J Plant Mol Breed (2020) 8 (2): 50 - 60
DOI: 10.22058/JPMB.2022.548053.1251

RESEARCH ARTICLE

# Expression analysis of *SiSOD* gene family during *Sesamum indicum* L. seed germination under various abiotic stresses

Seyyed Hamidreza Hashemipetroudi*, Esmaeil Bakhshandeh

Genetics and Agricultural Biotechnology Institute of Tabarestan (GABIT), Sari Agricultural Sciences and Natural Resources University (SANRU), Sari, Iran.

**ABSTRACT:** Sesame (*Sesamum indicum* L.) seed is a rich source of oil and protein, which could be used for cooking or primary source for some industrial applications. Seed germination is the most fundamental stage of a plant's life cycle, which is significantly influenced by various abiotic stresses. As a first report, the study attempted to evaluate the effect of environmental factors (i.e., low, optimum and high temperatures (*T*), water potential ($\psi$) and salinity) on eight superoxide dismutase (*SOD*) gene expressions (two Mn-SOD, two Cu/Zn-SOD and four Fe-SOD) during sesame germination. Results showed that all studied treatments remarkably influenced germination characteristics of sesame ($P \leq 0.05$). In general, the negative impact of each stress on sesame germination could be ranked as $\psi$ > salt stress > high *T* > low *T*, indicating that the germination was more influenced by $\psi$ than salt stress and *T*. There was a strong association between the decrease in germination parameters (relative to the optimal *T*) and the decrease in *SiSOD* expression under various stresses. Our results discovered that the *SiSODs* expression patterns were stress-specific. However, when subjected to the same stress, the majority of *SiSOD* genes displayed similar expression patterns. The findings of this study could lead to a better understanding of *SODs* role in other plants and the mechanisms involved in plants' stress responses, especially during their early stages of development.

**KEYWORDS**: Salinity and drought stresses; Seed germination; Sesame; Genome-wide identification

## INTRODUCTION

Sesame (*Sesamum indicum* L.) seed is a rich source of oil and protein [19]. With a high level of quality, its oil could be used for cooking and/or as a primary source of some industrial applications [32]. The optimum regions for producing this plant are tropical and subtropical areas in the world and some parts of southern Iran [34]. Sesame is considered as a drought-tolerant species (i.e., down to −1.23 MPa) and a moderate-tolerant species to salt stress (i.e., up to 210 mM NaCl) during the germination stage [6].

Seed germination (SG) is the most fundamental stage of plant's early life cycle. It is significantly influenced by various abiotic stresses, such as temperature (*T*), water potential ($\psi$), salinity [4]. Temperature is known as an important factor influencing SG characteristics in plants [8]. Both the germination percentage (GP) and the germination rate (GR) usually increase linearly with *T* (from the base *T* ($T_b$) to optimum *T* ($T_o$)) and then decrease linearly and/or curvedly at $T$s > $T_o$ until $T_c$ where will completely be stopped [1, 2, 6, 23]. Water deficit is also the most critical factor influencing SG characteristics. Indeed, germination characteristics significantly decreased at reduced $\psi$ [5]. Salinity has been considered another important factor that can limit SG

*Corresponding author (✉):irahamidreza@yahoo.com
Received: 2 February 2022/ Revised: 24 March 2022
Accepted: 9 April 2022

and/or cause seed death in some cases, such as accumulations of salt ions (i.e., $Na^+$ or $Cl^-$) within the seed, resulting in a reduction of $\psi$ that can influence enzymes, organelles and metabolic cell activities at the germination stage [20, 26]. All noted factors affect the germination alone and or in combination form, so the seeds population responds differently to the environment variations.

The overproduction and accumulation of reactive oxygen species (ROS) are common attributes of different cells under various abiotic stresses (i.e., $T$, $\psi$ and salinity). Proteins, DNA, lipids, and carbohydrates can suffer oxidative damage when reactive oxygen species (ROSs) are present [3, 28]. However, plants had robust enzymatic and non-enzymatic antioxidant defense systems, which can operate as a concert for limiting the cascades of uncontrolled oxidation utilizing scavenging of ROS [10]. Superoxide dismutase (SOD), catalase, and ascorbate peroxidase are three common scavengers that can effectively help plants for enzymatic scavenging of ROS in their cells. Understanding enzymes activity during SG is therefore of important ecological relevance.

Superoxide dismutase is the first antioxidant defense system against ROS effects. Indeed, it can eliminate superoxide ($O_2^-$) by its catalyzing into hydrogen peroxide ($H_2O_2$) and oxygen ($O_2$) [9, 10]. SODs inhibit $O_2^{\bullet}$ accumulation, hence reducing the risk of $OH^{\bullet}$ formation via the Haber-Weiss process. The 10,000-fold speed advantage of the Haber-Weiss reaction over the spontaneous dismutation process has long been proven. This means that the antioxidant enzyme SOD is vital to aerobic organisms, such as plants [21, 35].

Emerging next-generation sequencing (NGS) technology revolutionized biology by providing reference genome sequences that enable genome-wide investigations into gene function and expression and genomic organization [13]. As a result, distinct isoforms of *SODs* were identified, cloned, and described in various plant species. For instance, a genome-wide investigation of the *SOD* coding sequence in the rice and arabidopsis genomes found eight *OsSODs* and nine *AtSODs* genes, respectively. [35]. Abiotic stimuli as salinity, cold, and drought stress, as well as different developmental stages, were found to alter the expression profile of *AtSODs.* (i.e., seed, vegetative and panicle stages). However, three groups of SOD isoenzymes with different metal cofactors, including iron (Fe-SOD), manganese (Mn-SOD) and copper/zinc (Cu/Zn-SOD), have been reported with different subcellular localization [35].

Based on our knowledge, no study investigated the effect of $T$ (e.g., low, optimum and high), $\psi$ and salinity stress on antioxidant enzymes activity (i.e., *SOD*) during sesame SG. Therefore, the aims of this study were: (i) to study the effect of $T$, $\psi$ and salinity on SG characteristics of sesame and (ii) to investigate *SOD* gene family expression patterns in the above-mentioned abiotic stresses.

## MATERIALS AND METHODS

### Treatments

Sesame seeds (cv. 'Yellowhite') were produced in Sari city, Iran, and kept at 5°C before use. The viability of the seed was > 95% (assessed by the ISTA protocol) at the start of the experiment. Treatments were three constant $T$s (25 °C as a low $T$, 36 °C as an optimum $T$ and 42 °C as a high $T$), one level of $\psi$ (–1.13 MPa, prepared using the polyethylene glycol 8000 which was considered as drought tolerance threshold value for this cultivar) [6], and one level of salinity (150 mM, prepared using the NaCl which was considered as salinity tolerance threshold value for this cultivar) (unpublished data). Distilled water was used for the $T$ experiments (i.e., low, optimum and high $T$s). The $\psi$ was made by [18] method. At water and salt stress conditions, sesame seeds were also germinated at 36°C as $T_o$ [6].

### Seed germination test

Five replicates of 100 seeds were cultivated on two sheets of germination filter paper within ten cm Petri dishes containing seven ml of distilled water and or test solutions for each treatment. The dishes related to each treatment were put into a thin plastic bag for avoiding water evaporation from the dishes during the experiments, then they were randomly placed within an incubator (± 0.5°C precision). Sesame seeds were counted several times a day, depending on the studied treatments. The seed with radicle length > 0.2 cm was counted as germinated seed and then removed from the dished after each recording time. The experiments were stopped in each Petri dish when no new germinated seed was seen in three consecutive days. The maximum GP, $GR_{50}$ (=$1/t_{50}$) and $t_{50}$ (the time taken to reach the $50^{th}$ percentile of

maximum GP) were determined by Germin program proposed by Soltani and Maddah [29].

### Identification of SODs sesame genome

The query word "*S indicum* superoxide dismutase" was searched in Entrez Gene, a gene-specific database, at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene). The results containing *SOD* genes were selected, and their CDS, protein and genomic sequences were retrieved from the sesame representative genome (RefSeq assembly accession: GCF_000512975.1 (S_indicum_v1.0)) for *in silico* analysis. The results of protein sequences were verified on the SMART program (http://smart.embl-heidelberg.de/smart/set_mode.cgi?NORMAL=1) for the presence of the *SOD* domain. The arabidopsis SODs gene family expression profiling at the SG stage was explored and displayed using the Arabidopsis eFP browser tool [16]. Primer-BLAST method was used for designing specific primers to *SiSOD* gene family. To avoid genomic DNA amplification, primers were designed to span exon junctions so that the reverse primer spanned an exon junction, and PCR product size was restricted between 50 and 190 nucleotides. In order to specificity checking, primer pair parameters were set as follows: database; RefSeq mRNA; organism; *S. indicum* [11]. Primer pairs with similar Tm (varying from 59 to 63°C) were designed with 18-21 lengths and GC content ranging from 40 to 55 percent. The length of the amplicons varied between 83 and 186 bp. Supplementary Table 1 lists the primer sequences as well as their GenBank accession codes. The target gene expressions were normalized by the geometric mean of four reference genes, including *SiUBQ*, *SiGAPDH*, *SiTUB* and *SiACT* obtained from our previous study [27].

### qPCR analysis

Total RNA was isolated from germinated seed (at least 0.2 cm radicle long) treated by various environmental factors (i.e., NaCl, PEG, low T, optimum T and high T) using the Threezol (Riragene, Iran) and NucleoSpin RNA Plant kit (Macherey-Nagel, Germany) with some modification. Briefly, 100 mg of the homogenized powder (in liquid nitrogen, –196 °C) was transferred to the Eppendorf tube containing 1 ml of the Threezol reagent and incubated at 60 °C for 5 min. After adding 200 µl of chloroform, kept the lysate at room temperature for 5 min and then centrifuged at 10000 rpm for 10 min. The aqueous supernatant was transferred to new 1.5 ml microcentrifuge tube and was added 350 µl of RAP buffer (plus 3.5 µl ß-mercaptoethanol). Next, the mixed supernatant was filtrated by NucleoSpin filter by centrifuge at 10000 rpm for 1 min. According to the manufacturer's instructions, the supernatant was subsequently processed using NucleoSpin RNA plant column kit.

BioSpectrometers (Eppendorf, Germany) were used to verify the quality and amount of RNA samples by measuring absorbance at OD 260/280. A 1.2 percent agarose gel was used to evaluate the integrity of the RNA. The cDNA was produced using the RevertAid First Strand cDNA Synthesis Kit according to the manufacturer's instructions (Thermo Scientific, USA). The 1:5 diluted cDNA reactions were then kept at –20°C. In a CFX96 real-time PCR equipment, the Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific, USA) was utilized for RT-qPCR analysis (Bio-Rad, USA). All PCR reactions were subjected to dissociation curve analysis with continuous fluorescence monitoring from 55 to 95 °C after amplification. To assess primer specificity, melt curve analysis and end-point PCR on a 3 percent agarose gel were used. Each primer master mix included at least one non-template control (NTC). Three biological replications were used in each experiment. The relative gene expression ratio was also calculated using the $2^{-\Delta\Delta CT}$ method [25].

### Statistical analysis

All statistical analyses were conducted using the Statistical Analysis System ver. 9.4 [14], and the figures were drawn by the Excel ver. 2013 software. The means were also compared using the least significant difference (LSD) test at the 0.05 probability level.

## RESULTS

### Seed germination characteristics affected by different abiotic stresses

All studied stresses (i.e., low $T$, high $T$, $\psi$ and salinity) significantly influenced GP, GR and $T_{50}$ of sesame ($p < 0.05$) (Fig. 1). Maximum GP decreased significantly under the stress conditions compared to the control (optimum T, To), except at low T (i.e., 25°C), which was similar to the value obtained at $T_o$. Indeed, the decrease in GP was 34, 35 and 70% at high $T$, salt and water stresses

compared to the control, respectively (Fig. 1a). This is in agreement with the results reported by others in chicory (*Cichorium intybus* L.) [33], watermelon (*Citrullus vulgaris*) [4], *Retama raetam* [1] and camelina (*Camelina sativa* L.) [12]. The highest $GR_{50}$ observed at $T_o$ (0.1107 $h^{-1}$) which was 52, 53, 77 and 89% higher than those obtained at low $T$ (0.0532 $h^{-1}$), high $T$ (0.0520 $h^{-1}$), salt (0.0248 $h^{-1}$) and water (0.0122 $h^{-1}$) stress, respectively (Fig. 1b). Likewise, the $t_{50}$ value significantly increased by 2.0, 2.1, 4.8 and 9.1-folds at low $T$, high $T$, salt and water stresses, respectively, compared with the control condition ($T_o$). The amount of $t_{50}$, therefore, was estimated to be 9.04 h at $T_o$ and 18.8, 19.2, 43.5 and 82.0 h for the low $T$, high $T$, salt and water stresses, respectively (Fig. 1c). In general, the negative impact of each stress on SG characteristics of sesame was ranking $\psi$ > high $T$ = salt stress > low $T$ for maximum GP, $\psi$ > salt stress > high $T$ = low $T$ for both $GR_{50}$ and $t_{50}$. These findings agree with previous studies that showed SG in various species was more influenced by $\psi$ than $T$ and salt stress [5]. In other studies, Tilaki *et al.* [31] in alfalfa and Luan *et al.* [17] in sunflower reported that SG of these species was more influenced by $\psi$ than salt stress in the same values of stress level. This may be due to the uptake of salt ions (e.g., $Na^+$ and $Cl^-$) by the seed, which could lead to maintaining $\psi$ gradient allowing higher water uptake during SG and then better SG in higher levels of salt stress. Also, the decrease in sesame seed germination at high $T$ was due to the thermoinhibition phenomenon, which could be introduced as an adaptive strategy under this condition [24].

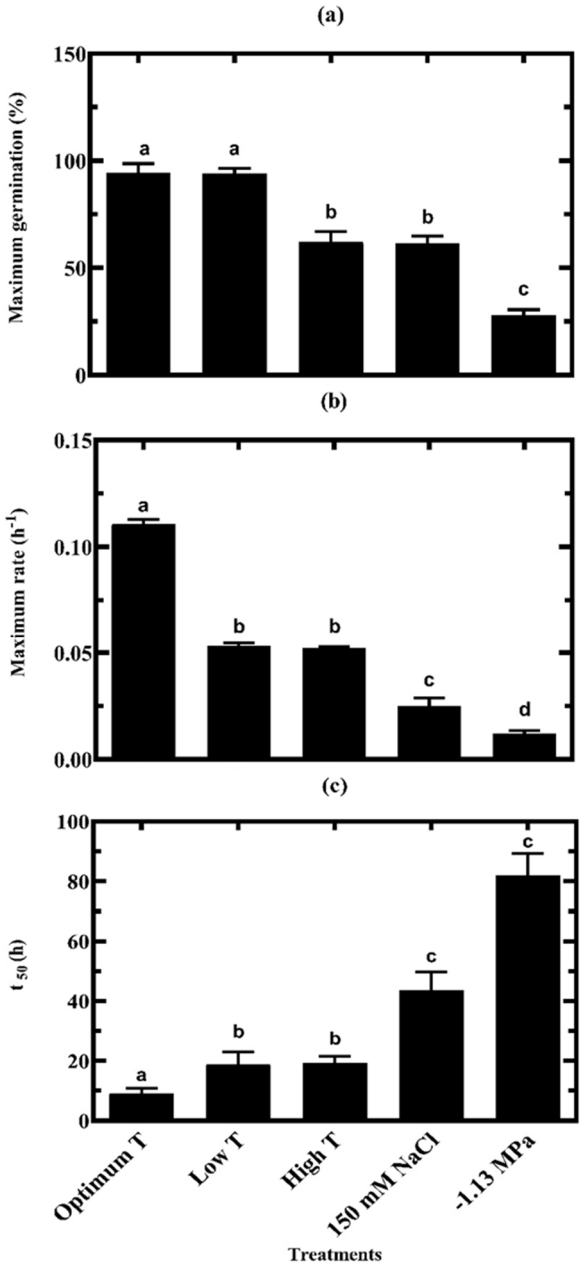


**Figure 1.** Maximum germination percentages (a), germination rate for the 50th percentile (b) and $t_{50}$ (c) of sesame affected by various abiotic stresses.

## Gene expression analysis

The most well-documented plant species for plant genomics, functional genomics, and transcriptomics is *A. thaliana*, and studies of *A. thaliana* gene function serve as the foundation for forming hypotheses and conducting experiments involving other plant species [16]. To understand the function and regulation of the *SODs* gene family members due to their important role against ROS effects as the first antioxidant defense system, the high-resolution profile of the *A. thaliana* transcriptome data (based on RNA sequencing) at the germination stage were obtained from Arabidopsis eFP Browser (Fig. 2). As shown in Fig. 2, the highest expression value was observed for two genes of *AtCSD1* and *AtCSD2* (copper/zinc superoxide dismutase). Most *AtSOD*s expressions were spatially (tissue-specific) and temporarily (three time-points) regulated during seed germination. Similar results were obtained from *O. sativa* gene family members. Indeed, the expression profile unveiled that *OsSODs* genes were differentially expressed in response to various stress treatments such as cold, drought, salt and heat [35].

Abiotic factors like drought, high/low temperature and salinity pose a serious threat to yield of sesame, thus expression analysis of *SiSOD* genes under these conditions were studied. We explored expression patterns

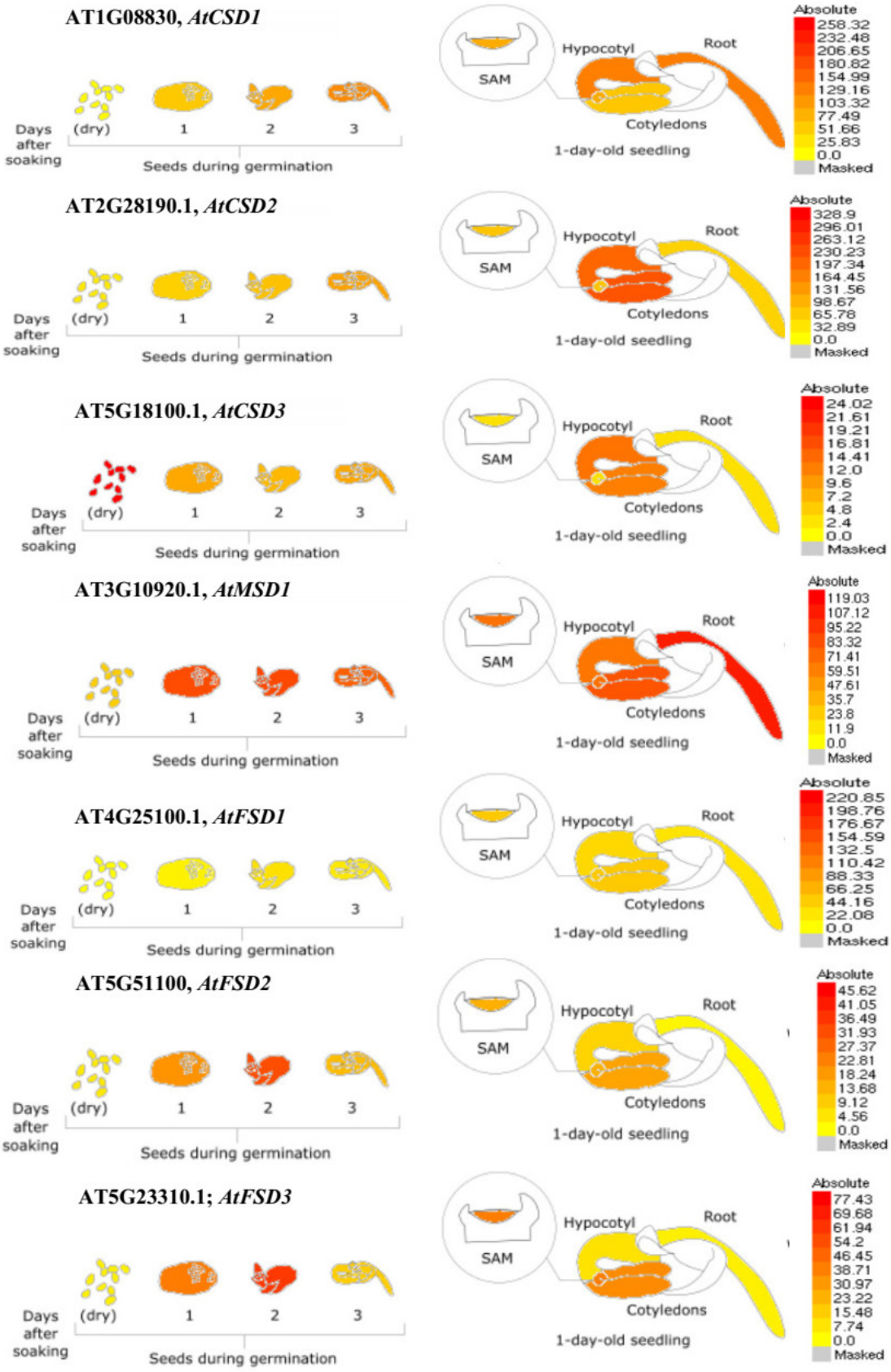


**Figure 2.** Visualization of *AtSOD* expression at seed germination stage from the Arabidopsis eFP browser program in the Araport11 data set. CSD, copper/zinc superoxide dismutase; ATMSD, manganese superoxide dismutase; FSD, Fe superoxide dismutase.

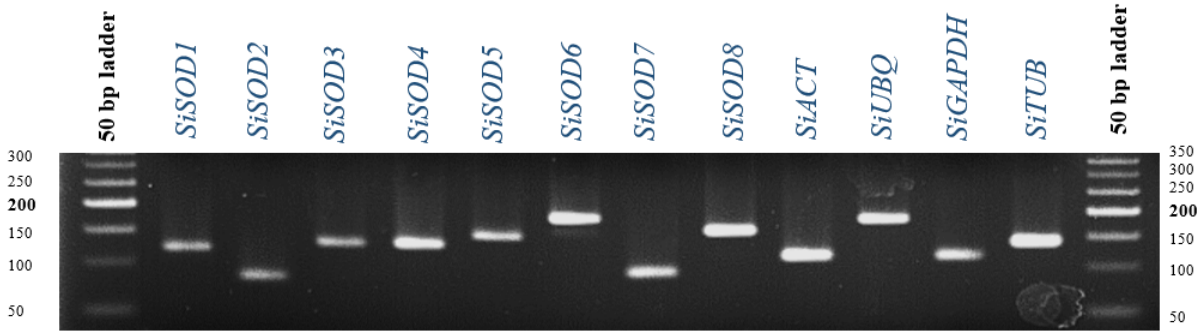


**Figure 3.** The amplicon profile of eight *SiSOD* genes and four reference genes on 3% agarose gel.

of eight *SODs* family members, including *SiSOD1, SiSOD2, SiSOD3, SiSOD4, SiSOD5, SiSOD6, SiSOD7* and *SiSOD8* in various abiotic stresses by using RT-qPCR. In this study, reverse primer spanned an exon junction to guarantee non-amplification of gDNA contaminations. The annealing temperature of the primers was set at 60°C, and a single sharp peak in the melt curve analysis without any primer-dimer confirmed the specificity of the primers (data not shown). The size of the amplicons associated with the target and reference genes was also validated by agarose gel electrophoresis in three percent gels (Fig. 3). A 95 percent confidence interval was used to establish statistically significant fold changes ($P \leq 0.05$). Gene expression patterns are depicted in Fig. 4. In general, *SiSOD* genes were expressed differentially under different abiotic stresses; however, the expression patterns of eight genes across each abiotic stress were highly similar (Fig. 4). Upregulation of expression was observed in all members of *SOD* gene family at Low *T*, while *SiSOD* gene expressions were downregulated at the other studied treatments (except for *SiSOD7* and *SiSOD8* genes that were upregulated in high *T* ($P < 0.05$)). The mRNA expression level of *SiSOD4* at water stress exhibited the lowest level of gene expression among all studied treatments/genes with 12.1-folds downregulation. All *SiSOD* gene family members showed downregulation in both water and salt stresses. According to the RT-qPCR expression data, the heat map depicted the differences in transcript abundance of the eight *SiSODs* between the treatments under investigation. (Fig. 5). Also, the *SiSODs* genes were clustered together based on their expression induction or reduction under all studied stresses.

As the initial line of defense against ROS, SODs work in cooperation with catalase and peroxidases to eliminate $O_2^{\bullet-}$ and $H_2O_2$ from the cell environment, respectively [7]. SODs also operate as a signaling molecule in a variety of plant signaling pathways and are engaged in a variety of plant developmental processes [35]. The coordinated activation or reduction of *SOD* gene expression revealed the behavior of *SOD* genes during stress physiology, whether a specific *SOD* regulates the pathway or crosstalks with other genes to modify the stress-responsive pathway. [35]. In this study, the transcript levels of the *SiSOD* gene family members were quantified to see how they changed in response to external treatments. As shown in Fig. 4, all the studied genes had a similar pattern in all given treatments except for *SiSOD7* and *SiSOD8* genes at high *T*. In contrast to mature plants, where the *SOD* gene shows increased expression in response to abiotic stress, the *SiSOD* gene showed largely decreased expression during the SG of sesame. Analysis of *HvSODs'* expression profiles revealed that several genes in this family expressed in a tissue-specific manner. Expression levels of five genes, namely *HvCSD2*, *HvCSD3*, *HvFSD1*, *HvFSD2*, and *HvMSD1*, fluctuated significantly during growth and development [36]. The expression profile of eight rapeseeds (*Brassica napus* L.) *BnSOD* gene family revealed considerably upregulated under salt, cold, waterlogging, and drought conditions [30]. In a genome-wide analysis of the wheat (*Triticum aestivum*), *TaSOD* gene family were variably expressed the same environmental stresses, as well as the expression changes of the same gene under different environmental stresses (NaCl, mannitol, and PEG) [15]. Interactions between trans-acting factors and *SOD* cis-regulatory elements may influence the expression level of *SOD* gene family.

Analysis of *LsSOD* family promoter regions revealed that the majority of cis-elements are involved in plant development, stress response, and hormone production [22]. Future research on cis regulatory elements in the promoter regions of the *SOD* gene family in sesame could reveal important details about how these genes are regulated.

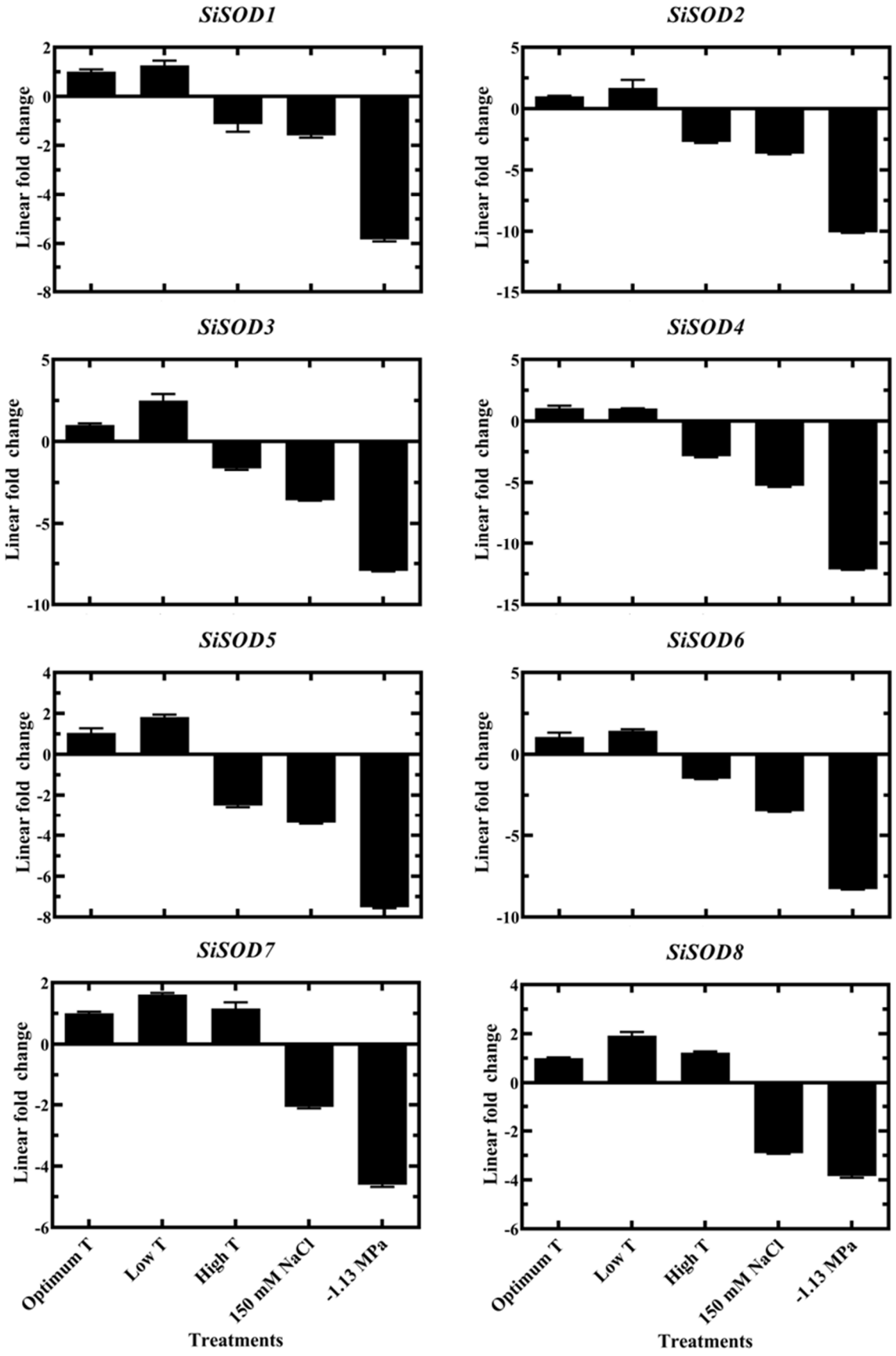


**Figure 4.** Expression profile of *SiSOD* gene family in various stress conditions.

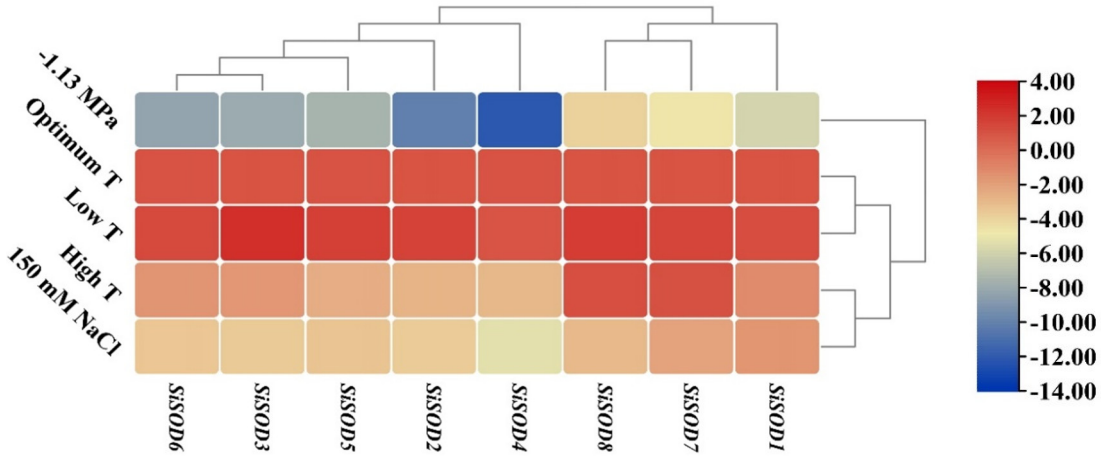


**Figure 5.** Heat-map of *SiSOD* gene expression against various abiotic stresses. The highest gene expression values are shown in red, while the lowest values are shown in blue.

## CONCLUSION

The current work aimed to obtain insight into the differential expression of *SiSODs* during the sesame germination stage in response to various abiotic stresses. Similar expression patterns were found in salt stress (ionic and osmotic effects) and water stress (osmotic effects), reflecting that osmotic effect rather than ionic effect may trigger these expression responses. To acquire a better knowledge of the mechanism influencing the expression settings of the *SiSOD* gene family, examining the enzymatic activity of these genes during the germination stage might be beneficial. To learn more about the *SiSOD* gene regulation in general, and how these genes are controlled spatially and temporally in particular, further research into the expression of these genes at different developmental stages is required. The findings of this study might enhance the understanding of *SODs* involvement in other plants, as well as the processes behind stress responses in plants, particularly during their early stages of development.

## ACKNOWLEDGEMENTS

This research is supported by the Genetics and Agricultural Biotechnology Institute of Tabarestan (GABIT) and Sari Agricultural Sciences and Natural Resources University (SANRU) research Grant (T263-98). The authors also gratefully acknowledge use of the services and facilities of the GABIT during this research.

### Conflicts of Interest

No conflict of interest was declared.

## REFERENCES

[1] Abdellaoui, R., Boughalleb, F., Zayoud, D., Neffati, M., and Bakhshandeh, E. 2019. Quantification of *Retama raetam* seed germination response to temperature and water potential using hydrothermal time concept. Environmental and Experimental Botany, 157: 211-216.

[2] Alvarado, V. and Bradford, K. 2002. A hydrothermal time model explains the cardinal temperatures for seed germination. Plant, Cell & Environment, 25(8): 1061-1069.

[3] Apel, K. and Hirt, H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol., 55: 373-399.

[4] Bakhshandeh, E., Atashi, S., Hafeznia, M., Pirdashti, H., and Teixeira da Silva, J.A. 2015. Hydrothermal time analysis of watermelon (*Citrullus vulgaris* cv.'Crimson sweet') seed germination. Acta Physiologiae Plantarum, 37(1): 1738.

[5] Bakhshandeh, E., Gholamhosseini, M., Yaghoubian, Y., and Pirdashti, H. 2020. Plant growth promoting microorganisms can improve germination, seedling growth and potassium uptake of soybean under drought and salt stress. Plant Growth Regulation, 90(1): 123-136.

[6] Bakhshandeh, E., Jamali, M., Afshoon, E., and Gholamhossieni, M. 2017. Using hydrothermal time concept to describe sesame (*Sesamum indicum* L.) seed germination response to temperature and water potential. Acta Physiologiae Plantarum, 39(11): 250.

[7] Bayanati, M., Tehranifar, A., Razavi, K., Nemati, S., Lohrasebi, T., and Ahmadi, N. 2019. Expression patterns analysis of SOD genes in responses to ethylene-induced oxidative stress in rose (*Rosa hybrida*) during flower

development. South African Journal of Botany, 127: 265-270.

[8] Bewley, J.D., Bradford, K., and Hilhorst, H. 2013. Seeds: physiology of development, germination and dormancy. 3rd edn. Springer, New York, p 407.

[9] Blokhina, O., Virolainen, E., and Fagerstedt, K.V. 2003. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Annals of botany, 91(2): 179-194.

[10] Gill, S.S. and Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant physiology and biochemistry, 48(12): 909-930.

[11] Hashemipetroudi, S.H., Nematzadeh, G., Ahmadian, G., Yamchi, A., and Kuhlmann, M. 2018. Assessment of DNA contamination in RNA samples based on ribosomal DNA. JoVE (Journal of Visualized Experiments), (131): e55451.

[12] Hosseini Sanehkoori, F., Pirdashti, H., and Bakhshandeh, E. 2021. Quantifying water stress and temperature effects on camelina (*Camelina sativa* L.) seed germination.

[13] Hutchins, J.R. 2019. Genomic Databases.

[14] Institute, S. 2014. SAS 9.4 Output delivery system: User's guide. SAS institute.

[15] Jiang, W., Yang, L., He, Y., Zhang, H., Li, W., Chen, H., Ma, D., and Yin, J. 2019. Genome-wide identification and transcriptional expression analysis of superoxide dismutase (SOD) family in wheat (*Triticum aestivum*). PeerJ, 7: e8062.

[16] Klepikova, A.V., Kasianov, A.S., Gerasimov, E.S., Logacheva, M.D., and Penin, A.A. 2016. A high resolution map of the Arabidopsis thaliana developmental transcriptome based on RNA-seq profiling. The Plant Journal, 88(6): 1058-1070.

[17] Luan, Z., Xiao, M., Zhou, D., Zhang, H., Tian, Y., Wu, Y., Guan, B., and Song, Y. 2014. Effects of salinity, temperature, and polyethylene glycol on the seed germination of sunflower (*Helianthus annuus* L.). The Scientific World Journal, 2014.

[18] Michel, B.E. and Radcliffe, D. 1995. A computer program relating solute potential to solution composition for five solutes. Agronomy Journal, 87(1): 126-130.

[19] Najeeb, U., Mirza, M., Jilani, G., Mubashir, A., and Zhou, W. 2012. Sesame, in Technological Innovations in Major World Oil Crops, Volume 1, Gupta, S.K., Editor. Springer. p. 131-145.

[20] Parmoon, G., Moosavi, S.A., and Siadat, S.A. 2018. How salinity stress influences the thermal time requirements of seed germination in *Silybum marianum* and *Calendula officinalis*. Acta physiologiae plantarum, 40(9): 175.

[21] Pospíšil, P., Prasad, A., and Rác, M. 2019. Mechanism of the formation of electronically excited species by oxidative metabolic processes: role of reactive oxygen species. Biomolecules, 9(7): 258.

[22] Rehman, S., Rashid, A., Manzoor, M.A., Li, L., Sun, W., Riaz, M.W., Li, D., and Zhuge, Q. 2022. Genome-Wide Evolution and Comparative Analysis of Superoxide Dismutase Gene Family in Cucurbitaceae and Expression Analysis of *Lagenaria siceraria* Under Multiple Abiotic Stresses. Frontiers in genetics, 12: 784878.

[23] Rowse, H. and Finch-Savage, W. 2003. Hydrothermal threshold models can describe the germination response of carrot (*Daucus carota*) and onion (*Allium cepa*) seed populations across both sub-and supra-optimal temperatures. New Phytologist, 158(1): 101-108.

[24] Sanehkoori, F.H., Pirdashti, H., and Bakhshandeh, E. 2021. Quantifying water stress and temperature effects on camelina (*Camelina sativa* L.) seed germination. Environmental and Experimental Botany, 186: 104450.

[25] Schmittgen, T.D. and Livak, K.J. 2008. Analyzing real-time PCR data by the comparative CT method. Nature protocols, 3(6): 1101-1108.

[26] Seal, C.E., Barwell, L.J., Flowers, T.J., Wade, E.M., and Pritchard, H.W. 2018. Seed germination niche of the halophyte *Suaeda maritima* to combined salinity and temperature is characterised by a halothermal time model. Environmental and Experimental Botany, 155: 177-184.

[27] Shakeri, S., Kazemitabar, S.K., and Hashemi, S.H. 2015. Study of Reference Genes in Sesame Leaves under Salt Stress by Real-Time PCR Method. Crop Biotechnology, 4(8): 1-10.

[28] Sharma, S.S. and Dietz, K.-J. 2009. The relationship between metal toxicity and cellular redox imbalance. Trends in plant science, 14(1): 43-50.

[29] Soltani, A. and Maddah, V. 2010. Simple applied programs for education and research in agronomy. Iranian Scientific Society of Agroecol. Publ.

[30] Su, W., Raza, A., Gao, A., Jia, Z., Zhang, Y., Hussain, M.A., Mehmood, S.S., Cheng, Y., Lv, Y., and Zou, X. 2021. Genome-wide analysis and expression profile of superoxide dismutase (SOD) gene family in rapeseed (*Brassica napus* L.) under different hormones and abiotic stress conditions. Antioxidants, 10(8): 1182.

[31] Tilaki, G.A.D., Behtari, B., and Behtari, B. 2009. Effect of salt and water stress on the germination of alfalfa (Medicago sativa L.) seed. Поволжский экологический журнал, (2): 158-164.

[32] Tunde-Akintunde, T. and Akintunde, B. 2004. Some physical properties of sesame seed. Biosystems Engineering, 88(1): 127-129.

[33] Vahabinia, F., Pirdashti, H., and Bakhshandeh, E. 2019. Environmental factors' effect on seed germination and seedling growth of chicory (*Cichorium intybus* L.) as an important medicinal plant. Acta physiologiae plantarum, 41(2): 27.

[34] Valiki, S.R.H., Ghanbari, S., Golmohammadzadeh, S., and Kiasari, K.R. 2015. Effect of different plant density on growth and yield of three cultivars of sesame (*Sesamum indicum* L.). in Biological Forum. Research Trend.

[35] Yadav, S., Gill, S.S., Passricha, N., Gill, R., Badhwar, P., Anjum, N.A., Francisco, J.-B.J., and Tuteja, N. 2019. Genome-wide analysis and transcriptional expression pattern-assessment of superoxide dismutase (SOD) in rice and Arabidopsis under abiotic stresses. Plant gene, 17: 100165.

[36] Zhang, X., Zhang, L., Chen, Y., Wang, S., Fang, Y., Zhang, X., Wu, Y., and Xue, D. 2021. Genome-wide identification of the SOD gene family and expression analysis under drought and salt stress in barley. Plant Growth Regulation, 94(1): 49-60.

.

# تجزیه و تحلیل بیان خانواده ژن *SiSOD* در مرحله جوانه‌زنی کنجد (*Sesamum indicum* L.) تحت تنش‌های غیرزیستی مختلف

سیدحمیدرضا هاشمی پطرودی *، اسماعیل بخشنده

پژوهشکده ژنتیک و زیست فناوری کشاورزی طبرستان، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ساری، ایران

*نویسنده مسئول: irahamidreza@yahoo.com

## چکیده

بذر کنجد (*Sesamum indicum* L.) یک منبع غنی از روغن و پروتئین است که می‌تواند برای پخت و پز یا منبع اولیه برای برخی کاربردهای صنعتی استفاده شود. جوانه‌زنی بذر مهم‌ترین مرحله چرخه زندگی یک گیاه است که به طور قابل ملاحظه‌ای تحت تأثیر تنش‌های مختلف غیرزیستی قرار می‌گیرد. بنابراین، این مطالعه با هدف بررسی اثر عوامل محیطی مختلف (دماهای پایین، مطلوب و بالا، پتانسیل آب ($\psi$) و شوری) بر بیان هشت ژن از خانواده سوپراکسید دیسموتاز (دو Mn-SOD، دو Cu/Zn-SOD و چهار Fe-SOD) در مرحله جوانه‌زنی کنجد به عنوان اولین گزارش انجام شد. نتایج نشان داد که تمامی تیمارهای مورد مطالعه به طور معنی‌داری بر خصوصیات جوانه‌زنی این گیاه تأثیرگذار بودند. به‌طورکلی، اثر منفی هر تنش بر جوانه‌زنی کنجد را می‌توان به صورت $\psi$ > تنش شوری > دمای بالا > دمای پایین رتبه‌بندی کرد، که نشان‌دهنده تأثیر منفی بیشتر $\psi$ نسبت به تنش‌های شوری و دما بر جوانه‌زنی کنجد می‌باشد. همبستگی بالا بین کاهش (نسبت به دمای بهینه) در پارامترهای جوانه‌زنی و کاهش بیان ژن‌های *SiSOD* در شرایط مختلف تنش مشاهده شد. نتایج نشان داد الگو بیان هر یک از ژن‌های *SiSODs* در یک تنش خاص تقریبا مشابه بود در حالی‌که الگوی بیان متفاوتی را در تنش‌های مختلف (بیان تنش-اختصاصی) نشان دادند. یافته‌های این مطالعه می‌تواند منجر به درک بهتر نقش ژن *SOD* در سایر گیاهان و مکانیسم‌های دخیل در پاسخ‌های تنش گیاهان، به‌ویژه در مراحل اولیه رشد آن‌ها شود.

**کلمات کلیدی:** تنش شوری و خشکی؛ جوانه‌زنی بذر؛ کنجد، بررسی گستره ژنومی.