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Full Length Article

Cloning, Sequence Analysis and Expression Profile of a Chloroplastic Copper/Zinc Superoxide Dismutase Gene from Lentil

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Abstract

Superoxide dismutase is a major components of antioxidant defense system against harmful reactive oxygen species. It scavenges superoxide radicals in specific cell components. In this research, *Cu/Zn-SOD* cDNA has been cloned and identified from lentil. The full length of *LcCu/Zn-SOD* was 877 bp containing a 609 bp ORF encoding a polypeptide of 202 amino acids with a predicted molecular weight of 20.55 kDa and predicted isoelectric point of 6.10. LcCu/Zn-SOD is most likely localized in chloroplasts and contains a conserved amino acids segment coordinating copper and zinc binding and conserved cysteine residues essential for disulfide-bond formation. Similarity analysis indicated that LcCu/Zn-SOD shared considerable sequence similarity with chloroplastic Cu/Zn-SOD protein from other plant species. Expression analysis of *LcCu/Zn-SOD* was examined in two-week old lentil seedlings subjected to drought stress, hydrogen peroxide and phytohormonal stimuli. Results of qRT-PCR showed that *LcCu/Zn-SOD* was early upregulated in response to drought condition and was upregulated after treating seedlings with hydrogen peroxide. Spraying lentil seedlings with SA resulted in downregulation of *LcCu/Zn-SOD* during 1 and 2 h of treatment and the expression increased after 4 h of treatment. The phytohormone ABA significantly altered the expression of this gene with 5-fold upregulation at 2 h compared to untreated seedlings. In response to JA treatment, *LcCu/Zn-SOD* was early and significantly upregulated at 1 h of treatment for *LcCu/Zn-SOD* (2.3 fold) The results of the study indicate that *LcCu/Zn-SOD* is possibly involved in lentil responses and defense to drought conditions and signaling molecules.

Keywords: Drought; Lentil; Oxidative stress; Phytohormone; Superoxide dismutase

Introduction

Plants are frequently affected by environmental adversities including abiotic and biotic stresses. The accumulation of reactive oxygen species (ROS) is the inevitable result of this exposure in plant cells. ROS impose oxidative damage to major macromolecules and therefore, are highly reactive and toxic at high concentrations (Caverzan *et al*. 2016; Zhang *et al*. 2022). The plant cell can equilibrate between production and detoxification of ROS by enzymatic and non-enzymatic mechanisms. The non-enzymatic antioxidant compounds can be water soluble such as ascorbate, phenolic compounds, flavonoids and glutathione, and lipid-soluble such as carotenoids and α-tocopherols (Racchi 2013).

Enzymatic antioxidants include different enzymes produced by plant cells under different type of stress conditions (Awad *et al*. 2021). These enzymes are superoxide dismutase (SOD), catalase, ascorbate peroxidase, glutathione reductase, and glutathione peroxidase (Apel and Hirt 2004; Abu-Romman 2016a).

The SOD is one of the most important metalloenzyme. It represents an effective components of antioxidant enzyme defense in plant cells against harmful ROS (Odat 2018). This enzyme is responsible for scavenging superoxide radicals in specific cell components (Scandalios 2005). In plants, SODs can be categorized into three classes based on their metal co-factor: copper-zinc SOD (Cu/Zn-SOD), iron SOD and manganese SOD (Fink and Scandalios 2002). These SODs groups are targeted to different subcellular compartments. Cu/Zn-SODs are targeted to cytoplasm and chloroplasts, Mn-SODs are targeted to peroxisomes and mitochondria, while Fe-SODs are localized in chloroplasts (Bowler *et al*. 1994).

Several SOD genes have been cloned from different plant species (Kaminaka *et al*. 1997; Abu-Romman and Shatnawi 2011). Expression level of SODs genes was reported to be induced in response to different environmental stresses (Abercrombie *et al*. 2008; Feng *et al*. 2016). Enhanced up-regulation of *Mn-SOD* expression was recorded in wheat plants subjected to H_2O_2, osmotic and salinity-induced oxidative stresses (Kaouthar *et al*. 2016).

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Fungal elicitor and wounding greatly increased *Cu/Zn-SOD* expression in pea plants (Kasai *et al*. 2006). Plants overexpressing *SOD* genes possess enhanced tolerance against oxidative damages (Gupta *et al*. 1993). For example, transgenic Arabidopsis plants overexpressing wheat *Mn-SOD* gene possessed high proline accumulation and reduced H_2O_2 levels (Kaouthar *et al*. 2016). Tolerance to drought conditions was recorded in transgenic Arabidopsis and rice seedlings overexpressing *Mn-SOD* genes from jojoba (Liu *et al*. 2013) and pea (Wang *et al*. 2005), respectively. Negi *et al*. (2015) showed that overexpression of peanut *Cu/Zn-SOD* gene enhanced tolerance of tobacco plants to drought and salinity stresses. Moreover, tobacco plants overexpressing mangrove exhibited reduced ROS generation in the chloroplast (Jing *et al*. 2015).

Lentil (*Lens culinaris* Medik*)* belongs to Fabaceae family, is a self-pollinating plant and an annual cool season legume. The morphological feature of lentil can be described as slender, branched with hairy leaves and stems and can reach 15-75 cm in height. The leaves are compound and alternate with 10-15 leaflets (Yadav *et al*. 2007). Lentil is mainly cultivated for its seeds which are rich in proteins, carbohydrates, vitamins, fibers and micro- and macro-nutrients (Ozdemir *et al*. 2015; Thavarajah *et al*. 2016). In different developing countries, lentil is rainfed-cultivated as a pulse crop and is important in the staple diet in Asia (Grusak 2009). India and Canada are the major lentil-producing countries, occupying 57% of global production (FAOSTAT 2016). Weak agronomic management, limited productivity potentials of landraces, and environmental stresses are the major limiting factors to successful lentil production (Erskine *et al*. 1993). The critical abiotic stresses limiting lentil production are drought, heat and salinity stresses (Van Hoorn *et al*. 2001; Shrestha *et al*. 2006; Bhandari *et al*. 2016). On the other hand, vascular wilt, rust, and blight diseases are the major pathogens affecting lentil plants worldwide (Eujayl *et al*. 1998; Ford *et al*. 1999; Pouralibaba *et al*. 2016).

With the exception of alfalfa, soybean, and pea (Eujayl *et al*. 1998; Ford *et al*. 1999; Pouralibaba *et al*. 2016), little is known about the antioxidant defense genes in other legumes. Therefore, the present work was aimed at characterizing copper/zinc superoxide dismutase (*LcCu/Zn-SOD*) gene from lentil.

Materials and Methods

Plant material and treatments

Seeds of the lentil (*Lens culinaris* Medik) cultivar Jordan 2 were planted in plastic pots (14×14 cm) filled with peatmoss and perlite and were irrigated with distilled water for two weeks under greenhouse conditions. Gene expression profile of *LcCu/Zn-SOD* was investigated in two-week old seedlings subjected to the following different treatments:

- Drought stress: by withholding irrigation for different time intervals
- Hormonal treatment: seedlings were sprayed with abscisic acid (100 μM), jasmonic acid (100 μM), or salicylic acid (1 m*M*).
- Hydrogen peroxide (H_2O_2): seedlings were sprayed with 10 m*M* H_2O_2.

For drought stress, leaves were collected at zero-time, 3 d and 6 d of treatment. For hormonal and H_2O_2, leaves were collected at zero time, 1, 2 and 4 h of treatment. The collected leaves were frozen in liquid nitrogen and stored at -20°C for RNA extraction.

RNA isolation and cDNA synthesis

Total RNA was isolated from frozen lentil leaves by using Spectrum™ Plant Total RNA Kit (Sigma) according to the protocol supplied by the manufacturer, then RNA concentration and purity was measured by spectrophotometer 260 and 280 nm (Biochrom, Cambridge). Furthermore, the quality of RNA in agarose gel was checked under U.V light. The first-strand cDNA was prepared from two µg of lentil leaf RNA using primeScript™MasterMix (Takara, Japan). All cDNA samples were stored at -20°C for gene expression analysis.

Cu/Zn-SOD gene cloning

For the purpose of identifying the full-length open reading frame (ORF) of *LcCu/Zn-SOD* gene from lentil, a candidate gene approach was followed. A pair of specific primers (5'-CAATGGCTTCACAAACTCTCGT-3') (sense, Cu/Zn-SOD F) and (5'- TTAGGGAAGAAACACACCTGACT-3') (antisense, Cu/Zn-SOD R) were chosen based on the sequence of pea *Cu/Zn-SOD* gene (GenBank accession No. X56435.1). The PCR reaction was carried out using iNtRON i-MAXTM II (iNtRON, Korea), and the PCR condition was as follow: 95ºC for 5 min, followed by 35 cycles of 95ºC for 30 s, 54ºC for 30 s and 72ºC for 2 min, then 72ºC for 10 min. PCR products were separated on 1% agarose gels. The specific PCR amplicon (877 bp) was cut from the gel and purified using GeneJET Gel Extraction Kit (Thermo Scientific, USA). The resulting purified products were cloned using PGEM®-T Easy Vector (Promega, USA) and sent for sequencing.

Bioinformatics analysis

ExPASY Translate tool (http://web.expasy.org/translate/) was used to obtain the protein sequences of lentil Cu/Zn-SOD and ProtParam tool (http://web.expasy.org/protparam/) was employed to analyze the LcCu/Zn-SOD protein physical and chemical parameters. LcCu/Zn-SOD protein targeting was predicted using ProtComp 9.0 online tool (http://linux1.softberry.com/berry.phtml?topic=protcompplandgroup=programsandsubgroup=proloc) and TargetP (http://www.cbs.dtu.dk/services/TargetP/) (Emanuelsson *et al*. 2000). Similarity analyses were performed using BLAST

(http://www.ncbi.nlm.nih.gov/BLAST/) (Altschul *et al*. 1990) and conserved protein domains were predicted by searching the NCBI Conserved Domain Database (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) (Marchler-Bauer *et al*. 2009). Multiple sequence alignment analysis was carried out using Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalw2/) (Larkin *et al*. 2007). Phylogenetic analysis of Cu/Zn-SOD proteins was performed by neighbor-joining algorithm using MEGA 7 program (Kumar *et al*. 2016) and branch confidence was assessed by bootstrapping analysis with 1000 replicates.

Gene expression analyses using quantitative RT-PCR

The analyses of gene expression of *LcCu/Zn-SOD* gene were performed by using of qRT-PCR. Specific primers for the reaction were designed using of Primer 3 software. Primer pairs for qRT-PCR of *Cu/Zn-SOD* (sense: 5'-GTGACCTGGGAAACATAGTTGC-3'; antisense: 5'-TTCATGTCCACCCTTTCCGAG-3') amplified a product of 152 bp amplicon. Actin gene (GenBank accession No.) was used as an internal control (sense: 5'-ATACCCCTGCCATGTATGTAGC-3'; antisense: 5'-AGCCAGATCAAGACGAAGGATG-3'). A total of 25 μL of PCR reaction mixture were contained 10 μL of KAPA SYBR ®FAST universal qPCR Kit (KAPA, USA), 0.4 μL of each specific primer (10 μM), 120 ng/μL of diluted cDNA as a template and RNase-free water was added to make up the final volume of 25 μL. Amplifications were performed for 2 min of an initial denaturation at 95°C, 45 cycles of 10 sec at 95°C, 25 sec at 54°C, 25 sec at 60°C at which fluorescent was acquired. Final extension step was performed for 2 min at 60°C.

All RT-PCR were runs and analyzed using three replicates for each sample. To measure the level of gene expression, fold difference was calculated using $2^{-\Delta\Delta CT}$ (Livak and Schmittgen 2001). Differences between interval times of treatment means were determined by least significant difference (LSD) at 5% confidence interval.

Results

Cloning and bioinformatics analyses of *Cu/Zn-SOD* gene in lentil

To identify a *Cu/Zn-SOD* gene from lentil, a candidate gene approach was followed. Based on pea *Cu/Zn-SOD* gene (GenBank accession No. X56435.1) sequence, a pair of specific primers were designed and used to amplify lentil *Cu/Zn-SOD* gene using lentil cDNA as a templet, cDNA used in PCR amplification was prepared from RNA isolated from lentil seedling subjected to drought stress. PCR amplification of *Cu/Zn-SOD* with *LcCu/Zn-SOD* F and *LcCu/Zn-SOD* R primers resulted in 877 bp product which was then purified and cloned in PGEM®-T Easy Vector and sequenced. The lentil *Cu/Zn-SOD* gene was designated as *LcCu/Zn-SOD*.

The full length of *LcCu/Zn-SOD* cDNA is 877 bp, and has a start and stop codon, indicating that *Lc Cu/Zn-SOD* gene is complete. This sequence consists of a complete open reading frame (ORF) of 609 bp with a 5'-UTR of 2 bp and a 3'-UTR of 266 bp (Fig. 1). The ORF of *Lc Cu/Zn-SOD* gene encodes a polypeptide of 202 amino acids (Fig. 1), with a predicted molecular weight of 20.55 kDa and a predicted isoelectric point of 6.10. The nucleotide sequence identified was submitted to the GenBank and has the accession number MK605637.

Protein targeting prediction for LcCu/Zn-SOD protein was performed using two online tools (TargerP 1.1 and ProtComp 9.0) in order to enhance the prediction efficiency. In plant cell, Cu/Zn-SOD protein can be localized to the cytosol and chloroplasts. The results showed that LcCu/Zn-SOD protein is probably localized in chloroplasts, which further indicates the presence of chloroplast transit peptide in this protein.

BLAST was used to investigate sequence similarity analysis of LcCu/Zn-SOD with related Cu/Zn-SODs from different plant species. LcCu/Zn-SOD shared considerable similarity percentages with chloroplastic Cu/Zn-SOD from Arabidopsis (83%), barrelclover (97%), chickpea (95%), common vetch (98%), faba bean (98%), pea (98%), and soybean (89%). This homology between LcCu/Zn-SOD with other plant chloroplastic proteins, further confirmed that the cloned gene encodes chloroplastic Cu/Zn-SOD.

Multiple sequence alignment was performed between LcCu/Zn-SOD and chloroplastic Cu/Zn-SOD proteins from pea (PsCu/Zn-SODII; CAA39819), common vetch (VsCu/Zn-SOD; AQM49974), chickpea (CaCu/Zn-SOD XP_004506078), barrelclover (MtCu/Zn-SOD XP_003606328.2) and soybean (GmCu/Zn-SOD XP_003538169). The analysis revealed the presence of conserved amino acids coordinating copper (at locations: His-94, -96, -111 and -168) and zinc (at locations: H-111, -119, -128 and Asp-131) binding. Moreover, the alignment indicated conserved cysteine residues (at locations: C-105 and C-194) involved in disulfide-bond formation (Fig. 2).

A neighbor-joining phylogenetic tree, with bootstrapping confidence values of 1000, was constructed using MEGA 7 program in order to investigate the phylogenetic relationship among different chloroplastic and cytoplasmic Cu/Zn-SOD protein homologs (Fig. 3). It was revealed that LcCu/Zn-SOD protein is clustered with chloroplastic Cu/Zn-SOD homologs and is closely related to proteins from the legume species: pea, common vetch, faba bean, and red clover.

Gene expression in response to drought stress and hydrogen peroxide

To explore the possible involvement of *Cu/Zn-SOD* gene in lentil responses and defense to abiotic-stress conditions, its expression of was examined in two-week old lentil seedlings subjected to drought stress and hydrogen peroxide (H_2O_2)

Fig. 1: Nucleotide sequence and deduced protein sequence of LcCu/Zn-SOD. The amino acids are designated with single-letter code below the middle nucleotide of each codon. Start codon is shaded in gray and stop codon is shaded in black

Fig. 2: Multiple sequence alignment of LcCu/Zn-SOD with related chloroplastic Cu/Zn-SOD proteins from different plant species. Black boxes represent amino acids coordinating copper and zinc binding. Gray boxes represent cysteine residues involved in disulfide-bond formation

using qRT-PCR. Drought stress was imposed by withholding irrigation, and the expression of *LcCu/Zn-SOD* gene was examined at 3 and 6 d of treatment. As shown in (Fig. 4), *LcCu/Zn-SOD* was upregulated only at 3 d of treatment and reached 2.24 fold. Downregulation of this gene was reached at 6 days with 30 fold compared to control plants.

Two-week old seedlings were sprayed with 10 m*M* H_2O_2, and the expression was examined at 1, 2 and 4 h of treatment (Fig. 5). qRT-PCR analysis showed *LcCu/Zn-SOD* was downregulated at 1 h of treatment. Enhanced expression of *LcCu/Zn-SOD* in response to H_2O_2 started at 2 h and reached 1.8 fold and then significantly peaked to 3.9 fold at 4 of treatment.

Gene expression in response to phytohormonal stimuli

The effects of phytohormonal stimuli (SA, ABA and JA) on *LcCu/Zn-SOD* gene expression was examined in two-week old lentil seedlings at 1, 2 and 4 h of treatment (Fig. 6). Treating seedling with SA (1 m*M*) resulted in downregulation of *LcCu/Zn-SOD* gene at 1 and 2 h of

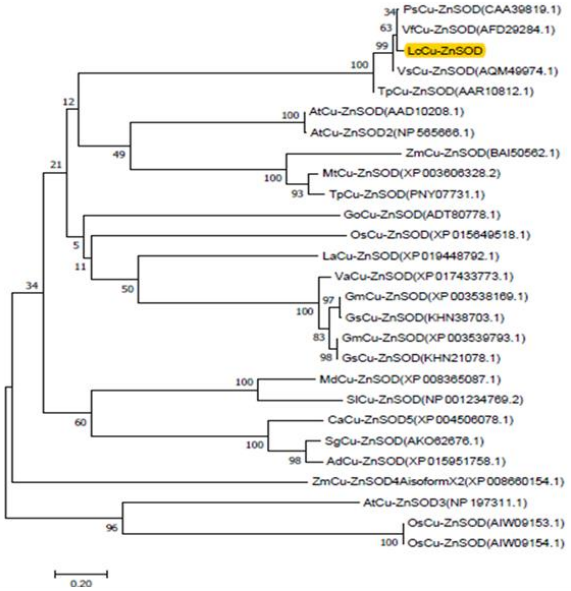

Fig. 3: Phylogenetic tree of Cu/Zn-SOD proteins from different plants. The phylogenetic tree was constructed by Mega 7 program. The bootstrap values indicate the number of times that each group occurred with 1000 replicates. GenBank accession numbers are indicated in parentheses

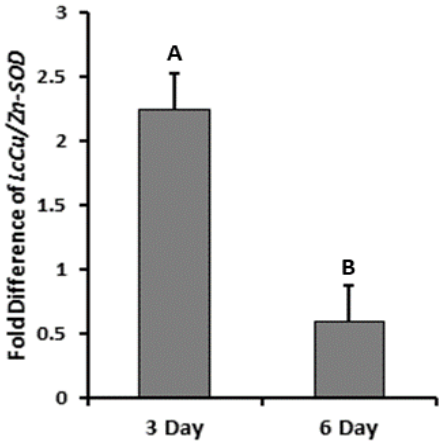

Fig. 4: Relative expression of *LcCu/Zn-SOD* in lentil seedlings subjected to drought stress for 3 and 6 d. The expression was normalized to *LcACT1* reference gene, and then were normalized with their expression in untreated control seedling (set to 1.0) at the corresponding time point. Data are means of three replicates ± SE. Significantly different values are indicated by different letters above the bars

treatment compared to untreated control. However, at 4 h of treatment, the expression of *LcCu/Zn-SOD* significantly peaked and reached 4.5 folds (Fig. 6). Expression profile of *LcCu/Zn-SOD* was investigated after spraying two-week old lentil seedlings with 100 µM of ABA. Fig. 6 showed downregulation of *LcCu/Zn-SOD* at 1 h and a significant upregulation at 2 h of treatment reaching 5.0 fold compared to control seedlings, clearly indication the positive signaling

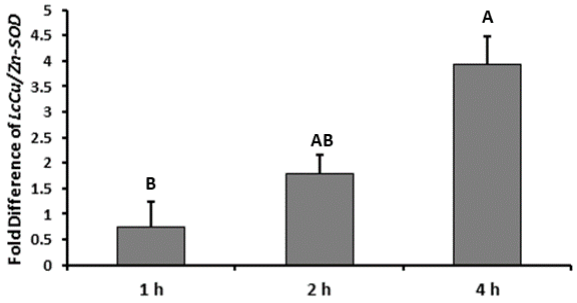

Fig. 5: Relative expression of *LcCu/Zn-SOD* in lentil seedlings treated with H_2O_2 for 1, 2 and 4 h. The expression was normalized to *LcACT1* reference gene, and then were normalized with their expression in untreated control seedling (set to 1.0) at the corresponding time point. Data are means of three replicates ± SE. Significantly different values are indicated by different letters above the bars

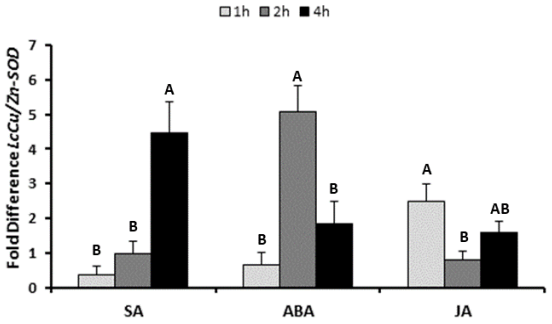

Fig. 6: Relative expression of *LcCu/Zn-SOD* in lentil seedlings treated with phytohormones for 1, 2, and 4 h. The expression level was normalized to *LcACT1* reference gene, and then were normalized with their expression in untreated control seedling (set to 1.0) at the corresponding time point. Data are means of three replicates ± SE. Significantly different values are indicated by different letters above the bars

action of ABA on *LcCu/Zn-SOD* gene.

Spraying lentil seedlings with JA (100 µM) significantly changed the expression of *LcCu/Zn-SOD* gene (Fig. 6). *LcCu/Zn-SOD* was early and significantly upregulated at 1 h of treatment which could be due to enhance ROS generation after JA treatment. The expression of this gene was then dropped to reach 20% and 1.6 folds for 2 and 4 h, respectively.

Discussion

In their natural habitats, crop plants are exposed to different stress factors. These stresses negatively impact plant growth, productivity, and development mainly by increasing ROS generation (Miller *et al.* 2008; Abu-Romman 2016b; Abdulfatah *et al.* 2021). In order to cope with enhanced ROS generation and the resulting oxidative damages, plants have developed in almost all cell compartments and effective anti-oxidative defense system (Halliwell 2006; Rajput *et al.* 2021). The first effective line of enzymatic defense in

different organelles against ROS-induced oxidative damage is represented by a group superoxide dismutases. These enzymes are capable of scavenging superoxide radicals (Eraslan *et al*. 2007).

Protein targeting prediction for LcCu/Zn-SOD protein indicated that this protein is probably to be localized in chloroplasts, which further indicates the presence of chloroplast transit peptide in this protein (Abu-Romman 2019). In plant cell, Cu/Zn-SOD protein can be localized to the cytosol and chloroplasts (Schinkel *et al.* 2001). The importance of this protein in protecting chloroplast from oxidative damages was proved in literature by different overexpression experiments, reduced chloroplast ROS generation was obtained in transgenic plants of tobacco overexpressing chloroplastic *Cu/Zn-SOD* from mangrove (Jing *et al*. 2015).

BLASTP was used to investigate sequence similarity analysis of LcCu/Zn-SOD with closely related proteins from different plants. The results indicated that the deduced protein sequence of LcCu/Zn-SOD displayed homology with other plant chloroplastic proteins, which further confirm that the cloned gene encode chloroplastic Cu/Zn-SOD.

Multiple sequence alignment was carried out using Clustal Omega between LcCu/Zn-SOD and different chloroplastic Cu/Zn-SOD proteins (Fig. 6). This analysis showed the presence of conserved amino acids coordinating copper and zinc binding, and conserved cysteine residues involved in disulfide-bond formation. The formation of disulfide bond between zinc and copper atoms functions in stabilizing the non-covalent connection in Cu/Zn-SOD isoenzyme (Fridovich 1989). It was shown that copper is the most critical atom for the Cu/Zn-SOD enzymatic activity, while zinc functions in maintaining the integrity and stability of the protein structure (Marino *et al*. 1995).

A phylogenetic tree was prepared to understand the phylogenetic relationship among different plant SOD proteins. The results revealed that LcCu/Zn-SOD protein is clustered with chloroplastic Cu/Zn-SOD homologs and is closely related to proteins from the legume species. Abu-Romman and Shatnawi (2011) showed that the phylogenetic tree of Cu/Zn-SOD protein separated cytoplasmic and chloroplastic proteins into two major clades.

Exploring expression analysis of defense genes in response to environmental stresses and signaling molecules presents a critical step toward understanding their functions. In this study, expression analyses of *LcCu/Zn-SOD* were investigated in response to drought stress, H_2O_2 and phytomormonal stimuli. Results of qRT-PCR showed that the expression profiles of this gene were altered in response to the treatments.

Different reports have shown increased SOD activity and expression in response to abiotic stress factors (Rehman *et al.* 2022). In different plant species, elevated SOD activity and gene expression were reported under osmotic and salinity stresses (Harinasut *et al*. 2003; Kukreja *et al.* 2005; Sharma and Dubey 2005; Eyidogan and Oz 2007; Gapińska *et al*. 2008; Jaleel *et al*. 2008; Manivannan *et al.* 2008). Moreover, halophytic plants have intrinsically elevated SOD activities used to operate their physiological and molecular adaptive responses under drought conditions (Bose *et al*. 2014). Feng *et al.* (2016) reported that promoters of different SOD genes contain cis-acting elements responsive for abiotic stresses. In literature, it was shown that overexpressing *SOD* genes has enhanced tolerance to environmental stresses and the associated oxidative damages. Transgenic tobacco overexpressing rice Cu/Zn-SOD exhibited improved drought and salinity tolerance (Badawi *et al*. 2004) and enhanced ozone tolerance in transgenic tobacco was achieved by overexpressing of pea *Cu/Zn-SOD* (Pitcher and Zilinskas 1996).

LcCu/Zn-SOD was early upregulated in response to drought condition (Fig. 4). Drought is a crucial abiotic stress associated with global climate change (Abu-Romman and Suwwan 2012). This stress condition negatively impacts photosynthetic efficiency in plants possible by impairing CO_2 exchange via stomatal closure (Alexieva *et al.* 2001). Chloroplasts and mitochondria represent the major locations of electron leakage leading to increased superoxide production. In photosystem I, the production of superoxide occurs in 4Fe-4S stromal complex. On the other hand, superoxide is generated in photosystem Π by H_2O_2 oxidation and by cytochrome and quinone receptor (Chen *et al*. 1995; Asada 1999; Foyer and Noctor 2009).

The expression of *LcCu/Zn-SOD* gene was examined at 1, 2 and 4 h of H_2O_2 treatment. This gene showed enhanced upregulation at 4 h of treatment (Fig. 5). Hydrogen peroxide is a non-radical ROS that imposes oxidative damages to membrane lipids (Bienert *et al.* 2006). Moreover, this compound was reported to act as a signaling molecule in activating molecular defense responses to oxidative stresses and mediate organelles crosstalk (Hernandez *et al*. 2010). The observed upregulation of *LcCu/Zn-SOD* after H_2O_2 treatment might indicate H_2O_2-derived signal(s) is involved in lentil *SOD* function. Hydrogen peroxide accumulation in maize leaves and was reported to increase SOD activities (Hu *et al*. 2006; Zhang *et al*. 2006). Moreover, Choudhary *et al*. (2012) reported that treating *Syzygium cumini* plant with H_2O_2 resulted in increased SOD activity.

Results of qRT-PCR showed elevated expression of both genes at 4 h of SA treatment (Fig. 6). Some reports have indicated that SA increased the generation of ROS. It was reported that maize plants pretreated with SA has increased antioxidant enzymes activities (Janda *et al*. 1999). The present results showed that *LcCu/Zn-SOD* was induced in response to ABA treatment (Fig. 6). Reports indicated that treating plants with ABA resulted in enhanced ROS generation and increases the activities and expression of antioxidant enzymes (Guan *et al*. 2000; Pei *et al*. 2000). *LcCu/Zn-SOD* was upregulated at 1 h of treatment, which could be due to enhance ROS generation after JA treatment (Fig. 6). Zhou *et al.* (2017) reported the presence MeJA responsive element (CGTCA-motif) in the promoter of different *Cu/Zn-SOD* genes in cucumber.

Conclusion

We isolated *Cu/Zn-SOD* gene from lentil with 877 bp in length containing a 609 bp ORF that encodes a protein of 202 amino acids. LcCu/Zn-SOD protein is targeted to chloroplast and shared high similarity percentages with chloroplastic Cu/Zn-SOD from different plant species. The expression of *LcCu/Zn-SOD* was enhanced by drought stress, H_2O_2 and hormonal treatments.

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Author Contributions

SA-R conceived and designed the experiments; MA-F performed the experiments; SA-R, NO, and MA-F analyzed the data and wrote the paper; SA-R edited and provided critical review of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable in this paper.

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