

Isolation and expression analysis of ascorbate peroxidase gene from lentil

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Abstract: Ascorbate peroxidase (APX) is an important enzyme that plays a pivotal role in the detoxification of cellular H₂O₂ and as a central component of AsA-GSH cycle. This enzyme belongs to the class I superfamily of heme-containing peroxidases in which iron plays an important role in its catalytic ability. In this study, the *APX* gene was cloned from lentil (*Lens culinaris* Medick) and was designated as *LcAPX1*. The full length of *LcAPX1* cDNA was 951 bp containing a 753 bp ORF encoding a protein of 250 amino acids with a predicted molecular mass of 27.08159 kDa and a theoretical isoelectric point of 5.52. The nucleotide sequence obtained was submitted to the GenBank under the accession number MH167389. The results of *in silico* prediction indicated that the *LcAPX1* is most likely localized in the cytoplasm. Moreover, similarity analysis using bioinformatics approach indicated that *LcAPX1* shared high sequence similarity with APX protein from other plant species and it is found that the enzyme contained a conserved APX active site in addition to a proximal heme-ligand motif. Based on phylogenetic analysis, *LcAPX1* protein was best clustered with the group of cytosolic APX proteins, and is most closely related to cytosolic APX proteins from legume plants. Additionally, the expression profiles of *LcAPX1* in 14-day old lentil seedling were analyzed in response to a variety of abiotic stresses and phytohormones treatments using quantitative reverse-transcription PCR (qRT-PCR). The expression analysis showed that the *APX* gene was upregulated under salinity conditions and treatment with hydrogen peroxide. Moreover, *LcAPX1* was upregulated in response to jasmonic acid and abscisic acid. On the other hand, *APX* expression was down regulated in response to methyl viologen and salicylic acid at all-time points examined. In conclusion, the overall results of this study indicate that the APX1 enzyme is likely involved in lentil defense/response to varied abiotic stresses and signaling molecules.

Keywords: Ascorbate peroxidase, lentil, oxidative stress, phytohormone, salinity.

Introduction

Environmental stresses, caused by abiotic and biotic factors, can be defined as any unfavorable conditions or external factors that impair plant metabolism, growth or development, and reduce crop yield (Negrão et al., 2017). Abiotic stresses are caused by non-living factors and in the form of harsh physical and chemical factors affecting the plants, such as drought, changes in temperatures, salinity, flooding, heavy metal toxicity, ozone, air pollution, UV-radiations, and agrichemicals (Cramer et al., 2011; Abu-Romman and Alzoubi, 2016; Ohama et al., 2017). On the other hand, biotic stresses are caused by interactions between the plant and other living organisms, like bacteria, fungi, insects, and other competing plants (Hasan et al., 2017; Gimenez et al., 2018).

The exposure of plants to various biotic and abiotic stress factors caused the accumulation and induction of Reactive Oxygen Species (ROS) (Gill and Tuteja, 2010). To avoid and reduce the harmful effects of ROS in plant cells under different stress factors, plants activate antioxidant systems which comprises both enzymatic and non-enzymatic mechanisms (Foyer and Noctor, 2011; Asrar et al., 2020). Ascorbate, glutathione, tocopherol, alkaloids, carotenoid and flavonoids, are non-enzymatic antioxidant protection activities in plants (Apel and Hirt, 2004; Foyer and Noctor, 2011). These compounds interact with ROS and act as a metabolic interface and modulate gene expression associated with abiotic stresses (Sharma et al., 2012; Asensi-Fabado et al., 2017).

The enzymatic mechanisms, used to scavenge ROS in plants, comprise different enzymes such as superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, monodehydroascorbate reductase, glutathione peroxidase, and dehydroascorbate reductase (Blokina et al., 2003; Scandalios, 2005; Abu-Romman, 2019). In higher plants, ascorbate peroxidase (APX; EC, 1.11.1.11) belongs to class I superfamily of heme-containing peroxidases in which iron plays an important role in the catalytic site of the enzyme (Lazzarotto et al., 2011). Studies at genome level demonstrated that APX isozymes in plants are encoded by multigenic families. *Arabidopsis* contain nine *APX* genes; whereas, rice has eight and tomato has seven *APX* genes (Chew et al., 2003; Teixeira et al., 2004; Najami et al., 2008). APX isoenzymes were reported to be localized in different subcellular compartments; namely chloroplast, cytoplasm, mitochondria, and peroxisome (Shigeoka et al., 2002; Madhusudhan et al., 2003; Sharma and Dubey, 2005). In higher plants APX is one of the most important antioxidant enzymes; is a central component of AsA-GSH cycle and it plays a pivotal role in the detoxification of cellular H₂O₂ (Maia et al., 2010; Sofo et al., 2015).

Several reports have shown the responsiveness of APX protein activity and gene expression upon exposure to environmental stresses. APX is differentially up-regulated in response to heavy metal toxicity, drought, salinity, chilling, UV irradiation, and heat stress (Koussevitzky et al., 2008; Yang et al., 2008; Maheshwari and Dubey, 2009; Abdulfatah et al., 2021a). Moreover, transgenic plants overexpression APX genes exhibited enhanced tolerance to certain abiotic stresses. Overexpression of poplar APX gene in tobacco plants enhanced salinity tolerance and reduced the paraquat induced oxidative damage (Li et al., 2009). Increased tolerance to heat stress was recorded in Arabidopsis plants overexpressing barley peroxisomal APX gene (Shi et al., 2001).

In legumes crops, APX genes were identified and characterized in cowpea (D'Arcy-Lameta et al., 2005), soybean (Jones et al., 1998), alfalfa (Wang et al., 2009), and in pea (Mittler and Zilinskas, 1992). However, in lentils APX gene has not yet been cloned. Therefore, we attempt to characterize the APX gene in lentils.

Results

Cloning and sequence analysis of lentil APX gene (LcAPX1)

To identify an APX gene from lentil, a candidate gene approach was followed. Based on pea APX gene (X62077) sequence, a pair of gene-specific primers were designed and used to amplify lentil APX gene using lentil cDNA as a template. PCR amplification of LcAPX with LcAPX F and LcAPX R primers resulted in 951 bp product which was then purified and cloned in PGEM®-T Easy Vector and sequenced. The lentil APX gene was designated as LcAPX1. The full length of LcAPX1 cDNA was 951 bp, and has a start and stop codon, indicating that LcAPX1 gene is complete. The sequence consists of a complete open reading frame (ORF) of 753 bp with a 5'-untranslated region of 81 bp, and a 3'-untranslated region of 117 bp. The ORF of LcAPX1 gene encodes a protein of 250 amino acids (Fig. 1), with a predicted molecular mass of 27.08159 kDa and a theoretical isoelectric point of 5.52. The nucleotide sequence obtained was submitted to the GenBank under the accession number MH167389.

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1      GAATTCGGCTTGTGCTCTCCTCGTGTCACTAGGGTTTAACCTCTTCCTTTTCTTCTTCTC
61     AGATTTTCGAGAATCGTTAGCTATGGGAAAATCTTACCCTACCGTTAGTGCCGATTACCAG
           M G K S Y P T V S A D Y Q
121    AAGGCTATTGAAAAGGCTAAGAGGAAGCTCAGAGGCTTCATCGCTGAGAAGAAATGCGCT
      K A I E K A K ƒ K L R G F I A E K K C A
181    CCTCTAATTCTCCGTTTGGCATGGCACTCTGCTGGTACTTTTGATTCGAAGACAAAGACT
      P L I L R L A W H S A G T F D S K T K T
241    GGTGGTCTTTTCGGAACCATTAAGCATCAAGCTGAGCTTGCTCATGGTGAACACGCGGC
      G G P F G T I K H Q A E L A H G A N S G
301    CTTGATATTGCTGTTAGGCTTTTGGAGCCTCTTAAGGAGCAATTCCCTATTGTGAGCTAT
      L D I A V R L L E P L K E Q F P I V S Y
361    GCTGATTTCTACCAGTTGGCTGGTGTGTTGCTGTTGAGGTCAGTGGTGGACCTGAAGTT
      A D F Y Q L A G V V A V E V T G G P E V
421    CCTTTCCACCCCGGTAGGGAGGACAAGCCCGAGCCACCACCAGAGGGTTCGCTTGCCTGAT
      P F H P G R E D K P E P P P E G R L P D
481    GCCACCAAGGGATCTGACCATTTGAGGGATGTGTTGGCAAAGCTATGGGGCTTAGTGAT
      A T K G S D H L R D V F G K A M G L S D
541    CAGGACATTGTTGCTCTATCTGGTGGTCAACCATTGGAGCTGCACACAAGGAGCGTTCT
      Q D I V A L S G G H T I G A A H K E R S
601    GGATTTGAGGGACCATGGACTTCCAATCCTCTCATTTTTGACAACCTACTACTTCACTGAG
      G F E G P W T S N P L I F D N S Y F T E
661    TTGTTGACTGGTGAGAAGGAAGGCCTTCTCAGTTGCCAAGTGATAAGGCACCTTTGTCT
      L L T G E K E G L L Q L P S D K A L L S
721    GATTCTGTATCCGCCCTCTTGTGAGAAATATGCAGCTGATGAAGATGCCTTCTTTGCT
      D S V F R P L V E K Y A A D E D A F F A
781    GATTATGCTGAAGCACACCCTAAGCTCTCCGAGCTTGGGTTTGCTGAAGCCTAAGTCACA
      D Y A E A H P K L S E L G F A E A *
841    GTTGGCTTCCGTTGTTTGGTGTGTTAGAGAGGCGCAGTGTCTGAATCTTTTACATAAATT
901    TCATAGACAATTGCTCTTATTTTCAATGTGATTCATCTTAGTTGGGTAGCA

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Fig. 1. cDNA sequence and deduced amino acid sequence of LcAPX1 from lentil. The amino acids sequence is designated with single-letter code below the middle nucleotide of each codon. Numbers indicate nucleotides. Start codon (ATG) is shaded in gray and stop codon (TAA) is indicated by an asterisk.

LcAPX1 subcellular localization was predicted *in silico* by using the online tools ProtComp 9.0 and TargetP 1.1. The prediction using TargetP excluded the possibility that LcAPX1 protein is chloroplastic. Moreover, ProtComp indicated that LcAPX1 is most probably localized in the cytoplasm.

BLAST and multiple sequence alignment analyses indicated that LcAPX1 shared high sequence similarity percentages with APX proteins from other plant species such as *Arabidopsis thaliana* APX1 (90%), soybean APX1 and APX2 (97%), tomato APX1 (92%), maize APX1 and APX2 (92%), sweet potato APX2 (93%), pea APX1 (98%), and alfalfa APX1 (98%).

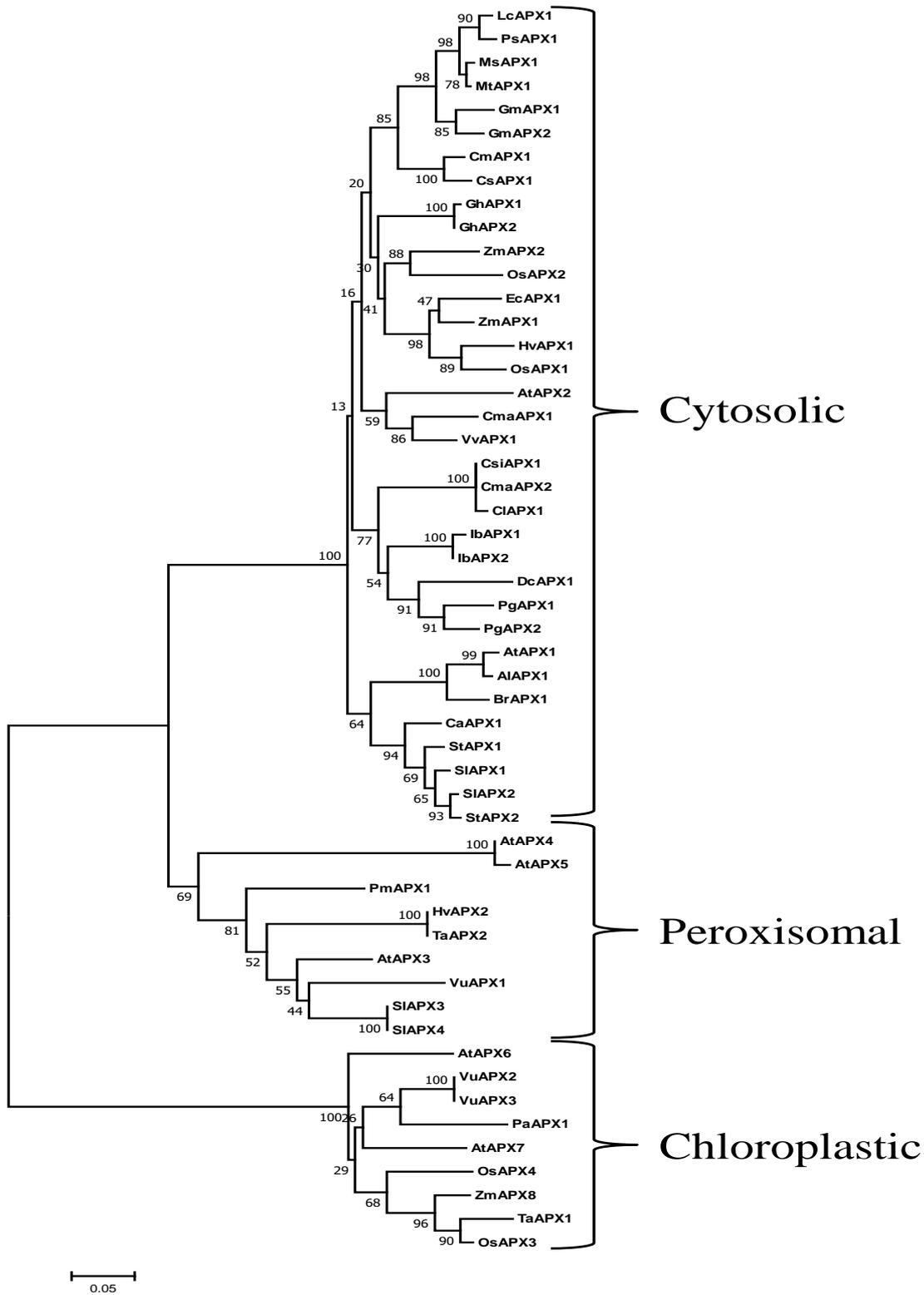


Fig. 3. Phylogenetic analysis of APX proteins from different plant species. The phylogenetic tree was performed by neighbor-joining algorithm implemented in the MEGA 7 program and branch confidence was assessed by bootstrapping analysis with 1000 replicates.

Expression analysis of *LcAPX1* gene

*Expression of *LcAPX1* in response to abiotic stresses*

To investigate the possible participation of *LcAPX1* in defense/responses under abiotic stresses, expression patterns of *LcAPX1* were examined in 14-day old lentil seedlings exposed to salinity, H₂O₂, and MV using qRT-PCR.

*Expression of *LcAPX1* in response to salinity*

The impact of salinity stress (150 mM NaCl) on *LcAPX1* expression was investigated over a period of 6 days. Upregulation in *LcAPX1* expression was recorded at 2 and 4 days. In the first 2 days, the expression level reached 1.666 fold, and then peaked by the end of 4 days at 1.813 fold. Down-regulation of *LcAPX1* was recorded at 6 days and reached 0.875 fold at 6 days compared to the untreated seedlings.

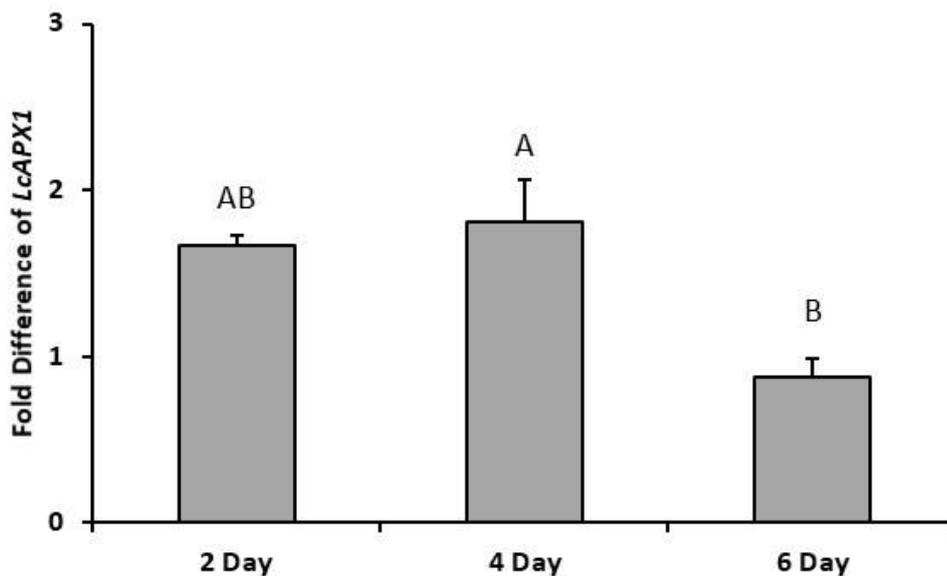


Fig. 4. Relative expression levels of *LcAPX1* in 14-day old seedlings subjected to salinity (150 mM NaCl) for 2, 4, and 6 days. The expression levels were measured by qRT-PCR. The expression of *LcAPX1* valued was normalized to *LcACT1* reference gene, and then were normalized with their expression in untreated control seedling (set to 1.0) at the corresponding time point. Data are means of three replicates \pm standard error (SE). Significantly different values are indicated by different letters above the bars.

Expression of *LcAPX1* in response to H_2O_2

In response to H_2O_2 treatment, *LcAPX1* expression was significantly altered. When lentil seedlings were treated with H_2O_2 , there was a 8.55 fold increase in *LcAPX1* expression level in the first 1 h and 1.390 fold in the next 2 h. At the end of 4 h, there was a down-regulation in *LcAPX1* expression to a level of 0.513 fold compared to untreated control. However, the difference in expression values at 2 and 4 h were not significant.

Expression of *LcAPX1* in response to MV

Lentil *APX1* expression was down-regulated in response to MV treatment over the period of 4 h after treatment. The expression at 1 h was found to be 0.209 fold and the level continued to fall to reach 0.113 fold at 2 h. The expression levels at these time points did not differ significantly. However, and at 4 h, the expression increased but remained down-regulated and scored 0.531 fold.

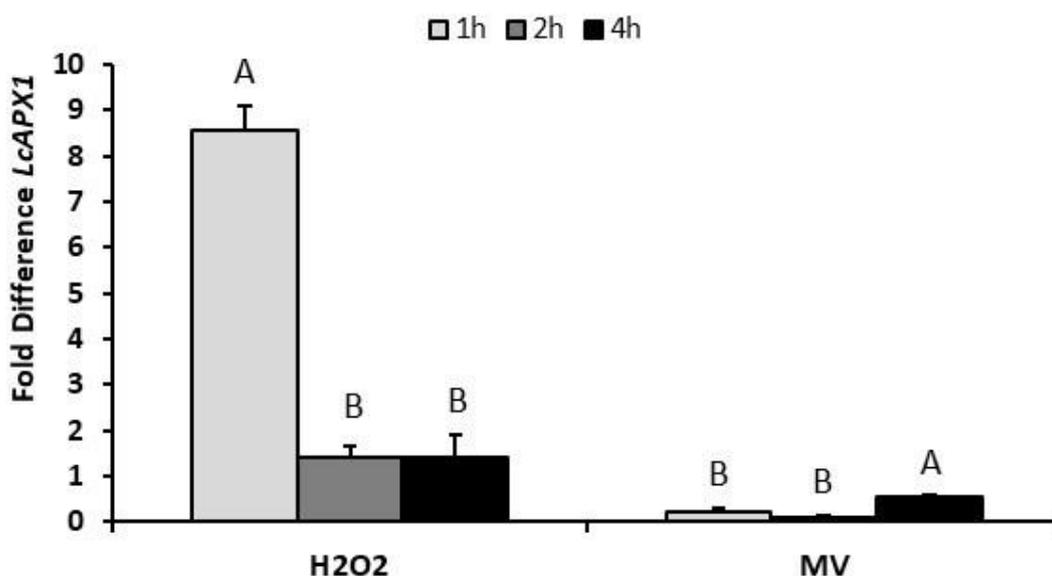


Fig. 5. Relative expression levels of *LcAPX1* in 14-day old seedlings subjected to exogenous application of A: H_2O_2 (10 mM) and B: methyl viologen (50 μ M) for 1, 2, and 4 h. The expression levels were measured by qRT-PCR. The expression of *LcAPX1* valued was normalized to *LcACT1* reference gene, 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 \pm standard error (SE). Significantly different values are indicated by different letters above the bars.

Expression of *LcAPX1* in response to phytohormones

To investigate the effect of phytohormones on *LcAPX1* expression patterns, three phytohormones; JA, ABA, and SA were used to treat 14-day old lentil plants, and the expression patterns of *LcAPX1* at transcription level were examined using qRT-PCR (Fig. 6). Upon JA treatment, *LcAPX1* was down-regulated after 1 and 2 h of treatment and reached 0.853 fold and 0.667 fold, respectively. However, and by the end of 4 h, the expression level was upregulated and was found to be 1.400 fold.

Treating lentil seedling with ABA led to an early upregulation in the level of *LcAPX1* expression and was measured at 3.245 fold after 1 h of treatment. On other hand, *LcAPX1* was down-regulated compared to untreated control after 2 and 4 h and were found to be 0.563 fold and 0.851 fold, respectively.

Expression analysis clearly indicated that SA resulted in *LcAPX1* down-regulation at all examined time point but with different extent. In response to SA treatment, *LcAPX1* expression level dropped down to its lowest point after 1 h of SA treatment and was measured at 0.162 fold. This level was not significantly different from the expression value recorded after 4 h (0.415 fold). On the other hand, at 2 h, *LcAPX1* expression started to increase but still down-regulated and was 0.951 fold.

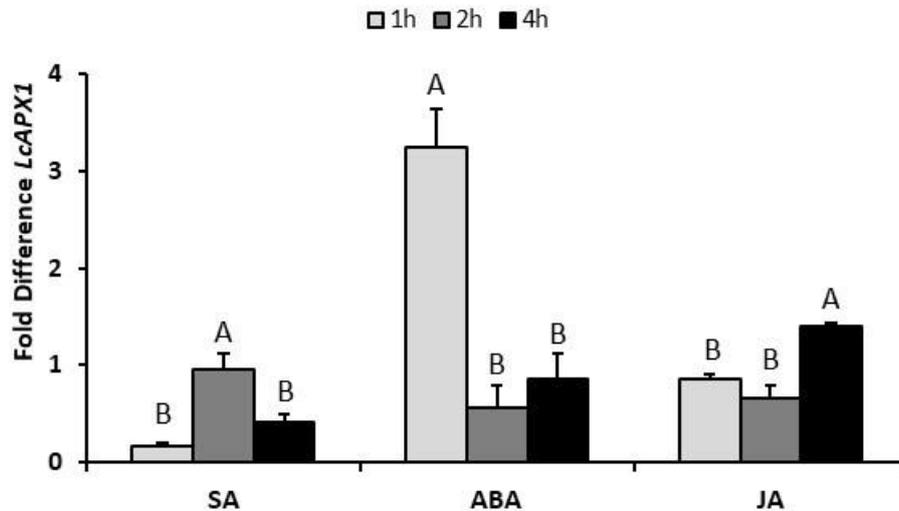


Fig. 6. Relative expression levels of *LcAPX1* in 14-day old seedlings subjected to exogenous application of A: JA (100 μ M), B: ABA (100 μ M), and C: SA (1 mM) for 1, 2, and 4 h. The expression levels were measured by qRT-PCR. The expression of *LcAPX1* valued was normalized to *LcACT1* reference gene, 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 \pm standard error (SE). Significantly different values are indicated by different letters above the bars.

Discussion

Cloning and sequence analysis of *LcAPX1*

The present study attempted to identify a member of this APX gene family in lentil. A full length cDNA was cloned and designated as *LcAPX1* (GenBank accession number: MH167389). The full length of *LcAPX1* cDNA is 951 bp, and the sequence has an in-frame stop codon (position 834) found downstream of the first ATG (position 82) suggesting that the cDNA is full length (Fig. 1).

Prediction of protein subcellular localization indicated that *LcAPX1* protein is most probably cytosolic, which implies the lack of chloroplast transit peptide and peroxisomal targeting signal from *LcAPX1* protein. APX isozymes are known to exhibit different subcellular localization based on the presence of organelle-specific targeting peptide and transmembrane domains. Members of the APX protein family can be targeted to chloroplasts, peroxisomes, mitochondria and cytoplasm (Sharma and Dubey, 2005; Teixeira et al., 2004).

Several reports have highlighted the importance of cytosolic APX as a key player in regulating H_2O_2 levels under abiotic stresses, such as drought, heat, high salt, wounding, and high light (Koussevitzky et al., 2008; Maruta et al., 2009; Wu et al., 2014). It has also been reported that cytosolic APX is also essential for proper functioning of chloroplastic APXs (Dayletova et al., 2005). Chloroplastic APX protects the photosynthetic apparatus against oxidative stress, whereas the cytosolic APX has a more general stress-protective function and can be activated by different kinds of stresses like salinity, drought, ozone, excessive light, heat, heavy metal and sulfur dioxide stress (Panchuk et al., 2005).

Protein BLAST indicated that the deduced amino acid sequence of *LcAPX1* displayed high sequence similarity with other plant cytosolic APX available from GenBank, which further indicates that *LcAPX1* is a cytosolic protein.

Multiple sequence alignment was accomplished using Clustal Omega (Fig. 2). Results showed that *LcAPX1* protein contained all the conserved regions of APX proteins. These comprise APX active site and the proximal heme-ligand motif. These functional motifs are required for the function, conformation, and structure of the APX proteins (Kjaersgard et al., 1997; Bhatt et al., 2013).

In an effort to characterize the phylogenetic relationship among different plant APX proteins, a neighbor-joining phylogenetic tree was constructed (Fig. 3). The resulting tree distributed plant APX proteins into three groups (cytosolic, peroxisomal, and chloroplastic APX proteins) which reflect the subcellular localization of these proteins to the corresponding compartment. The obvious divergence revealed by the APX phylogenetic tree between cytosolic, chloroplastic, and peroxisomal isoforms was in agreement with Li et al. (2007). A comprehensive molecular evolutionary analysis of the APX gene family was performed by Teixeira et al. (2004). They suggested that peroxisomal and cytosolic APX isoforms were evolved via duplication events of a non chloroplastic gene. Results from the present work indicated that LcAPX1 protein clustered with the group of cytosolic APX proteins. This result confirms the prediction of subcellular localization of LcAPX1.

Expression analysis of LcAPX1 gene

Plants are frequently exposed to different biotic and abiotic environmental stress factors (Bari and Jones, 2009; Negrão et al., 2017; Salman et al., 2017). As sessile organisms, and in order to respond and adapt to these stresses, plants developed various physiological and biochemical mechanisms (Asrar et al., 2020; Musallam et al., 2023). Plants adaptation to stress has been suggested to be mediated by both preexisting and induced defenses (Pastori and Foyer, 2002). Plant responses and defense are modulated by the activation of signal transduction pathways driven by phytohormone (Wasternack, 2007; Al-Momany and Abu-Romman, 2016), reactive oxygen species (ROS) (Mittler 2002), and nitric oxide (Sung and Hong, 2010). Previous studies have focused on changes in activity and gene expression for APX isozymes in higher plants subjected to environmental stresses, such as salt, extreme temperatures, high light, and ozone (Shigeoka et al., 2002). In the current study, transcriptional profiling of LcAPX1 was investigated to examine the effects of abiotic stresses (salinity, H₂O₂ and MV) and phytohormones (JA, ABA and SA) on LcAPX1 expression. Relative quantitative qRT-PCR showed that LcAPX1 was expressed in specific patterns under abiotic stresses and phytohormones (Fig. 4, 5 and 6).

Expression of LcAPX1 in response to abiotic stresses

Expression of LcAPX1 in response to salinity

Soil salinity is one of the major abiotic stresses affecting plant productivity in arid and semi-arid regions of the world (Al-Momany and Abu-Romman, 2023). There are two components affecting plants under high salt stress which are the osmotic stress and ionic stress (Demir and Kocakalican, 2002; Abdulfatah et al., 2021b). Salt stress affects all the major processes in plants such as growth, photosynthesis, protein synthesis, and lipid metabolism (Sobhanian et al., 2011; Abu-Romman and Suwwan, 2018).

LcAPX1 expression was monitored when lentil seedlings were subjected to salinity condition and results showed upregulation in LcAPX1 expression at 2 and 4 days of treatment (Fig. 4). Salinity stress also was shown to up-regulate the activity of cowpea VuAPX (Maia et al., 2010). Salinity stress up-regulated pea PsAPX (Hernandez et al., 1993, 1995, 1999). Kukreja et al. (2005) reported that the activity of APX increased in roots of *Medicago sativa* under NaCl treatment. Moreover, Rasool et al. (2013) reported that the enzymatic activity of APX in chickpea and the expression of CaAPX increased under salinity treatment. Thus, it could be concluded from the present study that LcAPX1 might be involved in plant oxidative stress responses resulting from salinity. In contrast, Park et al. (2004) reported that the sweet potato leaves treated with NaCl showed reduced expression of APX1. On the other hand, it has been shown in some plant species that the steady-state transcript levels of cytosolic APX were not affected by NaCl stress (Menezes-Benavente et al., 2004; Hong et al., 2007).

Expression of LcAPX1 in response to H₂O₂

H₂O₂ has been known in biological systems as a non-radical group of the ROS with the potential to damage biological macromolecules. This ROS species is produced by plants under normal conditions during the metabolic processes and acts as a key regulator in a broad range of physiological processes (Foreman et al., 2003; Quan et al., 2008). Furthermore, H₂O₂ is known to activate genes in defense signaling pathways (Kuśnierczyk et al., 2008; Hernandez et al., 2010; Al-Momany and Abu-Romman, 2014) and mediate molecular responses to biotic and abiotic stresses (Torres et al., 2002; Sharma et al., 2012). When accumulated at high concentrations, H₂O₂ can lead to oxidative stress in plants and trigger programmed cell death (Torres and Dangl, 2005).

LcAPX1 expression was rapidly induced by exogenous application of H₂O₂ on lentil seedlings (Fig. 5). Cowpea APX was also shown to be induced when plants were sprayed with H₂O₂ (Hasan et al., 2016). H₂O₂ treatment was very effective in increasing the enzymatic activity of APX in *Syzygium cumini* plant (Choudhary et al., 2012), and Lee et al. (1999) reported that the treatment of cultured soybean cells with exogenous H₂O₂ resulted in the alteration of cytosolic APX transcription levels. The upregulated of cytosolic LcAPX1 transcripts in response to H₂O₂ might indicate that H₂O₂ or a H₂O₂-derived signals are involved in LcAPX1 function to immediately detoxify H₂O₂, and therefore suggesting a possible role of LcAPX1 in oxidative stress responses.

Expression of LcAPX1 in response to MV

Methyl viologen (MV) is one of the commonly used reagents to study photooxidative stress in plants (Babatunde et al., 2014). MV generates superoxide anions and H₂O₂ in chloroplasts (Fujibe et al., 2004). The lower concentration of MV (1 μM) was sufficient to cause damage to photosynthetic apparatus, which could be seen by the responses of chlorophyll fluorescence parameters (Krieger-Liszkaya et al., 2011; Kasajima, 2017).

Several studies indicated that MV induces the photo accumulation of H₂O₂ and inactivates the stromal and thylakoid-bound APX due to the inhibitory effects of MV on the photoregeneration of AsA (Nakano and Asada, 1980). APX was shown to be inactivated when the concentration of AsA was lower than 20 μM, and therefore could not reduce H₂O₂ to water (Miyake and Asada, 1992; Mano et al., 2001). Furthermore, *in vivo* APX and SOD were shown to be largely inhibited by MV (Mano et al., 2001; Kim and Lee, 2005).

In this study, MV treatment down-regulated *LcAPX1* transcripts (Fig. 5), indicating that MV induced photooxidative stress in lentil, and the inactivation of *APX1* was attributed to the loss of AsA and accumulation of H₂O₂. In contrast, spinach treated with MV showed increased in cytosolic *APX* transcription (Yoshimura et al., 2000).

Transgenic plants over expressing *APX* showed elevated protection against MV-mediated oxidative stress (Kwon et al., 2002). These results have been reported in different plants such as in *Arabidopsis* (Abarca et al., 2001), *Hordeum glaucum* (Lasat et al., 1997), tobacco (Kwon et al., 2002) and pea (Allen et al., 1997).

Expression of *LcAPX1* in response to phytohormones

Plants are modulated by the activation of signal transduction pathways driven by phytohormone (Wasternack, 2007) and ROS (Mittler, 2002). Plant hormones regulate complex signaling networks involving developmental processes as well as plant responses to environmental stresses including biotic and abiotic stresses (Bari and Jones, 2009).

Expression of *LcAPX1* in response to JA

Jasmonates are lipid-derived signaling molecules stimulated by biotic and abiotic stresses (Wasternack, 2007) such as fungal elicitors (Gundlach et al., 1992) and wounding (Creelman et al., 1992). In addition, JA acts as an important signal transduction molecule involved in plant defense responses and can efficiently stimulate the production of secondary products in plant cells (Naill and Roberts, 2005).

Exogenous application of jasmonic acid was shown to alleviate the adverse effect of abiotic stresses in crop plants such as wounding in tobacco and soybean (Creelman et al., 1992; Seo et al., 2007), salt stress in barley (Walia et al., 2007) and water deficit in soybean (Reinbothe et al., 1992). Moreover, and in biotic stresses, JA contributes to enhanced resistance against insect herbivores and pathogens in tomato and *Arabidopsis* (Cooper and Goggin, 2005; Truman et al., 2007). In addition to its role in plant responses to stress, JA acts as a growth regulator influencing several developmental processes (Staswick et al., 1992; Creelman and Mullet, 1995; Berger et al., 1996; Saniewski et al., 1999).

The observed up-regulation of *LcAPX1* by exogenous application of JA (Fig. 6), can be explained by the increased production and accumulation of H₂O₂ in response to JA (Orozco-Cardenas and Ryan, 1999; Orozco-Cardenas et al., 2001). JA was shown to stimulate *APX* expression at the levels of mRNA (Örvar et al., 1997), protein (Rakwal et al., 1999) and enzyme activity (Maksymiec and Krupa, 2002).

Expression of *LcAPX1* in response to ABA

ABA plays a critical role in the modulation of plant responses and defense to different abiotic and biotic stresses (Bari and Jones, 2009; Atkinson and Urwin, 2012) and regulation of various developmental processes in plants (Christmann et al., 2006; Tuteja, 2007; Alazem and Lin, 2015).

LcAPX1 expression was early upregulated with ABA (Fig. 6) after 1 h of treatment, which suggests the presence of a signaling effect of ABA in the *LcAPX1* action. In agreement of these findings, the exogenous application of the ABA increased the expression of pea *APX1* (Mittler and Zilinskas, 1992), and rice *OsAPX1* and *OsAPX2* (Agrawal et al., 2003). Jiang and Zhang (2001) reported that treating maize with ABA resulted in increased activities of APX, suggesting that ABA can result in an oxidative stress in plants and expression of *ZmAPX* may be controlled by an ABA-dependent signaling pathway. In contrast, no effects on *APX* gene expression in *Brassica napus* in response to ABA (Vansuyt et al., 1997). Previous studies showed that exogenous application of ABA to different plant tissues enhanced the generation of ROS, including H₂O₂ (Guan et al., 2000; Pei et al., 2000), and this action increases the activities of antioxidative gene expression such as *APX* (Hu et al., 2006; Zhang et al., 2006). In addition, ABA can increase the content of redox ascorbate and glutathione metabolites (Foyer and Noctor, 2000; Jiang and Zhang, 2002).

Expression of *LcAPX1* in response to SA

SA is a plant signaling molecule which plays an important role in the defense responses against pathogen attack (Halim et al., 2006; Tuteja and Sopory, 2008) and induces the expression of pathogenesis-related proteins (Malamy et al., 1990). In addition, SA induce systemic acquired resistance in a variety of plants (Malamy and Klessig, 1992; Shirasu et al., 1997). Several studies support the roles of SA in modulating the plant response to several abiotic stresses (Khan et al., 2013; Alavi et al., 2014; Fayez and Bazaid, 2014). Moreover, SA affects a variety of developmental transitions (Martínez et al., 2004; Mohammed and Tarpley, 2013).

In this study, SA resulted in down-regulation of *LcAPX* (Fig. 6), suggesting that the exogenous application of SA increases the generation of ROS by inhibiting H₂O₂- detoxifying enzymes, including APX (Yuan and Lin, 2008; Barba-Espín et al., 2011). Furthermore, APX is post transcriptionally suppressed by SA (Mittler et al., 1998; Yuan and Lin, 2004). Moreover, Barba-Espín et al. (2011) reported that exogenous SA application decreased APX activity in pea plants, and inhibition of APX activity by SA was reported in tobacco (Durner and Klessig, 1996) and rice (Choudhury and Panda, 2004).

Materials and Methods

Plant material and treatment conditions

Seeds of lentil (*Lens culinaris* Medik. cv. Jordan 1) were grown in peatmoss and perlite in a ratio of 1:1 under greenhouse conditions. The seeds were irrigated with distilled water for two weeks. To investigate the gene expression patterns of APX gene, lentil seedlings were subjected to different treatments including abiotic stresses, signaling molecules and hormonal treatment. For salinity stress, plants were irrigated every two days with NaCl solution (150 mM) and leaf samples were collected at 0, 2, 4 and 6 days. For signaling molecules and hormonal treatments, different set of seedlings were sprayed with hydrogen peroxide (H₂O₂) (10 mM), methyl viologen (MV) (50 µM), jasmonic acid (JA) (100 µM), salicylic acid (SA) (1 mM), and abscisic acid (ABA) (100 µM) until leaf drop point. In all treatments, leaf samples were collected at 0, 1 h, 2 h, and 4 h of treatment. For both control and treatments, six leaves for each replicate were collected. The collected leaf tissues were quickly frozen in liquid nitrogen and stored at -20 °C for further analysis.

Isolation of total RNA and cDNA preparation

Total RNA was isolated from frozen leaf samples of lentil seedlings subjected to the above mentioned treatments using Spectrum™ Plant Total RNA Kit (Sigma). DNase treatment was performed on each binding column by adding 5 µl of (1 U/µl) DNase I (Zymo Research, USA) and 35 µl of DNase I digestion buffer. The concentration and purity of all RNA samples were assayed spectrophotometrically at 260 and 280 nm.

The first-strand cDNA was prepared from 500 ng of total RNA using primeScript™ MasterMix (Takara, Japan). The concentration and purity of all cDNA samples were assayed spectrophotometrically at 260 and 280 nm. cDNA samples were diluted to 1:10 with sterile RNase free water and stored at -20 °C for gene expression analysis.

Cloning of lentil APX Gene

In order to amplify the complete open reading frame (ORF) of APX gene from lentil, a candidate gene approach was followed. A pair of primers (5'-GAATTCGGCTTGTGCTCTCC -3') (sense, APX F) and (5'- TGCTACCCAAC TAAGATGAATCAC-3') (antisense, APX R) were designed based on the sequence of pea APX gene (GenBank accession No. X 62077.1). Lentil cDNA generated from two-week-old seedlings subjected to salinity (NaCl 150 mM) for two days, was used as a PCR template. The PCR reaction was performed in 0.2 ml microfuge tubes using the iNtRON i-MAXTM II system (iNtRON, Korea). A total of 25 µl of PCR reaction mixture containing about 500 ng template cDNA, 12.5 µl 2x master mix solution, 1 µl of each primer (10 µM) and distilled H₂O was added to make up the final volume of 25 µl. The condition of the PCR reaction was as follows: 95 °C for 5 min, 35 cycles of 95 °C for 30 s, 50 °C for 30 s and 72 °C for 2 min, then 72 °C for 10 min and 4 °C 30 min. PCR products were separated on 1 % low melting agarose gels. The single band of specific PCR product (951 bp) was cut from the gel with a sharp razor blade and purified using GeneJET Gel Extraction Kit (Thermo Scientific, USA) according to the manufacturer protocol. The eluted DNA concentration and purity were assayed using NanoDrop based on absorbance at 260 and 280 nm. The eluted DNA was stored at -20°C for cloning purposes. The purified PCR product was cloned using PGEM®-T Easy Vector (Promega, USA) and sequenced.

Bioinformatic analyses

The protein sequence of lentil APX (LcAPX) was obtained by ExPASy Translate tool (<http://web.expasy.org/translate/>), and ProtParam tool (<http://web.expasy.org/protparam/>) was employed to analyze the LcAPX protein physical and chemical parameters.

LcAPX subcellular localization 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/>). Similarity analyses were performed using BLAST algorithm

(<http://www.ncbi.nlm.nih.gov/BLAST/>), and protein domains were identified by searching the NCBI Conserved Domain Database (<http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>). Multiple sequence alignment analysis was carried out using Clustal Omega (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>). Phylogenetic analysis of APX proteins was performed by a neighbor-joining algorithm implemented in the MEGA 7 program, and branch confidence was assessed by bootstrapping analysis with 1000 replicates.

The following protein sequences (protein accession numbers in parentheses) were used to build the phylogenetic tree: *Arabidopsis thaliana* AtAPX1 (NP_001077481.1), AtAPX2 (NP_187575.2), AtAPX3 (NP_195226.1), AtAPX4 (NP_195321.1), AtAPX5 (NP_001328787.1), AtAPX6 (CAA67426), AtAPX7 (AAL07168); *Glycine max* GmAPX1 (NP_001237785.1), GmAPX2 (NP_001235587.1); *Eleusine coracana* EcAPX1 (AEI98602.1); *Prunus avium* PaAPX1 (APO15263.1); *Solanum lycopersicum* SlAPX1 (AAZ77770), SlAPX2 (AAZ77771), SlAPX3 (NP_001295260), SlAPX4 (ABA10744); *Daucus carota* DcAPX1 (AKH49594.1); *Brassica rapa* BrAPX1 (CCC55735.1); *Citrus maxima* CmAPX1 (ACM17463.1); *Gossypium hirsutum* GhAPX1 (ABR18607.1); *Plantago major* PmAPX1 (CAH59427.1); *Capsicum annuum* CaAPX1 (AAY21068.1); *Zea mays* ZmAPX1 (NP_001152249.1), ZmAPX2 (NP_0011105500), ZmAPX8 (AFW71783); *Arabidopsis lyrata* AlAPX1 (XP_002889670.1); *Hordeum vulgare* HvAPX1 (CAA06996.1), HvAPX2 (BAB62533.1); *Triticum aestivum* TaAPX1 (AAS80159.1), TaAPX2 (ABQ53157.1); *Solanum tuberosum* StAPX1 (XP_006366125.1), StAPX2 (BAC22953.1); *Ipomoea batatas* IbAPX1 (AAP42501.1), IbAPX2 (ALP06091.1); *Pisum sativum* PsAPX1 (CAA43992.1); *Vitis vinifera* VvAPX1 (ABX79340.1); *Medicago sativa* MsAPX1 (AIY27528.1); *Medicago truncatula* MtAPX1 (XP_003606510.1); *Cucumis melo* CmAPX1 (ABS42984.2); *Cucumis sativus* CsAPX1 (AGJ72850.1); *Citrus sinensis* CsiAPX1 (XP_006488195.10); *Citrus maxima* CmAPX2 (ACM17464.1); *Citrus limon* ClAPX1 (ADK38619.1); *Vigna unguiculata* VuAPX1 (AAS46016), VuAPX2 (AAS55853), VuAPX3

(AAS55852); *Oryza sativa* OsAPX1 (BAA08264), OsAPX2 (BAB17666), OsAPX3 (BAC79363.1), OsAPX4 (BAC793623.1); *Panax ginseng* PgAPX1 (ASK05453), PgAPX2 (ASK05454).

Gene expression analysis of lentil APX using quantitative RT-PCR

For the expression analysis, transcript levels from different treatments were monitored by quantitative RT-PCR using APX specific primers (5'- GGGGCTTAGTGATCAGGACATT-3') as sense primer and (5'- GCCTTCCTTCTCACCAGTCAA-3') as antisense primer. To verify equivalent loading of cDNA, *Actin* gene (GenBank accession No. MH167390) was used as an internal reference, which has low and stable gene expression in plants under abiotic stress, and *Actin* gene fragments were amplified using (5'- ATACCCCTGCCATGTATGTAGC - 3') as sense primer and (5'- AGCCAGATCAAGACGAAGGATG -3') as antisense primer. Primers were designed using the Primer3 program. A total of 20 µl of PCR reaction mixture were contained 10 µl of KAPA SYBR @FAST universal qPCR Kit (KAPA, U.S.A), 0.4 µl of 10 µM of each gene-specific primer, 150 ng/µl of diluted cDNA as a template, and RNase-free water was added to make up the final volume of 20 µl. Amplifications were performed for 2 min of an initial denaturation at 95 °C, 45 cycles of 10 s at 95 °C, 25 s at 57 °C, 25 s at 60 °C at which fluorescent was acquired. Final extension step was performed for 2 min at 60 °C (Biorad, U.S.A). All RT-PCR expressions were performed and analyzed in a completely randomized design (CRD) using three replicates for each sample. For measuring the level of gene expression, fold difference was calculated using the formula: $(\Delta C_T = C_T (\text{Target gene in treated sample}) - C_T (\text{Reference gene in treated sample})) - \Delta C_T = C_T (\text{Target gene in control sample}) - C_T (\text{Reference gene in control sample})$. $\Delta\Delta C_T = \Delta C_T (\text{treated}) - C_T (\text{control})$. Normalization target gene expression level = $2^{-\Delta\Delta C_T}$ (Livak

Statistical analysis

The bioassays were conducted with three replicates. The data from the experiments were subjected to analysis of variance (ANOVA) using SAS 9.4 for Windows. Differences between interval times of treatment means were determined by least significant difference (LSD) test at 5% confidence interval.

Conclusion

A full length cDNA of *LcAPX1* from lentil was cloned and characterized. The results showed that *LcAPX1* is a cytoplasmic protein and shared homology with cytoplasmic APXs from legumes. This gene is upregulated in response to salinity, H₂O₂, JA, and ABA. The present study provided the needed molecular results that can be used to elucidate the biological role of *LcAPX1* under stress conditions and in response to signaling molecules.

Author Contributions

SA-R conceived and designed the experiments; HR performed the experiments; SA-R, NO, and HR 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.

References

- Abarca D, Roldán M, Martín M, Sabater B (2001). *Arabidopsis thaliana* ecotype Cvi shows an increased tolerance to photo-oxidative stress and contains a new chloroplastic copper/zinc superoxide dismutase isoenzyme. *J Exp Bot.* 52(360):1417-1425. <https://doi.org/10.1093/jexbot/52.360.1417>
- Abdulfatah HF, Hassawi DS, Abu-Romman S (2021a). Differential physiological responses of three sesame genotypes to drought stress and the expression of antioxidant genes. *Ecol Environ Conserv.* 27:S47-S54. <https://www.envirobiotechjournals.com/EEC/v27octSupplIssue2021/EEC-7.pdf>
- Abdulfatah HF, Hassawi DS, Abu-Romman S (2021b). Physiological responses of sesame genotypes (*Sesamum indicum* L.) to salinity. *Indian J Ecol.* 48(17):403-409. https://www.researchgate.net/publication/358727150_Physiological_Responses_of_Sesame_Genotypes_Sesamum_indicum_L_to_Salinity
- Abu-Romman S (2019). Molecular cloning and gene expression analysis of chloroplastic copper/zinc superoxide dismutase gene in *Vicia sativa* L. *Res Crops.* 20(1):215-222. <https://doi.org/10.31830/2348-7542.2019.031>
- Abu-Romman S, Alzubi J (2016). Transcriptome analysis of *Arabidopsis thaliana* in response to cement dust. *Genes Genomics.* 38(9):865-878. <https://doi.org/10.1007/s13258-016-0432-4>
- Abu-Romman S, Suwwan MA (2018). Changes in osmotic potential, protein content and proline accumulation in response to NaCl salinity and phosphorus in cucumber microshoots grown on proliferation medium. *Res Crops.* 19(1):107-112. <https://doi.org/10.5958/2348-7542.2018.00018.9>

- Agrawal GK, Jwa NS, Iwahashi H, Rakwal R (2003). Importance of ascorbate peroxidases OsAPX1 and OsAPX2 in the rice pathogen response pathways and growth and reproduction revealed by their transcriptional profiling. *Gene* 322:93-103. <https://doi.org/10.1016/j.gene.2003.08.017>
- Alavi NSM, Arvin MJ, Kalantari KM (2014). Salicylic acid and nitric oxide alleviate osmotic stress in wheat (*Triticum aestivum* L.) seedlings. *J Plant Interact.* 9(1):683-688. <https://doi.org/10.1080/17429145.2014.900120>
- Alazem M, Lin NS (2015). Roles of plant hormones in the regulation of host-virus interactions. *Mol Plant Pathol.* 16(5):529-540. <https://doi.org/10.1111/mpp.12204>
- Allen RD, Webb RP, Schake SA (1997). Use of transgenic plants to study antioxidant defenses. *Free Radic Biol and Med.* 23(3):473-479. [https://doi.org/10.1016/S0891-5849\(97\)00107-X](https://doi.org/10.1016/S0891-5849(97)00107-X)
- Al-Momany B, Abu-Romman S (2014). Cloning and molecular characterization of a flavin-dependent oxidoreductase gene from barley. *J Appl Genet.* 55(4):457-468. <https://doi.org/10.1007/s13353-014-0227-8>
- Al-Momany B, Abu-Romman S (2016). Homologs of old yellow enzyme in plants. *Aust J Crop Sci.* 10(4):584-590. <https://doi.org/10.21475/ajcs.2016.10.04.p7555x>
- Al-Momany B, Abu-Romman S (2023). Cucumber and salinity. *Aust J Crop Sci.* 17(7):581-590. <https://doi.org/10.21475/ajcs.23.17.06.p3915>
- Apel K, Hirt H (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Ann Rev Plant Biol.* 55:373-399. <https://doi.org/10.1146/annurev.arplant.55.031903.141701>
- Asensi-Fabado M-A, Amtmann A, Perrella G (2017). Plant responses to abiotic stress: The chromatin context of transcriptional regulation. *Biochim Biophys Acta (BBA) – Gene Regul Mechanisms.* 1860(1):106-122. <https://doi.org/10.1016/j.bbagr.2016.07.015>
- Asrar H, Hussain T, Qasim M, Nielsen BL, Gul B, Khan MA (2020). Salt induced modulations in antioxidative defense system of *Desmostachya bipinnata*. *Plant Physiol Biochem.* 147:113-124. <https://doi.org/10.1016/j.plaphy.2019.12.012>
- Atkinson NJ, Urwin PE (2012). The interaction of plant biotic and abiotic stresses: from genes to the field. *J Exp Bot.* 63(10):352-3543. <https://doi.org/10.1093/jxb/ers100>
- Babatunde MM, Oladimeji AA (2014). Comparative study of acute toxicity of paraquat and galex to *Oreochromis niloticus*. *Intern J Adv Sci Techn Res.* 4(3):437-444. <https://rspublication.com/ijst/2014/june14/41.pdf>
- Barba-Espín G, Clemente-Moreno MJ, Alvarez S, García-Legaz MF, Hernández JA, Díaz-Vivancos P (2011). Salicylic acid negatively affects the response to salt stress in pea plants. *Plant Biol.* 13(6):909-917. <https://doi.org/10.1111/j.1438-8677.2011.00461.x>
- Bari R, Jones JD (2009). Role of plant hormones in plant defense responses. *Plant Mol Biol.* 69(4):473-488. <https://doi.org/10.1007/s11103-008-9435-0>
- Berger S, Bell E, Mullet JE (1996). Two methyl jasmonate-insensitive mutants show altered expression of *AtVsp* in response to methyl jasmonate and wounding. *Plant Physiol.* 111(2):525-531. <https://doi.org/10.1104/pp.111.2.525>
- Bhatt D, Saxena SC, Jain S, Dobriyal AK, Majee M, Arora S (2013). Cloning, expression and functional validation of drought inducible ascorbate peroxidase (*Ec-apx1*) from *Eleusine coracana*. *Mol Biol Rep.* 40(2):1155-1165. <https://doi.org/10.1007/s11033-012-2157-z>
- Blokhina O, Virolainen E, Fagerstedt KV (2003). Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Ann Bot.* 91(2):179-194. <https://doi.org/10.1093/aob/mcf118>
- Chew O, Whelan J, Millar AH (2003). Molecular definition of the ascorbate-glutathione cycle in Arabidopsis mitochondria reveals dual targeting of antioxidant defenses in plants. *J Biol Chem.* 278(47):46869-46877. <https://doi.org/10.1074/jbc.M307525200>
- Choudhary R, Saroha AE, Swarnkar PL (2012). Effect of abscisic acid and hydrogen peroxide on antioxidant enzymes in *Syzygium cumini* plant. *J Food Sci Technol.* 49(5):649-652. <https://doi.org/10.1007/s13197-011-0464-3>
- Choudhury S, Panda SK (2004). Role of salicylic acid in regulating cadmium induced oxidative stress in *Oryza sativa* L. roots. *Bulg J Plant Physiol* 30(3-4):95-110. http://www.bio21.bas.bg/ipp/gapbfiles/v-30/04_3-4_95-110.pdf
- Christmann A, Moes D, Himmelbach A, Yang Y, Tang Y, Grill E (2006). Integration of abscisic acid signalling into plant responses. *Plant Biol.* 8(3):314-325. <https://doi.org/10.1055/s-2006-924120>
- Cooper WR, Goggin FL (2005). Effects of jasmonate-induced defenses in tomato on the potato aphid, *Macrosiphum euphorbiae*. *Entomol Exp Appl.* 115(1):107-115. <https://doi.org/10.1111/j.1570-7458.2005.00289.x>
- Cramer GR, Urano K, Delrot S, Pezzotti M, Shinozaki K (2011). Effects of abiotic stress on plants: a systems biology perspective. *BMC Plant Biol.* 11(1):1-14. <https://doi.org/10.1186/1471-2229-11-163>
- Creelman RA, Mullet JE (1995). Jasmonic acid distribution and action in plants: regulation during development and response to biotic and abiotic stress. *Proc Natl Acad Sci USA.* 92(10):4114-4119. <https://doi.org/10.1073/pnas.92.10.4114>
- Creelman RA, Tierney ML, Mullet JE (1992). Jasmonic acid/methyl jasmonate accumulate in wounded soybean hypocotyls and modulates wound gene expression. *Proc Natl Acad Sci USA.* 89(11):4938-4941. <https://doi.org/10.1073/pnas.89.11.4938>
- D'Arcy-Lameta A, Ferrari-Iliou R, Contour-Ansel D, Pham-Thi AT, Zully-Fodil Y (2005). Isolation and characterization of four ascorbate peroxidase cDNAs responsive to water deficit in cowpea leaves. *Ann Bot.* 97(1):133-140. <https://doi.org/10.1093/aob/mcj010>
- Davletova S, Rizhsky L, Liang H, Shengqiang Z, Oliver DJ, Coutu J, Shulaev V, Schlauch K, Mittler R (2005). Cytosolic ascorbate peroxidase 1 is a central component of the reactive oxygen gene network of Arabidopsis. *Plant Cell.* 17(1):268-281. <https://doi.org/10.1105/tpc.104.026971>

- Demir Y, Kocaçalışkan I (2002). Effect of NaCl and proline on bean seedlings cultured in vitro. *Biol Plant*. 45(4):597-599. <https://doi.org/10.1023/A:1022343101727>
- Durner J, Klessig DF (1996). Salicylic acid is a modulator of tobacco and mammalian catalases. *J Biol Chem*. 271(45):28492-28501. <https://doi.org/10.1074/jbc.271.45.28492>
- Fayez KA, Bazaid SA (2014). Improving drought and salinity tolerance in barley by application of salicylic acid and potassium nitrate. *J Saudi Soc Agric Sci*. 13(1):45-55. <https://doi.org/10.1016/j.jssas.2013.01.001>
- Foreman J, Demidchik V, Bothwell JH, Mylona P, Miedema H, Torres MA, Linstead P, Costa S, Brownlee C, Jones JDG, Davies JM, Dolan, L (2003). Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. *Nature*. 422:442-446. <https://doi.org/10.1038/nature01485>
- Foyer CH, Noctor G (2011). Ascorbate and glutathione: the heart of the redox hub. *Plant Physiol*. 155(1):2-18. <https://doi.org/10.1104/pp.110.167569>
- Foyer CH, Noctor G (2000). Oxygen processing in photosynthesis: regulation and signaling. *The New Phytol*. 146:359-388. <https://doi.org/10.1046/j.1469-8137.2000.00667.x>
- Fujibe T, Saji H, Arakawa K, Yabe N, Takeuchi Y, Yamamoto KT (2004). A methyl viologen-resistant mutant of *Arabidopsis*, which is allelic to ozone-sensitive *rcd1*, is tolerant to supplemental ultraviolet-B irradiation. *Plant Physiol*. 134(1):275-285. <https://doi.org/10.1104/pp.103.033480>
- Gill SS, Tuteja N (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem*. 48(12):909-930. <https://doi.org/10.1016/j.plaphy.2010.08.016>
- Gimenez E, Salinas M, Manzano-Agugliaro F (2018). Worldwide research on plant defense against biotic stresses as improvement for sustainable agriculture. *Sustainability*. 10(2):391. <https://doi.org/10.3390/su10020391>
- Guan LM, Zhao J, Scandalios JG (2000). Cis-elements and trans-factors that regulate expression of the maize *Cat1* antioxidant gene in response to ABA and osmotic stress: H₂O₂ is the likely intermediary signaling molecule for the response. *Plant J*. 22(2):87-95. <https://doi.org/10.1046/j.1365-313x.2000.00723.x>
- Gundlach H, Müller MJ, Kutchan TM, Zenk MH (1992). Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. *Proc Natl Acad Sci USA*. 89(6):2389-2393. <https://doi.org/10.1073/pnas.89.6.2389>
- Halim VA, Vess A, Scheel D, Rosahl S (2006). The role of salicylic acid and jasmonic acid in pathogen defense. *Plant Biol*. 8(3):30-313. <https://doi.org/10.1055/s-2006-924025>
- Hasan H S, Al-Hadid KJ, Abu-Romman S (2017). *Sarcopoterium spinosum* (L.) Spach aqueous extract inhibits seed germination and seedling growth of winter wheat (*Triticum durum* Desf.). *Res Crops*. 18(2): 210-215. <https://doi.org/10.5958/2348-7542.2017.00035.3>
- Hasan SA, Irfan M, Masrahi YS, Khalaf MA, Hayat S (2016). Growth, photosynthesis, and antioxidant responses of *Vigna unguiculata* L. treated with hydrogen peroxide. *Cogent Food Agric*. 2(1):1-13. <https://doi.org/10.1080/23311932.2016.1155331>
- Hernandez JA, Campillo A, Jimenez A, Alarcon JJ, Sevilla F (1999). Response of antioxidant systems and leaf water relations to NaCl stress in pea plants. *New Phytol*. 141(2):241-251. <https://doi.org/10.1080/23311932.2016.1155331>
- Hernandez M, Fernandez-Garcia N, Diaz-Vivancos P, Olmos E (2010). A different role for hydrogen peroxide and the antioxidative system under short and long salt stress in *Brassica oleracea* roots. *J Expl Bot*. 61(2):521-535. <https://doi.org/10.1093/jxb/erp321>
- Hong CY, Hsu YT, Tsai YC, Kao CH (2007). Expression of ascorbate peroxidase 8 in roots of rice (*Oryza sativa* L.) seedlings in response to NaCl. *J Expl Bot*. 58(12):3273-3283. <https://doi.org/10.1093/jxb/erp321>
- Hu X, Zhang A, Zhang J, Jiang M (2006). Abscisic acid is a key inducer of hydrogen peroxide production in leaves of maize plants exposed to water stress. *Plant Cell Physiol*. 47(11):1484-1495. <https://doi.org/10.1093/pcp/pcl014>
- Jiang M, Zhang, J (2002). Role of abscisic acid in water stress-induced antioxidant defense in leaves of maize seedlings. *Free Radic Res*. 36(9):1001-1015. <https://doi.org/10.1080/1071576021000006563>
- Jones DK, Dalton DA, Rosell FI, Raven EL (1998). Class I heme peroxidases: characterization of soybean ascorbate peroxidase. *Arch Biochem Biophys*. 360(2):173-178. <https://doi.org/10.1006/abbi.1998.0941>
- Kasajima I (2017). Difference in oxidative stress tolerance between rice cultivars estimated with chlorophyll fluorescence analysis. *BMC Res Notes*. 10(1):168. <https://doi.org/10.1186/s13104-017-2489-9>
- Khan MIR, Khan NA (2013). Salicylic acid and jasmonates: approaches in abiotic stress tolerance. *J Plant Biochem Physiol*. 1(4):e113. <https://doi.org/10.4172/2329-9029.1000e113>
- Kim JH, Lee CH (2005). *In vivo* deleterious effects specific to reactive oxygen species on photosystem I and II after photo-oxidative treatments of rice (*Oryza sativa* L.) leaves. *Plant Sci*. 168(4):1115-1125. <https://doi.org/10.1016/j.plantsci.2004.12.012>
- Kjaersgard IV, Jespersen HM, Østergaard L, Welinder KG (1997). From sequence analysis of three novel ascorbate peroxidases from *Arabidopsis thaliana* to structure, function and evolution of seven types of ascorbate peroxidase. *Biochem J* 326(2):305-310. <https://doi.org/10.1042/bj3260305>
- Koussevitzky S, Suzuki N, Huntington S, Armijo L, Sha W, Cortes D, Shulaev V, Mittler R (2008). Ascorbate peroxidase 1 plays a key role in the response of *Arabidopsis thaliana* to stress combination. *J Biol Chem*. 283(49):34197-34203. <https://doi.org/10.1074/jbc.M806337200>
- Krieger-Liszkay A, Kós P B, Hideg É (2011). Superoxide anion radicals generated by methylviologen in photosystem I damage photosystem II. *Physiol Plant*. 142(1):17-25. <https://doi.org/10.1111/j.1399-3054.2010.01416.x>

- Kukreja S, Nandwal AS, Kumar N, Sharma SK, Unvi V, Sharma PK (2005). Plant water status, H₂O₂ scavenging enzymes, ethylene evolution and membrane integrity of *Cicer arietinum* roots as affected by salinity. *Biol Plant*. 49(2):305-308. <https://doi.org/10.1007/s10535-005-5308-4>
- Kuśnierczyk A, Winge PER, Jørstad TS, Troczyńska J, Rossiter J T, Bones AM (2008). Towards global understanding of plant defense against aphids—timing and dynamics of early *Arabidopsis* defense responses to cabbage aphid (*Brevicoryne brassicae*) attack. *Plant Cell Environ*. 31(8):1097-1115. <https://doi.org/10.1111/j.1365-3040.2008.01823.x>
- Kwon SY, Jeong YJ, Lee HS, Kim JS, Cho KY, Allen RD, Kwak SS (2002). Enhanced tolerances of transgenic tobacco plants expressing both superoxide dismutase and ascorbate peroxidase in chloroplasts against methyl viologen-mediated oxidative stress. *Plant Cell Environ*. 25(7): 873-882. <https://doi.org/10.1046/j.1365-3040.2002.00870.x>
- Lasat MM, DiTomaso JM, Hart JJ, Kochian LV (1997). Evidence for vacuolar sequestration of paraquat in roots of a paraquat-resistant *Hordeum glaucum* biotype. *Physiol Plant*. 99(2): 255-262. <https://doi.org/10.1111/j.1399-3054.1997.tb05410.x>
- Lazzarotto F, Teixeira FK, Rosa SB, Dunand C, Fernandes CL, de Vasconcelos Fontenele A, Silveria JAG, Verli H, Margis R, Margis-Pinheiro M (2011). Ascorbate peroxidase-related (APx-R) is a new heme-containing protein functionally associated with ascorbate peroxidase but evolutionarily divergent. *New Phytol*. 191(1):234-250. <https://doi.org/10.1111/j.1469-8137.2011.03659.x>
- Lee SC, Kang BG, Oh SE (1999). Induction of ascorbate peroxidase by ethylene and hydrogen peroxide during growth of cultured soybean cells. *Mol Cell*. 9(2):166-171. [https://doi.org/10.1016/S1016-8478\(23\)13525-4](https://doi.org/10.1016/S1016-8478(23)13525-4)
- Li YJ, Hai RL, Du XH, Jiang XN, Lu H (2009). Over-expression of a *Populus* peroxisomal ascorbate peroxidase (*PpAPX*) gene in tobacco plants enhances stress tolerance. *Plant Breed*. 128(4):404-410. <https://doi.org/10.1111/j.1439-0523.2008.01593.x>
- Li HB, Qin YM, Pang Y, Song WQ, Mei WQ, Zhu YX (2007). A cotton ascorbate peroxidase is involved in hydrogen peroxide homeostasis during fiber cell development. *New Phytol*. 175(3):462-471. <https://doi.org/10.1111/j.1469-8137.2007.02120.x>
- Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta$ CT method. *Methods*. 25(4):402-408. <https://doi.org/10.1006/meth.2001.1262>
- Madhusudhan R, Ishikawa T, Sawa Y, Shigeoka S, Shibata H (2003). Characterization of an ascorbate peroxidase in plastids of tobacco BY-2 cells. *Physiol Plant*. 117(4):550-557. <https://doi.org/10.1034/j.1399-3054.2003.00066.x>
- Maheshwari R, Dubey RS (2009). Nickel-induced oxidative stress and the role of antioxidant defense in rice seedlings. *Plant Growth Regul*. 59(1):37-49. <https://doi.org/10.1007/s10725-009-9386-8>
- Maia JM, De Macedo CC, Voigt EL, Freitas JBS, Silveira JAG (2010). Antioxidative enzymatic protection in leaves of two contrasting cowpea cultivars under salinity. *Biol Plant*. 54(1):159-163. <https://doi.org/10.1007/s10535-010-0026-y>
- Maksymiec W, Krupa Z (2002). Jasmonic acid and heavy metals in *Arabidopsis* plants—a similar physiological response to both stressors. *J Plant Physiol*. 159(5):509-515. <https://doi.org/10.1078/0176-1617-00610>
- Malamy J, Klessig DF (1992). Salicylic acid and plant disease resistance. *Plant J*. 2(5):643-654. https://doi.org/10.1007/1-4020-5184-0_12
- Malamy J, Carr JP, Klessig DF, Raskin I (1990). Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection. *Science*. 250(4983):1002-1004. <https://doi.org/10.1126/science.250.4983.1002>
- Mano JI, Ohno C, Domae Y, Asada K (2001). Chloroplastic ascorbate peroxidase is the primary target of methylviologen-induced photooxidative stress in spinach leaves: its relevance to monodehydroascorbate radical detected with *in vivo* ESR. *Biochim Biophys Acta (BBA)-Bioenerg*. 1504(2-3):275-287. [https://doi.org/10.1016/S0005-2728\(00\)00256-5](https://doi.org/10.1016/S0005-2728(00)00256-5)
- Martínez C, Pons E, Prats G, León J (2004). Salicylic acid regulates flowering time and links defense responses and reproductive development. *Plant J*. 37(2):209-217. <https://doi.org/10.1046/j.1365-313x.2003.01954.x>
- Maruta T, Tanouchi A, Tamoi M, Yabuta Y, Yoshimura K, Ishikawa T, Shigeoka S (2009). *Arabidopsis* chloroplastic ascorbate peroxidase isoenzymes play a dual role in photoprotection and gene regulation under photooxidative stress. *Plant Cell Physiol*. 51(2):190-200. <https://doi.org/10.1093/pcp/pcp177>
- Menezes-Benavente L, Teixeira FK, Kamei CLA, Margis-Pinheiro M (2004). Salt stress induces altered expression of genes encoding antioxidant enzymes in seedlings of a Brazilian indica rice (*Oryza sativa* L.). *Plant Sci*. 166(2):323-331. <https://doi.org/10.1016/j.plantsci.2003.10.001>
- Mittler R (2002). Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci* 7(9):405-410. [https://doi.org/10.1016/S1360-1385\(02\)02312-9](https://doi.org/10.1016/S1360-1385(02)02312-9)
- Mittler R, Zilinskas BA (1992). Molecular cloning and characterization of a gene encoding pea cytosolic ascorbate peroxidase. *J Biol Chem*. 267(30):21802-21807. [https://doi.org/10.1016/S0021-9258\(19\)36683-9](https://doi.org/10.1016/S0021-9258(19)36683-9)
- Mittler R, Feng X, Cohen M (1998). Post-transcriptional suppression of cytosolic ascorbate peroxidase expression during pathogen-induced programmed cell death in tobacco. *Plant Cell*. 10(3):461-473. <https://doi.org/10.1105/tpc.10.3.461>
- Miyake C, Asada K (1992). Thylakoid-bound ascorbate peroxidase in spinach chloroplasts and photoreduction of its primary oxidation product monodehydroascorbate radicals in thylakoids. *Plant Cell Physiol*. 33(5):541-553. <https://doi.org/10.1093/oxfordjournals.pcp.a078288>
- Mohammed AR, Tarpley L (2013). Effects of enhanced ultraviolet-B (UV-B) radiation and antioxidative-type plant growth regulators on rice (*Oryza sativa* L.) leaf photosynthetic rate, photochemistry and physiology. *J Agric Sci*. 5(5):115-128. <https://doi.org/10.5539/jas.v5n5p115>
- Musallam A, Abu-Romman S, Saddler MT (2023). Molecular characterization of Dehydrin in Azraq saltbush among related *Atriplex* species. *BioTech*. 12(2):27. <https://doi.org/10.3390/biotech12020027>

Naill MC, Roberts SC (2005). Cell cycle analysis of *Taxus* suspension cultures at the single cell level as an indicator of culture heterogeneity. *Biotechnol Bioeng.* 90(4):491-500. <https://doi.org/10.1002/bit.20446>

Najami N, Janda T, Barriah W, Kayam G, Tal M, Guy M, Volokita M (2008). Ascorbate peroxidase gene family in tomato: its identification and characterization. *Mol Genet Genom.* 279(2):171-182. <https://doi.org/10.1007/s00438-007-0305-2>

Nakano Y, Asada K (1980). Spinach chloroplasts scavenge hydrogen peroxide on illumination. *Plant Cell Physiol.* 21(8):1295-1307. <https://doi.org/10.1093/oxfordjournals.pcp.a076128>

Negrão S, Schmöckel SM, Tester M (2017). Evaluating physiological responses of plants to salinity stress. *Ann Bot.* 119(1):1-11. <https://doi.org/10.1093/aob/mcw191>

Ohama N, Sato H, Shinozaki K, Yamaguchi-Shinozaki K (2017). Transcriptional regulatory network of plant heat stress response. *Trends Plant Sci.* 22(1):53-65. <https://doi.org/10.1016/j.tplants.2016.08.015>

Orozco-Cárdenas ML, Narváez-Vásquez J, Ryan CA (2001). Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate. *Plant Cell.* 13(1):179-191. <https://doi.org/10.1105/tpc.13.1.179>

Orozco-Cardenas M, Ryan CA (1999). Hydrogen peroxide is generated systemically in plant leaves by wounding and systemin via the octadecanoid pathway. *Proc Natl Acad Sci USA.* 96(11):6553-6557. <https://doi.org/10.1073/pnas.96.11.6553>

Örvar BL, McPherson J, and Ellis BE (1997). Pre-activating wounding response in tobacco prior to high-level ozone exposure prevents necrotic injury. *Plant J.* 11(2):203-212. <https://doi.org/10.1046/j.1365-313x.1997.11020203.x>

Panchuk II, Zentgraf U, Volkov RA (2005). Expression of the *Apx* gene family during leaf senescence of *Arabidopsis thaliana*. *Planta.* 222(5):926-932. <https://doi.org/10.1007/s00425-005-0028-8>

Park SY, Ryu SH, Jang IC, Kwon SY, Kim JG, Kwak SS (2004). Molecular cloning of a cytosolic ascorbate peroxidase cDNA from cell cultures of sweetpotato and its expression in response to stress. *Mol Genet Genom.* 271(3):339-346. <https://doi.org/10.1007/s00438-004-0986-8>

Pastori GM, Foyer CH (2002). Common components, networks, and pathways of cross-tolerance to stress. The central role of "redox" and abscisic acid-mediated controls. *Plant Physiol.* 129(2):460-468. <https://doi.org/10.1104/pp.011021>

Pei ZM, Murata Y, Benning G, Thomine S, Klüsener B, Allen GJ, Grill E, Schroeder JI (2000). Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. *Nature.* 406(6797):731-734. <https://doi.org/10.1038/35021067>

Quan LJ, Zhang B, Shi WW, Li HY (2008). Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. *J Integ Plant Biol.* 50(1):2-18. <https://doi.org/10.1111/j.1744-7909.2007.00599.x>

Rakwal R, Agrawal GK, Yonekura M (1999). Separation of proteins from stressed rice (*Oryza sativa* L.) leaf tissues by two-dimensional polyacrylamide gel electrophoresis: Induction of pathogenesis-related and cellular protectant proteins by jasmonic acid, UV irradiation and copper chloride. *Electrophoresis.* 20(17):3472-3478. [https://doi.org/10.1002/\(SICI\)1522-2683\(19991101\)20:17<3472::AID-ELPS3472>3.0.CO;2-0](https://doi.org/10.1002/(SICI)1522-2683(19991101)20:17<3472::AID-ELPS3472>3.0.CO;2-0)

Rasool S, Ahmad A, Siddiqi TO, Ahmad P (2013). Changes in growth, lipid peroxidation and some key antioxidant enzymes in chickpea genotypes under salt stress. *Acta Physiol Plant.* 35(4):1039-1050. <https://doi.org/10.1007/s11738-012-1142-4>

Reinbothe S, Reinbothe C, Lehmann J, Parthier B (1992). Differential accumulation of methyl jasmonate-induced mRNAs in response to abscisic acid and desiccation in barley (*Hordeum vulgare*). *Physiol Plant.* 86(1):49-56. <https://doi.org/10.1093/oxfordjournals.pcp.a029244>

Salman M, Salameh N, Abu-Romman S (2017). Germination and seedling growth of barley as affected by 'Artemisia annua' water extract. *Plant Omics.* 10(1):1-6. <https://doi.org/10.21475/poj.10.01.17.241>

Saniewski M, Ueda J, Miyamoto K (1999). Interaction of ethylene with jasmonates in the regulation of some physiological processes in plants. In *Biology and biotechnology of the plant hormone ethylene II*. Springer, Dordrecht pp: 173-180. <https://doi.org/10.1093/jxb/erj103>

Scandalios JG (2005). Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. *Braz J Med Biol Res.* 38(7):995-1014. <https://doi.org/10.1590/s0100-879x2005000700003>

Seo S, Katou S, Seto H, Gomi K, Ohashi Y (2007). The mitogen-activated protein kinases WIPK and SIPK regulate the levels of jasmonic and salicylic acids in wounded tobacco plants. *Plant J.* 49(5):899-909. <https://doi.org/10.1111/j.1365-313X.2006.03003.x>

Sharma P, Dubey RS (2005). Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. *Plant Growth Regul.* 46(3):209-221. <https://doi.org/10.1007/s10725-005-0002-2>

Sharma P, Jha AB, Dubey RS, Pessarakli M (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J Bot.* 2012:1-26. <https://doi.org/10.1155/2012/217037>

Shi WM, Muramoto Y, Ueda A, Takabe T (2001). Cloning of peroxisomal ascorbate peroxidase gene from barley and enhanced thermotolerance by overexpressing in *Arabidopsis thaliana*. *Gene.* 273(1):23-27. [https://doi.org/10.1016/s0378-1119\(01\)00566-2](https://doi.org/10.1016/s0378-1119(01)00566-2)

Shigeoka S, Ishikawa T, Tamoi M, Miyagawa Y, Takeda T, Yabuta Y, Yoshimura K (2002). Regulation and function of ascorbate peroxidase isoenzymes. *J Exp Bot.* 53(372):1305-1319. <https://doi.org/10.1093/jexbot/53.372.1305>

Shirasu K, Nakajima H, Rajasekhar VK, Dixon RA, Lamb C (1997). Salicylic acid potentiates an agonist-dependent gain control that amplifies pathogen signals in the activation of defense mechanisms. *Plant Cell.* 9(2):261-270. <https://doi.org/10.1105/tpc.9.2.261>

- Sobhanian H, Aghaei K, Komatsu S (2011). Changes in the plant proteome resulting from salt stress: toward the creation of salt-tolerant crops. *J Proteom.* 74(8):1323-1337. <https://doi.org/10.1016/j.jprot.2011.03.018>
- Sofa A, Scopa A, Nuzzaci M, Vitti A (2015). Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses. *Int J Mol Sci.* 16(6):13561-13578. <https://doi.org/10.3390/ijms160613561>
- Staswick PE, Su W, Howell SH (1992). Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an *Arabidopsis thaliana* mutant. *Proc Natl Acad Sci USA.* 89(15):6837-6840. <https://doi.org/10.1073/pnas.89.15.6837>
- Sung CH, Hong JK (2010). Sodium nitroprusside mediates seedling development and attenuation of oxidative stresses in Chinese cabbage. *Plant Biotechnol Rep.* 4(4):243-251. <https://doi.org/10.1080/17429145.2021.2024286>
- Teixeira FK, Menezes-Benavente L, Margis R, Margis-Pinheiro M (2004). Analysis of the molecular evolutionary history of the ascorbate peroxidase gene family: inferences from the rice genome. *J Mol Evol.* 59(6):761-770. <https://doi.org/10.1007/s00239-004-2666-z>
- Torres MA, Dangl JL (2005). Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. *Curr Opin Plant Biol.* 8:397-403. <https://doi.org/10.1016/j.pbi.2005.05.014>
- Torres MA, Dangl JL, Jones JD (2002). *Arabidopsis* gp91^{phox} homologues *AtrbohD* and *AtrbohF* are required for accumulation of reactive oxygen intermediates in the plant defense response. *Proc Natl Acad Sci USA.* 99(1):517-522. <https://doi.org/10.1073/pnas.012452499>
- Truman W, Bennett MH, Kubigsteltig I, Turnbull C, and Grant M (2007). *Arabidopsis* systemic immunity uses conserved defense signaling pathways and is mediated by jasmonates. *Proc Natl Acad Sci USA.* 104(3):1075-1080. <https://doi.org/10.1073/pnas.0605423104>
- Tuteja N, Sopory SK (2008). Chemical signaling under abiotic stress environment in plants. *Plant Signal Behav.* 3(8):525-536. <https://doi.org/10.4161/psb.3.8.6186>
- Vansuyt G, Lopez F, Inzé D, Briat JF, Fourcroy P (1997). Iron triggers a rapid induction of ascorbate peroxidase gene expression in *Brassica napus*. *FEBS Lett.* 410(2-3):195-200. [https://doi.org/10.1016/s0014-5793\(97\)00587-5](https://doi.org/10.1016/s0014-5793(97)00587-5)
- Walia H, Wilson C, Condamine P, Liu X, Ismail AM, Close TJ (2007). Large-scale expression profiling and physiological characterization of jasmonic acid-mediated adaptation of barley to salinity stress. *Plant Cell Environ.* 30(4):410-421. <https://doi.org/10.1111/j.1365-3040.2006.01628.x>
- Wang WB, Kim YH, Lee HS, Kim KY, Deng XP, Kwak SS (2009). Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. *Plant Physiol Biochem.* 47(7):570-577. <https://doi.org/10.1016/j.plaphy.2009.02.009>
- Wasternack C (2007). Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Ann Bot.* 100(4):681-697. <https://doi.org/10.1093/aob/mcm079>
- Wu G, Wang G, Ji J, Gao H, Guan W, Wu J, Guan C, Wang Y (2014). Cloning of a cytosolic ascorbate peroxidase gene from *Lycium chinense* Mill. and enhanced salt tolerance by overexpressing in tobacco. *Gene.* 543(1):85-92. <https://doi.org/10.1016/j.gene.2014.03.061>
- Yang Y, Han C, Liu Q, Lin B, Wang J (2008). Effect of drought and low light on growth and enzymatic antioxidant system of *Picea asperata* seedlings. *Acta Physiol Plant.* 30(4):433-440. <https://doi.org/10.1007/s11738-008-0140-z>
- Yoshimura K, Yabuta Y, Ishikawa T, Shigeoka S (2000). Expression of spinach ascorbate peroxidase isoenzymes in response to oxidative stresses. *Plant Physiol.* 123(1):223-234. <https://doi.org/10.1104/pp.123.1.223>
- Yuan S, Lin HH (2004). Transcription, translation, degradation and circadian clock. *Biochem Biophys Res Commun.* 321(1):1-6. <https://doi.org/10.1016/j.bbrc.2004.06.093>
- Yuan S, Lin HH (2008). Minireview: role of salicylic acid in plant abiotic stress. *Z Naturforsch C.* 63(5-6):313-320. <https://doi.org/10.1515/znc-2008-5-601>
- Zhang A, Jiang M, Zhang J, Tan M, Hu X (2006). Mitogen-activated protein kinase is involved in abscisic acid-induced antioxidant defense and acts downstream of reactive oxygen species production in leaves of maize plants. *Plant Physiol.* 141(2):475-487. <https://doi.org/10.1104/pp.105.075416>