291. https://doi.org/10.1007/s11248-007-9099-6

Schmidt, A., Gube, M., Schmidt, A., Kothe, E., 2009. In silico analysis of nickel containing superoxide dismutase evolution and regulation. J. Basic Microbiol, 49:109118. https://doi.org/10.1002/jobm.200800293

Smeets, K., Ruytinx, J., Semane, B., Van Belleghem, F., Remans, T., Van Sanden, S., Cuypers, A., 2008. Cadmium-induced transcriptional and enzymatic alterations related to oxidative stress. Environ Exp Bot, 63:18.

Smirnoff, N. and Arnaud, D., 2019. Hydrogen peroxide metabolism and functions in plants. New Phytol, 221:11971214. https://doi.org/10.1111/nph.15488

Smith, M. and Doolittle, R., 1992. A comparison of evolutionary rates of the two major kinds of superoxide dismutase. J. Mol. Evol, 34:175184. https://doi.org/10.1007/BF00182394

Steffens, B., 2014. The role of ethylene and ROS in salinity, heavy metal, and flooding responses in rice. Front Plant Sci, 5:685. https://doi.org/10.3389/fpls.2014.00685

Sultana, R., Khurram, B., Akihiro, M., Maho, T and Motoaki, S., 2016. Transcriptomic analysis of soil-grown *Arabidopsis thaliana* roots and shoots in response to a drought stress. Frontiers in Plant ence, 7, 180-. https://doi.org/10.3389/fpls.2016.00180

Tsang, E.W., Bowler, C., Hrouart, D., Van Camp, W., Villarroel, R., Genetello, C., Inz, D., 1991. Differential regulation of superoxide dismutases in plants exposed to environmental stress. Plant Cell, 3:783792. https://doi.org/10.1105/tpc.3.8.783

Tyagi, S., Singh, S.P. and Upadhyay, S.K., 2019. Role of Superoxide Dismutases (SODs) in Stress Tolerance in Plants. In Molecular Approaches in Plant Biology and Environmental Challenges. Springer, Singapore. ISBN:978-981-15-0689-5

Wang, F.Z., Wang, Q.B., Kwon, S.Y., Kwak, S.S., Su, W.A., 2005. Enhanced drought tolerance of transgenic rice plants expressing a pea manganese superoxide dismutase. J Plant Physiol, 162:465472. https://doi.org/10.1016/j.jplph.2004.09.009

Wu, G.L., Cui, J., Tao, L., Yang, H., 2010. Fluroxypyr triggers oxidative damage by producing superoxide and hydrogen peroxide in rice (*Oryza sativa*). Ecotoxicology, 19:124-132. https://doi.org/10.1007/s10646-009-0396-0

Xia, X.M., Wang, W., Yuan, R., Deng, F.N., Shen, F.F., 2015. Superoxide dismutase and its research in plant stress-tolerance. Mol Plant Breeding, 13:26332646.

Xie, Z., Sun, X., Wang, Y., Luo, Y., Xie, X., Su, C., 2014. Response of growth and superoxide dismutase to enhanced arsenic in two Bacillus species. Ecotoxicology, 23:19221929. https://doi.org/10.1007/s10646-014-1318-3

Zhou, Y.J., Zhang, S.P. and Liu, C.W., 2009. The protection of selenium on ROS-mediated apoptosis by mitochondria dysfunction in cadmium-induced LLC-PK1 cells. Toxicol Vitro, 23:288294. https://doi.org/10.1016/j.tiv.2008.12.009

Zhu, J.K., 2016. Abiotic stress signaling and responses in plants. Cell, 167:313324. https://doi.org/10.1016/j.cell.2016.08.029