Pak. J. Bot., 50(5): 1769-1774, 2018.

EFFECTS OF HYDROGEN PEROXIDE ON INITIAL GROWTH AND ENZYMATIC ANTIOXIDANT SYSTEM OF *LACTUCA SATIVA* L (ASTERACEAE)

RODRIGO MIRANDA MORAES[1], MARÍLIA CARVALHO[1], FERNANDA CARLOTA NERY[2], PLINIO RODRIGUES SANTOS FILHO[3], MARINA LIMA NOGUEIRA[1] AND SANDRO BARBOSA[1*]

[1]*Universidade Federal de Alfenas; Departamento de Ciências da Natureza, Alfenas–MG, Brazil*
[2]*Universidade Federal de Lavras; Departamento de Biologia, Lavras – MG, Brazil*
[3]*Universidade Federal de Alfenas; Departamento de Bioquímica, Alfenas –MG, Brazil*
**Corresponding author's email: sandro.barbosa@unifal-mg.edu.br*

Abstract

Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), can function as signaling molecules in basic cellular processes in plants, or cause oxidative damage to biomolecules when in excess. The objective of this study was to evaluate H_2O_2 phytotoxicity on germination, growth, initial development and cell cycle, in addition to the response of the enzymatic antioxidant system of *L. sativa*. For this purpose, *L. sativa* seeds were exposed to H_2O_2 solutions at the following concentrations: 0.0, 1.0, 2.5, 5.0, 7.5 and 10 mM for a period of 7 days. After 24 hours of exposure to H_2O_2, there was a significant reduction in germination percentage from 2.5 mM. With the increase in H_2O_2 concentration, there was a reduction in root length, and seedling fresh and dry biomass. However, dry biomass was more affected by H_2O_2 when compared to fresh biomass. H_2O_2 significantly reduced mitotic index, but did not induce significant chromosomal abnormalities. The treatment with 7.5 mM H_2O_2 led to a significant proline accumulation. On the other hand, the activity of superoxide dismutase and ascorbate peroxidase increased significantly only at a concentration of 2.5 and above 5.0 mM, respectively. There was no significant activity of the enzymes catalase and guaiacol peroxidase. Therefore, the exposure to H_2O_2 resulted in proline accumulation, and an increase in antioxidant enzyme activity, in response to H_2O_2 increase to maintain redox homeostasis inside the cell.

Keywords: Lettuce, Bioassay, Mitotic index, Reactive oxygen species, Plant metabolism.

Introduction

Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), are metabolites constantly produced under normal conditions of the aerobic plant metabolism (Foyer & Noctor, 2009), and can function as signaling molecules in basic cellular processes (Sharma *et al.,* 2012). These ROS may act as second messengers, mediating responses to different biotic and abiotic stimuli (Yi *et al.,* 2014). However, they are detrimental when in excess, causing oxidative damage to biomolecules and, ultimately, cell death (Quan *et al.,* 2008).

H_2O_2 is considered moderately reactive, with a relatively long intracellular half-life (1 ms) (Karuppanapandian *et al.,* 2011), and therefore considered the most stable among ROS. Among its main physiological functions, the involvement in stomatal movement, root gravitropism, tolerance to oxygen deficiency, cell wall strengthening, senescence, phytoalexin production and cell cycle control can be highlighted (Yi *et al.,* 2014; Pokora *et al.,* 2017). However, since it has the ability to diffuse freely through the membranes, H_2O_2 can have a deleterious action, once it participates in the formation reaction of the hydroxyl radical ($OH^•$), the most reactive oxidant in the ROS family (Karuppanapandian*et al.,* 2011). Thus, excess H_2O_2 can inactivate enzymes by oxidation and cause damage to nucleic acids, proteins, carbohydrates and lipids (Gill & Tuteja, 2010; Mittler *et al.,* 2011).

For proper regulation of intra and extracellular ROS levels, plants have developed a complex antioxidant metabolism that includes both enzymatic and non-enzymatic activities (Sharma *et al.,* 2012). The coordinated activity of these antioxidants regulates detoxification and/or elimination of ROS by reducing oxidative damage (Choudhury *et al.,* 2013). Thus, there must be a balance between production and elimination of ROS, to regulate their metabolic signaling function, or to determine their role as cell-damaging molecules (Puthur, 2016).

In phytotoxicity studies, the use of bioassays with higher plants sensitive to the toxic agent is common. In this context. *Lactuca sativa* L. is widely used as a test organism (Reigosa *et al.,* 2013; Moraes *et al.,* 2015; Santos *et al*., 2017), due to its sensitivity to chemical agents, rapid germination, linear growth over a wide pH range and low sensitivity to osmotic potentials (Gomes *et al.,* 2012). Its use is also justified by the wide cultivation around the world and its representativeness in human food. Considering the lack of studies relating the application of H_2O_2 to *L. sativa*, and aiming to understand the performance of this compound and stress tolerance mechanisms, the objective of this study was to evaluate H_2O_2phytotoxicity on germination, growth, initial development and cell cycle, in addition to the enzymatic antioxidant activity of *L. sativa*.

Material and Methods

Plant material and treatments: For the evaluation of germination percentage (G %), root length (RL), fresh (FB) and dry biomass (DB), four replicates with 30 *L. sativa* seeds, cv. Grand Rapids were used. The seeds were randomly distributed in Petri dishes lined with a double layer of Whatman no. 2 paper, and moistened with 3 mL of H_2O_2 solution. The tested concentrations of H_2O_2 were 1.0, 2.5, 5.0, 7.5 and 10 mM, and distilled water was used as a negative control. For the quantification of proline, enzymatic antioxidant activity and lipid peroxidation, four

replicates with 50 seeds and 5 mL of H_2O_2 solution were used. For the cytogenetic characteristics: mitotic index (MI) and occurrence of chromosomal abnormalities (CA), three replicates with 30 seeds and 3 mL of H_2O_2 solution were used; 4,000 cells were analyzed per treatment. The plates containing the seeds were kept in a BOD germination chamber at 20 °C with a 12-hour photoperiod (Simões *et al.,* 2013). Both pH and osmotic potential of the solutions were evaluated using, respectively, a digital pH meter (TecnoponmPA 210 – Piracicaba - SP) and an automatic osmometer (Advanced Instruments - 3320 Micro-Osmometer – Norwood, MA).

Evaluation of phytotoxicity and cytotoxicity: G% was evaluated 24 and 48 hours after exposure to H_2O_2, counting the seeds that had a root protrusion of ± 0.1 cm. RL was obtained by the measurement of ten roots randomly selected at the end of the seventh day of cultivation, with the aid of a digital caliper. These plants were then weighed on an analytical scale (Marte, Shimadzu AY 220) for the determination of FB and placed in a forced air circulation oven (Nova Ética 400 ND) at 45ºC until constant weight for the determination of DB. For the determination of MI and AC, *L. sativa* root tips were obtained 24 hours after exposure to each treatment. The roots were fixed in Carnoy solution, hydrolyzed in 5M HCl at 25°C and stained with Schiff's reagent. Cytogenetic preparations were made according to Pereira *et al.,* (2013), and 4,000 cells were evaluated per treatment. MI consisted of counting the cells during the cell cycle, and was determined according to Moraes *et al.,*(2015).

Enzymatic antioxidant activity and proline content: For the extraction of antioxidant enzymes, 0.2 g fresh seedlings were macerated in liquid nitrogen, with 30% polyvinylpolypyrrolidone, and homogenized in 1.5 mL extraction buffer. The homogenates were centrifuged at 15.000 rpm for 10 minutes at 4°C, and the supernatants were collected for enzymatic analyses of superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and guaiacol peroxidase (POD) (Biemelt *et al.,* 1998). The activity of SOD (EC 1.15.1.1) was determined by the ability of the enzyme to inhibit the photochemical reduction of nitro blue tetrazolium (NBT), proposed by Giannopolitis and Ries (1977). A unit of SOD was defined as the ability of the enzyme to reduce 50% NBT. The activity of APX (EC 1.11.1.11) was determined by decreasing the absorbance of ascorbate (ε = 2.8 mM^{-1} cm^{-1}) at 290 nm every 15 seconds for 3 minutes, according to Nakano and Asada (1981). The activity of CAT (EC 1.11.1.6) was determined by decreasing the absorbance at 240 nm, every 15 seconds for 3 minutes, monitored by the consumption of hydrogen peroxide, as described by Havir & MicHale (1987). The activity of POD (EC 1.11.1.7) was determined by the increase in absorbance at 470 nm, observed in the formation of tetraguaiacol for 3 minutes (ε = 26.6 mM^{-1} cm^{-1}), according to Fang & Kao (2000). Proline content was determined at seven days of cultivation, according to the method described by Torello & Rice (1986). Color intensity was measured at 520 nm in a spectrophotometer (Halo SB-10 Dynamica Scientific) and the absorbances obtained were compared with the standard curve of proline.

Lipid peroxidation: Lipid peroxidation was determined by the quantification of thiobarbituric acid reactive species (TBARS), as described by Buege&Aust (1978). Readings were determined in a spectrophotometer (Halo SB-10 Dynamica Scientific) at 540 nm.The concentration of the MDA/TBA complex was calculated by the following equation: [MDA] = 540 / (ξ.b), where: ξ (extinction coefficient = 1.56 x 10^{-5} cm^{-1}); b (optical length = 1).

Statistical analysis: The experimental design was completely randomized, with six treatments (0.0, 1.0, 2.5, 5.0, 7.5 and 10 mM H_2O_2) and four replicates for phytotoxicity and biochemical parameters, and three replicates for cytological parameters. Data were submitted to analysis of variance and means were compared by the Scott-Knott test at 5% significance. The variables that did not meet the assumptions of normality and/or homogeneity of the residues were transformed by the square root.

Results

The pH values found in the solutions of the different treatments with H_2O_2 ranged from 5.36 to 5.57, while the values of osmotic potentials ranged from -0.09 to -1.30 MPa.

After 24 hours of exposure to H_2O_2, the concentrations 5.0, 7.5 and 10 mM significantly reduced seed germination percentage (G%) by 30%, 78% and 96%, respectively, in relation to the control treatment (Fig. 1A). However, at 48 hours, all treatments showed germination above 80%, not significantly different from each other; thus, the use of a positive control treatment was not required. Therefore, it was possible to observe that the germination of *L. sativa* seeds was more sensitive to H_2O_2 in the first 24 hours, when compared to the 48-hour exposure time (data not shown).

Root length (RL) of *L. sativa* seedlings was significantly reduced from the treatment with 1.0 mM H_2O_2 (Fig. 1B). Thus, the highest H_2O_2 concentrations (7.5 and 10 mM), resulted in a lower RL, with 3.36 and 2.02 mm, respectively.

The treatment with H_2O_2 reduced mitotic index (MI) at all tested concentrations (Fig. 1C). The reductions were 36.1%, 38.1%, 36.4%, 23.7% and 18.7% at the concentrations 1.0, 2.5, 5.0, 7.5 and 10 mM H_2O_2, respectively, when compared to the control treatment.

Seedling fresh biomass (FB) was significantly reduced only when exposed to concentrations of 7.5 and 10 mM H_2O_2 (Fig. 1D). However, concentrations from 1.0 mM H_2O_2 were sufficient to result in a decrease in dry biomass (DB) (Fig. 1D). Therefore, the DB of *L. sativa* seedlings was significantly more affected by the different H_2O_2 concentrations in relation to the FB, which was more sensitive to its phytotoxic action.

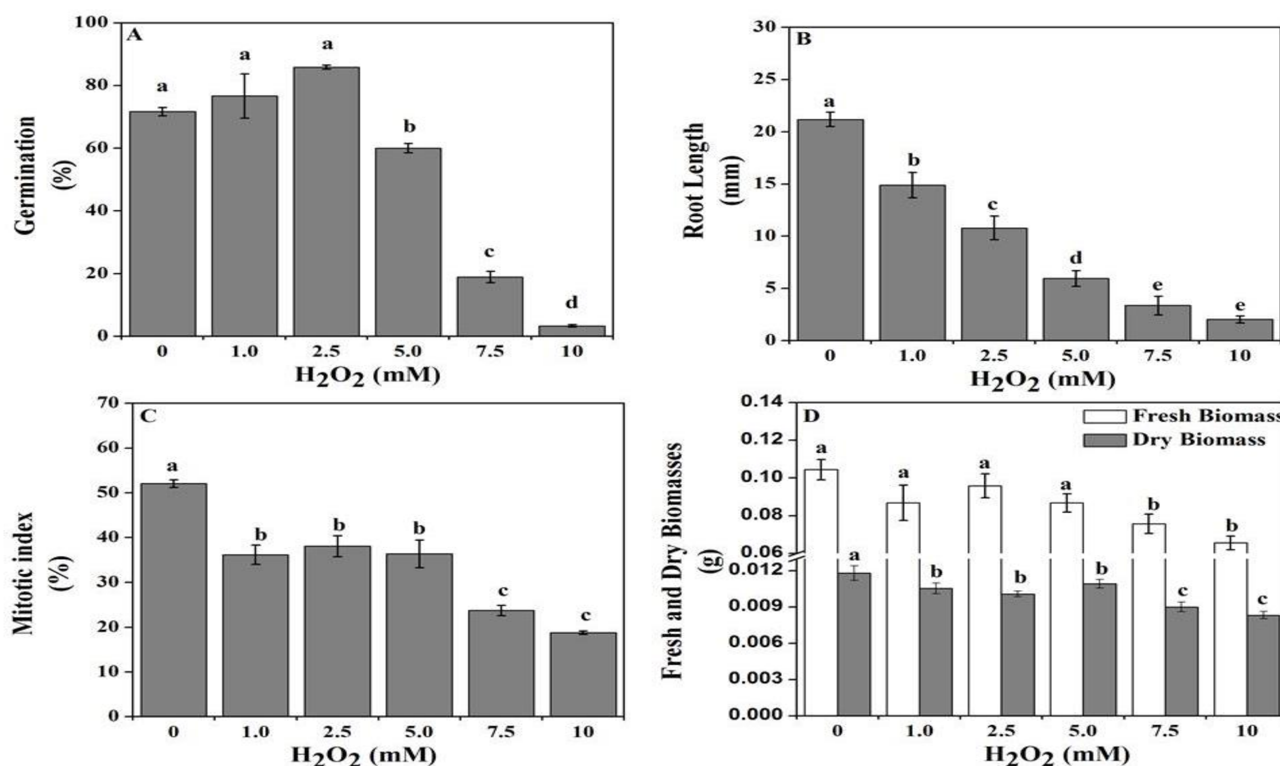

Fig. 1. (A) Germination percentage (G%) in 24 hours; (B) Root length (mm); (C) Mitotic index (%); (D) Fresh and Dry biomass (g) of *L. sativa* in relation to H_2O_2 concentrations. Bar: Standard error of 4 replicates.

There was a greater proline accumulation at 7.5 and 10 mM H_2O_2 (Fig. 2). However, the H_2O_2 concentrations used were not sufficient to cause a significant increase in lipid peroxidation and malondialdehyde levels (data not shown).

After 7 days of exposure to H_2O_2, the activity of superoxide dismutase (SOD) in *L. sativa* seedlings exposed to the treatment with 2.5 mM H_2O_2 increased significantly by 20% in relation to the control, differing from the other concentrations tested (Fig. 3A). In addition, there was a significant increase in the activity of ascorbate peroxidase (APX) by 78% at 5.0 mM H_2O_2 in relation to the other treatments (Fig. 3B). Thus, APX and SOD significantly increased their activity at intermediate H_2O_2 concentrations (2.5 and 5.0 mM) but were inhibited at the highest concentrations (7.5 and 10 mM). However, there was no significant activity of the enzymes catalase and guaiacol peroxidase (POD) (data not shown).

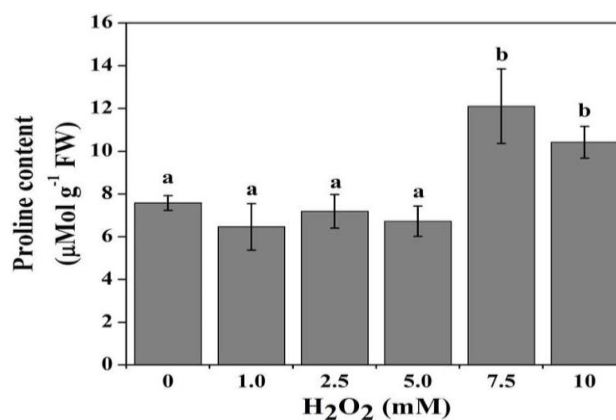

Fig. 2. Proline content in *L. sativa* seedlings exposed to different H_2O_2 concentrations. Bar: Standard error of 4 replicates.

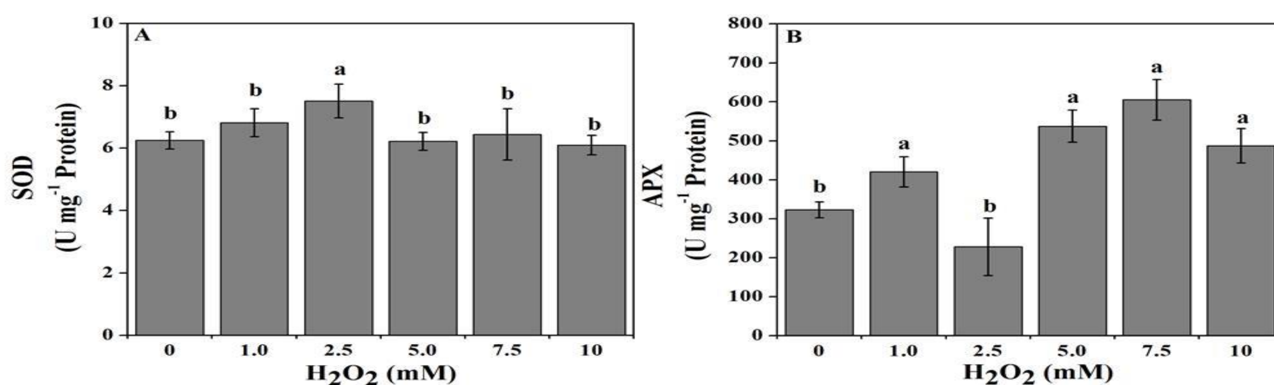

Fig. 3. Activity of (A) superoxide dismutase (SOD) and (B) ascorbate peroxidase (APX) in *L. sativa* seedlings exposed to different H_2O_2 concentrations. Bar: Standard error of 4 replicates.

Discussion

According to Borella&Pastorini (2011), lettuce seeds tolerate a wide range of pH variation (from 4 to 10) for germination. According to Gatti *et al.,* (2014), solutions with osmotic potential of up to -0.2 MPa do not significantly interfere in germination, growth and initial development of *L. sativa*. In plant bioassays, it is important to control the different variables, in addition to the requirement to standardize the procedures, to better understand the results obtained in this type of study (Souza Filho *et al.,* 2010).

Seed germination is a parameter widely used in plant bioassays (Moraes *et al.,* 2015), although it does not demonstrate other aspects of the germination process, such as delays, since it only involves final results (Silva & Aquila, 2006). Changes in the germination pattern may result in changes in membrane permeability, DNA transcription and translation, second messenger functionality, respiration due to oxygen sequestration, conformation of receptor proteins and enzymes, or the combination of these factors (Ferreira & Aquila, 2000). In this context, although H_2O_2 is a potentially toxic reactive oxygen species (ROS), it has aroused special interest due to its ability to lead to seed germination in different plant species (Ishibashi *et al.,* 2012). In *L. sativa* seeds, it leads to ruptures in the endosperm (Zhang *et al.,* 2014), which allows greater mobilization of reserves to the embryonic axis (Verma *et al.,* 2015), resulting in germination. However, in this study, H_2O_2 had no effect on germination – on the contrary, it delayed this process in the first 24 hours, suggesting its phytotoxic effect at the highest concentrations tested.

Root length (RL) is another variable widely used in phytotoxicity bioassays (Simões *et al.,* 2013; Moraes *et al.,* 2015). Since the primary root is the first organ to emerge from seed germination, its growth and development are directly affected by environmental conditions such as temperature, humidity, gravity, availability of nutrients and other substances in the environment (Santisree *et al.,* 2011). In this context, the inhibitory effect of H_2O_2 on the elongation of the *Arabidopsis* primary root was demonstrated by its exogenous delivery (Dunand *et al.,* 2007). Thus, the reduction in *L. sativa* RL, verified in this study, may be related to the reduction in the percentage of dividing cells in the meristematic region, since this region is responsible for root growth (Adam & El-Ashry, 2010; Pereira *et al.,* 2013). This inhibitory effect may occur due to an imbalance between the production of ROS and antioxidant activity, and may cause a rapid increase in intracellular ROS levels, called "oxidative burst" (Sharma *et al.,* 2012), which results in oxidative damage to cell structures (Kim & Kwak, 2010).

According to Alvim *et al.,* (2011), the increase or decrease in mitotic index (MI) can be used as cytotoxicity parameters. In this context, according to Pokora*et al.,* (2017), changes in intracellular concentrations of H_2O_2 are responsible for modulating the expression of genes required for cell cycle progression. Thus, under normal conditions, H_2O_2 seems to coordinate growth and plant development, in addition to regulating the cell cycle (Slesak *et al.,* 2007). However, according to Tsukagoshi (2012), the exogenous H_2O_2 supply may have a repressing effect on the expression of cell cycle-related genes, reducing the size of the root meristem. Thus, the increase in H_2O_2 concentrations had led to a cytotoxic action on the meristematic cells of *L. sativa* root tips, altering the cell division pattern, which directly reflected in the reduction in RL and plant biomass.

Cytogenetic analyses with detection and quantification of chromosomal abnormalities (CA) have been used to evaluate the mutagenic potential of contaminants (Alvim *et al.,* 2011). Chromosomal breaks, c-metaphases, bridges, binucleate and micronucleated cells are the most frequently observed chromosomal changes (Matsumoto *et al.,* 2006). However, for this study, there was no significant difference in the occurrence of CA, indicating that H_2O_2 did not have clastogenic potential.

The reduction in dry biomass (DB) as a function of the increase in H_2O_2 concentration is possibly due to the stomatal closure caused by ROS signaling, resulting in a lower carbon fixation and a smaller DB increase (Schock *et al.,* 2014). Important processes of cellular metabolism have been associated with H_2O_2, such as stomatal movement, whose closure causes a decrease in the carbon fixation rate via the Calvin cycle (Puthur, 2016). In addition, peroxidase associated with the polymerization of lignin precursors may restrict cell elongation due to the use of H_2O_2 as a substrate resulting cell wall thickening (Passardi *et al.,* 2004). As a consequence, cell wall peroxidases may be involved in the regulation of root growth, involving the metabolism of H_2O_2 so that, at higher H_2O_2 concentrations (7.5 and 10 mM), there may hae been an increase in lignin at the root tips, preventing water absorption. According to Jafariyan*et al.,* (2016), the pretreatment of wheat seeds with H_2O_2 increased water absorption and content in seedlings, yielding higher biomass. However, the biomass differences in seedlings presented in this study can also be attributed to the germination delay observed during the first 24 hours of exposure to H_2O_2.

According to Yang *et al.,* (2009), the exogenous application of H_2O_2 results in a series of signal transduction events, leading to a rapid proline accumulation. This amino acid is often associated with antioxidant metabolism, besides being involved in the inhibition of programmed cell death, since it acts as an osmoprotector, protein stabilizer, and as a lipid peroxidation inhibitor (Sharma *et al.,* 2012; Puthur, 2016). In addition, the increase in proline content may be related to a plant stress response to avoid water loss, since this amino acid is important in osmotic adjustment in plant cells (Paixão *et al.,* 2014). However, in this study, the increase in proline content as a function of the increase in H_2O_2 concentrations was not able to reduce biomass loss, especially at concentrations higher than 5.0 mM H_2O_2.

The growth restriction of *L. sativa* seedlings is possibly related to the phytotoxic effects of ROS, which can lead to changes in the structural conformation of proteins, instability and cell membrane permeability, with modifications that are detrimental to plant physiology (Lamhamdi *et al.,* 2011). The toxic effect of H_2O_2 is linked to enzyme inactivation by the oxidation of their thiol groups, and damage to proteins, carbohydrates, lipids and nucleic acids (Gill & Tuteja, 2010; Mittler *et*

al., 2011). In this context, since the cellular redox state is precisely regulated by processes of production and removal of ROS in different cell compartments (Kapoor *et al.,* 2015), antioxidant enzymes were characterized by increasing their activity in plant tissues as a response to the oxidative stress induced by the high H_2O_2 concentration. Thus, not only are the biological effects of H_2O_2 dependent on its concentration, but also on its production site, plant developmental stage and the previous plant exposure to other types of stress, as discussed by Pokora *et al.,* (2017).

Although H_2O_2 accumulation impairs the function and integrity of membranes due to the fact that they affect polyunsaturated fatty acids and cause lipid peroxidation (Farmer & Mueller, 2013), there was no significant increase in lipid peroxidation and malondialdehyde levels with increasing H_2O_2 concentrations. This indicates that none of the H_2O_2 concentrations tested affected the structure of the cell membranes. However, the maintenance of cell membrane integrity is possibly due to the increased enzyme activity of the antioxidant system. Thus, compartmentalization of antioxidant enzymes may have important consequences for the maintenance of adequate H_2O_2 levels (Gill & Tuteja, 2010; Kapoor *et al.,* 2015). In addition, proline accumulation in the tissues is also a proof that the cell constantly tries to minimize oxidative stress. However, in multicellular organisms, increased expression of antioxidant enzymes is not the only cellular response to increased H_2O_2 concentration (Maia *et al.,* 2012).

Conclusions

The exposure of *L. sativa* seeds to H_2O_2 was phytotoxic, resulting in a lower germination percentage, especially in the first 24 hours. In addition, higher H_2O_2 concentrations (7.5 and 10 mM) reduced root length, fresh and dry biomass, besides the activity of APX and SOD. This ROS was also cytotoxic; however, it was not clastogenic.

Therefore, exposure to H_2O_2 resulted in proline accumulation at the highest concentrations and also in an increase in the activities of antioxidant enzymes at intermediate concentrations, in response to the increase in H_2O_2 to maintain redox homeostasis inside the cell. This shows that *L. sativa* did not have an efficient antioxidant system to remove ROS generated mainly at concentrations of 7.5 and 10 mM H_2O_2.

Acknowledgments

The authors thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior [Coordination for the Improvement of Higher Education Personnel] Grant AUX PE–PNPD-2297.2011), CNPq (Conselho Nacional de DesenvolvimentoCientífico e Tecnológico [National Counsel of Technological and Scientific Development]), and FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais [Minas Gerais State Research Foundation]) for funding and research grants awarded to complete the present study.

References

Adam, F. and Z. El-Ashry. 2010. Evaluation of genotoxicity of 4-n-Nonylphenol using *Vicia faba* L. *J. Biol. Sci.,* 10: 368-372.

Alvim, L.B., F. Kummrow, L.A. Beijo, C.A. Lima and S. Barbosa. 2011. Avaliação da citogenotoxicidade de efluentes têxteis utilizando *Allium cepa* L./Evaluation of the cytogenotoxicity of textile effluents using *Allium cepa* L. *Revista Ambiente & Água,* 6: 255.

Biemelt, S., U. Keetman and G. Albrecht. 1998. Re-aeration following hypoxia or anoxia leads to activation of the antioxidative defense system in roots of wheat seedlings. *Plant Physiol.,*116: 651-658.

Borella, J. and L.H. Pastorini. 2011. Influência alelopática de *Phytolacca dioica* L. na germinação e crescimento inicial de tomate e picão-preto. *Biotemas,* 22: 67-75.

Buege, J.A. and S.D. Aust. 1978. Microsomal lipid peroxidation. *Methods in Enzymol.,* 52: 302-310.

Choudhury, S., P. Panda, L. Sahoo and S.K. Panda. 2013. Reactive oxygen species signaling in plants under abiotic stress. *Plant Signaling & Behavior,* 8: 23681.

Dunand, C., M. Crèvecoeur and C. Penel. 2007. Distribution of superoxide and hydrogen peroxide in Arabidopsis root and their influence on root development: possible interaction with peroxidases. *New Phytologist,* 174: 332-341.

Fang, W.C. and C.H. Kao. 2000. Enhanced peroxidase activity in rice leaves in response to excess iron, copper and zinc. *Plant Sci.,* 158: 71-76.

Farmer, E.E. and M.J. Mueller. 2013. ROS-mediated lipid peroxidation and RES-activated signaling. *Ann. Rev. Plant Biol.,* 64: 429-450.

Ferreira, A.G. and M.E.A. Aquila. 2000. Alelopatia: uma área emergente da ecofisiologia. *Revista Brasileira de Fisiologia Vegetal,* 12: 175-204.

Foyer, C.H. and G. Noctor. 2009. Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. *Antiox. & Redox Signal,* 11: 861-905.

Gatti, A.B., L.K. Takao, V.C. Pereira, A.G. Ferreira, M.I.S. Lima and S.C.J. Gualtieri. 2014. Seasonality effect on the allelopathy of cerrado species. *Brazil. J. Biol.,* 74: S064-S069.

Giannopolitis, C.N. and S.K. Ries. 1977. Superoxide dismutases I. Occurrence in higher plants. *Plant Physiol.,* 59: 309-314.

Gill, S.S. and N. Tuteja. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. & Biochem.,* 48: 909-930.

Gomes, L., F. Silva, S. Barbosa and F. Kummrow. 2012. Ecotoxicity of sludges generated by textile industries: a review. *Ecotoxi. and Environm. Contamin.,* 7: 89-96.

Havir, E.A. and N.A. McHale. 1987. Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. *Plant Physiol*., 2: 450-455.

Ishibashi, Y., Y. Koda, S.H. Zheng, T. Yuasa and M. Iwaya-Inoue. 2012. Regulation of soybean seed germination through ethylene production in response to reactive oxygen species. *Ann. Bot.*, 111: 95-102.

Jafariyan, T. and M.J. Zarea. 2016. Hydrogen peroxide affects plant growth promoting effects of *Azospirillum*. *J. Crop Sci. & Biotechnol*., 19: 167-175.

Kapoor, D., R. Sharma, N. Handa, H. Kaur, A. Rattan, P. Yadav and R. Bhardwaj. 2015. Redox homeostasis in plants under abiotic stress: role of electron carriers, energy metabolism mediators and proteinaceous thiols. *Frontiers in Environ. Sci.*, 3: 13.

Karuppanapandian, T., J.C. Moon, C. Kim, K. Manoharan and W. Kim. 2011. Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms. *Aust. J. Crop Sci*., 5: 709.

Kim, Y.H. and S.S. Kwak. 2010. The role of antioxidant enzymes during leaf development. *Reactive Oxygen Species and Antioxidants in Higher Plants*, p. 129.

Lamhamdi, M., A. Bakrim, A. Aarab, R. Lafont and F. Sayah. 2011. Lead phytotoxicity on wheat (*Triticum aestivum* L.) seed germination and seedlings growth. *Comptes Rendus Biologies*, 334: 118-126.

Maia, J.M, S.L. Ferreira Silva, E.L. Voigt, C.E.C. Macêdo, L.F.A. Ponte and J.A.G. Silveira. 2012. Atividade de enzimas antioxidantes e inibição do crescimento radicular de feijão caupi sob diferentes níveis de salinidade. *Acta Botanica Brasilica,* 26(2): 342-349.

Matsumoto, S.T., M.S. Mantovani, M.I.A. Malaguttii, A.L. Dias, I.C. Fonseca and M.A. Marin-Morales. 2006. Genotoxicity and mutagenicity of water contaminated with tannery effluents, as evaluated by the micronucleus test and comet assay using the fish *Oreochromis niloticus* and chromosome aberrations in onion root-tips. *Genetics & Mol. Biol.,* 29: 148-158.

Mittler, R., S. Vanderauwera, N. Suzuki, G. Miller, V.B. Tognetti, K. Vandepoele and F. Van Breusegem. 2011. ROS signaling: the new wave? *Trends in Plant Sci.*, 16: 300-309.

Moraes, R.M., S. Barbosa, P.R. dos Santos Filho, M. Carvalho and M. de Lima Nogueira. 2015. Effects of copper on physiological and cytological aspects in *Lactuca sativa* L. *Revista Brasileira de Biociências*, 13: 115-121.

Nakano, Y. And K. Asada. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant & Cell Physiol*., 22: 867-880.

Paixão, C.L., D.D.S. de Jesus, A. Dias de Azevedo, E.D.F. de Santana, F. de Santana, R. da Bahia and C. das Almas. 2014. Caracterização fisiológica e bioquímica de genótipos de girassol com tolerância diferenciada ao estresse hídrico. *Enciclopédia Biosfera,* 10: 2011-2022.

Passardi, F., C. Penel and C. Dunand. 2004. Performing the paradoxical: how plant peroxidases modify the cell wall. *Trends in Plant Sci*., 9: 534-540.

Pereira, M.P., F.J. Pereira, L.C. de Almeida Rodrigues, S. Barbosa and E.M. De Castro. 2013. Fitotoxicidade do chumbo na germinação e crescimento inicial de alface em função da anatomia radicular e ciclo celular. *Revista Agro@mbiente On-line*, 7: 36-43.

Pokora, W., A. Aksmann, A. Baścik-Remisiewicz, A. Dettlaff-Pokora, M. Rykaczewski, M. Gappa and Z. Tukaj. 2017. Changes in nitric oxide/hydrogen peroxide content and cell cycle progression: Study with synchronized cultures of green alga *Chlamydomonas reinhardtii*. *J. Plant Physiol*., 208: 84-93.

Puthur, J.T. 2016. Antioxidants and cellular antioxidation mechanism in plants. *S. Indian J. Biol. Sci*., 2: 14-17.

Quan, L.J., B. Zhang, W.W. Shi and H.Y. Li. 2008. Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. *J. Integrat. Plant Biol*., 50: 2-18.

Reigosa, M., A.S. Gomes, A.G. Ferreira and F. Borghetti. 2013. Allelopathic research in Brazil. *Acta Botanica Brasilica*, 27: 629-646.

Santisree, P., S. Nongmaithem, H. Vasuki, Y. Sreelakshmi, M.G. Ivanchenko and R. Sharma. 2011. Tomato root penetration in soil requires a coaction between ethylene and auxin signaling. *Plant Physiol*., 156: 1424-1438.

Santos, S.C., U.A. Oliveira, L.O.R. Trindade, M.D.O. Assis, J.M.S. Campos, E.G. Salgado and S. Barbosa. 2017. Genotypes selection for plant bioassays using *Lactuca sativa* L. and *Allium cepa* L. *Pak. J. Bot*., 49: 2201-2212.

Schock, A.A., A. Ramm, E.G. Martinazzo, D.M. Silva and M.A. Bacarin. 2014. Crescimento e fotossíntese de plantas de pinhão-manso cultivadas em diferentes condições de luminosidade. *R. Bras. Eng. Agríc. Ambiental*., 18: 3-9.

Sharma, P., A.B. Jha, R.S. Dubey and M. Pessarakli. 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J. Bot*., 2012.

Silva, F.M. and M.E.A. Aquila. 2006. Potencial alelopático de espécies nativas na germinação e crescimento inicial de *Lactuca sativa* L. (Asteraceae). *Acta Botanica Brasilica*,20: 61-69.

Simões, M.S., R.H. Madail, S. Barbosa and M. de Lima Nogueira. 2013. Padronização de bioensaios para detecção de compostos alelopáticos e toxicantes ambientais utilizando alface. *Biotemas*, 26: 29-36.

Slesak, I., M. Libik, B. Karpinska, S. Karpinski and Z. Miszalski. 2007. The role of hydrogen peroxide in regulation of plant metabolism and cellular signalling in response to environmental stresses. *Acta Biochimica Polonica-English Edition*, 54: 39.

Souza Filho, A.P.S., E.S.C. Gurgel, M.S.M. Queiroz and J.U.M. Santos. 2010. Atividade alelopática de extratos brutos de três espécies de Copaifera (Leguminosae-Caesalpinioideae). *Planta Daninha*, 28: 743-751.

Torello, W. and L. Rice. 1986. Effects of NaCl stress on proline and cation accumulation in salt sensitive and tolerant turfgrasses. *Plant & Soil*, 93: 241-247.

Tsukagoshi, H. 2012. Defective root growth triggered by oxidative stress is controlled through the expression of cell cycle-related genes. *Plant Sci.*, 197: 30-39.

Verma, G., S. Mishra, N. Sangwan and S. Sharma. 2015. Reactive oxygen species mediate axis-cotyledon signaling to induce reserve mobilization during germination and seedling establishment in *Vigna radiata*. *J. Plant Physiol.*, 184: 79-88.

Yang, S.L., S.S. Lan and M. Gong. 2009. Hydrogen peroxide-induced proline and metabolic pathway of its accumulation in maize seedlings. *J. Plant Physiol*., 166: 1694-1699.

Yi, H., X. Liu, M. Yi and G. Chen. 2014. Dual role of hydrogen peroxide in Arabidopsis guard cells in response to sulfur dioxide. *Adv. Toxicol*., v. 2014.

Zhang, Y., B. Chen, Z. Xu, Z. Shi, S. Chen, X. Huang and X. Wang. 2014. Involvement of reactive oxygen species in endosperm cap weakening and embryo elongation growth during lettuce seed germination. *J. Exp. Bot*., 65: 3189-3200.

(Received for publication 28 October 2017)