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Effects of drought stress during seed development and subsequent accelerated ageing on wheat seed mitochondrial ultra-structure, seedling antioxidant enzymes, and malondialdehyde

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Abstract

In this experiment, wheat plants were exposed to drought stress during seed development and then we worked on the obtained seeds. The seed mitochondrial ultra-structure, and antioxidant enzymes in seedling were investigated after exposure to seed accelerated ageing. Drought stress during seed development induced some changes in mitochondrial ultra-structure. Encountering seed development to drought stress decreased antioxidant enzymes activity but increased H_2O_2 in the seedling. Seed accelerated ageing declined antioxidant enzyme activity but increased malondialdehyde. Also, mitochondrial ultra-structure showed heavy damage after accelerated ageing. Most damages in mitochondrial ultra-structure were observed in the seeds obtained from drought-stressed plants then exposed to accelerated ageing. A swollen form of mitochondria with less crista was observed in the seeds that were exposed to accelerated ageing. Damage to mitochondria may be the main cause for seed ageing. However, the activity of antioxidant enzymes is a key factor in order to repair the damage and inhibit the lipid peroxidation resulted in maintaining cell membrane integrity in aged wheat seeds.

Keywords: Accelerated ageing, Antioxidant defense, Late-season drought stress, Seed physiology, *Triticum aestivum* L.

Introduction

Drought stress is the most important abiotic stress that seriously reduces the production in arid and semi-arid regions of the world. Most wheat-producing regions in the world face water shortages in a part of the growing season (Lipiec *et al*., 2013). Wheat is one of the most important crops for humans and is grown in different regions of the world under different weather conditions. In the main area of Iran, early summer drought and heat reduce grain-filling period and thus decrease the quality of the seeds. Late season drought stress in irrigated wheat fields causes a significant decrease in the quality and quantity of grain yield (Najafian, 2009).

A better understanding of seed deterioration leads to new ways of producing, harvesting, storing, and protecting the seeds for prolonged periods. The successful production of agriculture is highly dependent on seed quality. This quality is reduced by seed deterioration (Deepa *et al*., 2013). Seed ageing is one of the main problems for the quality and vitality of seeds during storage in different environments. Seed ageing may be due to the accumulation of oxygen free radicals and the damage caused by them, which leads to lipid peroxidation, impaired synthesis of RNA and proteins, and DNA degradation during storage of seeds (Chen *et al*., 2013; McDonald, 1999). Mitochondria plays an important role in producing scavengers of oxygen free radicals in seed germination and provide the energy needed for this process. Mitochondria also plays a very important role in resistance and tolerance against stress (Xin *et al.*, 2014).

Germination is a complex phenomenon that involves drastic changes in metabolic activities and genetic programming of seeds. Like any other phenomenon that requires energy, it looks that mitochondria are the main center of the manufacturer and provider of intracellular ATP for germination (Bewley, 1997). The mitochondrion is one of the main targets of damaging stresses that are probably due to high turnover or transformation of oxygen free radicals. Mitochondria are the primary source of oxygen free radicals in plant cells and thus the first target of oxidative damage that can spread to other organelles also during the process of deterioration and ageing (Jacoby *et al.*, 2012).

Oxygen free radicals are highly reactive and in the absence of protective mechanisms can seriously disrupt normal metabolism and lead to oxidative damage to lipids, proteins, and nucleic acids. Production of oxygen

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free radicals can be limited by the antioxidant system including antioxidant compounds, such as ascorbate, salicylate, glutathione, tocopherol and antioxidant enzymes such as superoxide dismutase, ascorbic peroxidase, peroxidase, and catalase (Foyer and Noctor, 2003).

In this study, we investigated the effects of drought during seed development and subsequent accelerated ageing on seed mitochondrial ultra-structure and also seedling antioxidant enzymes, MDA, and H_2O_2 content of wheat. Because we wanted to study the effects of drought stress during seed development on seed deterioration and physiological quality in resulted seedling, so we exposed a part of the seeds to AAT. In addition, we studied late-season drought stress effects on antioxidants in the seeds only, which has been published previously (Eisvand *et al.*, 2016).

Materials and methods

Plant material and treatments: Wheat seeds (*Triticum aestivum* L. cv. Pishtaz) were prepared from a previous experiment had studied on effects of late-season drought. In this experiment, plant density was 400 seeds per square meters and fertilizers (NPK and microelements) were applied according to the soil test. The field is located in the Agricultural Research Center, Kermanshah, Iran. Two irrigation regimes, control, and late season drought stress were used. Irrigation was stopped at booting stage in drought regime (these plants are called drought-stressed plants in this paper and their seeds as well). The seeds were harvested in 11/07/2013. Seeds were sealed in plastic bags and stored for 5 months at 3°C in the seed laboratory of Lorestan University. Our experiments on the seeds were carried out in the seed laboratory of Lorestan University, Khorram Abad, Iran, in 2014. The viability of the seed lot was tested before starting experiment as a standard germination test, i.e. 400 seeds were selected randomly and tested in four replicates (ISTA 2012), it was 100%.

Seed accelerated ageing: Seeds were subjected to accelerated ageing condition (40±1°C and 100% RH) for 72 hrs. according to Delouche and Baskin (Delouche and Baskin, 1973). Seeds were put over a mesh at 2cm above water in a plastic box (20×20×15 cm) containing distilled water (5 cm depth of water). Then the box tightly sealed and transferred to an incubator at 40±1°C for 72 h. Then, they were transferred to room temperature (25°C) for 12 hrs., divided into two parts and used for germination test and biochemical evaluations.

Seed germination test to producing seedling: After sterilization with hypochlorite 1% for 2 minutes and washing with distilled water, 150 seeds of each treatment were sowed in three petri dishes like the top of the paper method. The petri dishes were watered with distilled water, labeled and transferred to germinator (20°C, 75% RH, dark) for 8 days. Radicle emergence about 0.2 cm were considered as a criterion for germination (ISTA 2012).

Seedling malondialdehyde (MDA) content: At the end of germination test, 10 of 6-day old seedlings selected randomly and 500 mg fresh weight of the whole seedling were freeze-dried and homogenized by hand, with 5.0 mL of 5% trichloro acetic acid (TCA) at 4°C and centrifuged at 10000×g for 15 mins. The MDA determination was done according to the Heath and Packer by spectrophotometer at 532 nm and 600 nm (Heath and Packer, 1968).

Seedling hydrogen peroxide (H_2O_2) determination: Sampling (500 mg of the whole seedling) was done similar to what was mentioned for MDA. The H_2O_2 content was determined according to Patterson *et al.* (Patterson *et al.*, 1984). Absorbance change of the titanium peroxide complex was recorded at 415 nm. Absorbance values were quantified using a standard curve generated from H_2O_2 concentrations.

Seed mitochondrial Transmission Electron Microscopy (TEM): Preparation of wheat seeds for TEM was carried out according to Borek *et al* (Borek *et al.*, 2009). Seeds were fixed in Karnowsky half-strength fixative. Postfixation was conducted in 1% OsO_4. The samples were stained in 2% aqueous solution of uranyl acetate. Dehydration was performed in a series of acetone solutions. The objects were embedded in the epoxy resin of low viscosity. Ultrathin sections were prepared on Ultrotome III (LKB), stained in 5% uranyl acetate and 0.5% lead citrate and observed under the transmission electron microscope, TEM-CM30 Philips.

Enzyme Extraction: In order to extract the enzymes and for evaluoting them, seedlings (0.3g FW) were frozen and then ground in a 4 mL solution containing 50 mM phosphate buffer (pH 7.0), 1% (w/v) polyvinyl poly pyrrolidone (13:1 buffer volume/FW). The homogenate was centrifuged at 15000×g for 30 mins. and the supernatant was collected and used for enzyme assays. Preparations for enzyme extraction and enzyme assay were carried out at 4°C.

Catalase activity was estimated by the method of Cakmak and Horst at A240 (Cakmak and Horst, 1991). The reaction mixture included 100 µl enzyme extraction, 500 µl 10 mM H_2O_2, and 1.4 mL 25 mM sodium phosphate buffer. The decrease in absorbance was recorded for 1 min. The enzyme activity was expressed as units mg^{-1} protein.

Peroxidase was determined by the oxidation of guaiacol in the presence of H_2O_2. The increase in A470 was recorded for 1 mins. (Ghanati *et al.*, 2002). The reaction mixture was including 100 µl crude enzymes, 500 µl 5 mM H_2O_2, 500 µl 28 mM guaiacol, and 1.9 mL 60 mM potassium phosphate buffer (pH 6.1).

Superoxide dismutase activity was determined according to the method of Giannopolitis and Ries (Giannopolitis and Ries, 1977). The reaction mixture contained 100 µl 1 mM riboflavin, 100 µl 12 mM l-methionine, 100 µl 0.1 mM EDTA (pH 7.8), 100 µl 50 mM Na_2CO_3 (pH 10.2), and 100 µl 75 mM nitroblue tetrazolium (NBT) in 2.3 ml 25 mM sodium phosphate buffer (pH 6.8), with 200 µl crude enzyme extraction in

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a final volume of 3 mL. Glass test tubes that contained the mixture was illuminated with a fluorescent lamp (120 W). Identical tubes that were not illuminated served as controls. After illumination for 15 mins., the absorbance was measured at 560 nm.

Ascorbate peroxidase activity was assayed following the method of Nakano and Asada (Nakano and Asada, 1981). The reaction buffer solution contained 50 mM K-P buffer (pH 7.0), 0.5 mM AsA, 0.1 mM H_2O_2, 0.1 mM EDTA, and enzyme extraction in a final volume of 0.7 mL. The reaction was started by the addition of H_2O_2 and the activity was measured by observing the decrease in absorbance at 290 nm for 1 min.

Glutathione reductase activity was assayed by the method of Goldberg and Spooner (Goldberg and Spooner, 1983). The reaction mixture contained 100 µl of enzyme extraction, 2.5 mL of 120 M phosphate buffer (pH 7.2) and 0.1 mL of both EDTA (0.015 mol/L) and oxidised glutathione (0.065 mol/L). After 5 mins. 0.05 mL of NADPH (9.6 mol/L) was added and mixed thoroughly. The reaction was monitored after every 15 sec at 340 nm. The enzyme activity was expressed as unit mg^{-1} protein.

Native polyacrylamide gel electrophoresis (Native-PAGE): Native PAGE was used to study the isozymes patterns of APX, CAT, and SOD according to Laemmli (Laemmli, 1970) except that SDS was omitted and the gels were supported by 10% glycerol. Gel staining for APX, CAT and SOD were carried out according to Rao *et al.* (Rao *et al.*, 1996), Robertson *et al.* (Robertson *et al.*, 1987) and Beachamp and Faridovic (Beauchamp and Fridovich, 1971), respectively.

Statistical analysis: The one-way analysis of variance (ANOVA) was used for data analysis and the means were compared by least significant difference (LSD) test using SAS 9.1 software. Differences at $P<0.05$ were considered significant. Standard error was used for error bar in the graph.

Results and discussion

Germination results have been published in the previous paper (Eisvand *et al.*, 2016). Germination of seeds obtained from drought-stressed plants compared to the control was lower. In addition, accelerated ageing reduced the germination of both seeds (obtained from normal and drought-stressed plants). The seeds obtained from drought-stressed plants were more sensitive to ageing and germination loss (Eisvand *et al.*, 2016).

Mitochondria ultrastructure was examined by transmission electron microscopy (TEM) (Fig. 1). In non-aged seeds from control plants, in a normal cell, after water absorption, mitochondria were well recognizable and distinct. In contrast, mitochondria of the aged seeds showed fundamental changes. Normally, under control treatment, the mitochondrion was detectable with many well-developed cristae in comparison with drought-stressed ones. Mitochondria in non-aged seeds resulted from unstressed plants were oval-formed with well-developed cristae (Fig. 1A). However, with the ageing of the seeds, the mitochondria showed morphological changes and cristae were very little, thin and not easily detectable (Fig. 1B). In seeds from the drought-stressed plant, mitochondria were larger and swollen form with very low cristae (Fig. 1C) When these seeds exposed to AA, mitochondria were more swollen and no cristae were detected (Fig. 1D).

When seeds were produced under drought stress then exposed to accelerated ageing; resulted seedlings had the most MDA content compared to the non-aged ones (Fig. 2). However, MDA content for both seedlings obtained from drought stressed and control seeds were the same without the accelerated ageing.

Seedlings obtained from drought-stressed seeds have more H_2O_2 content compared with non-drought-stressed. Also, accelerated ageing increased H_2O_2 content of seed from both control and stressed plants. The maximum content of H_2O_2 was obtained in drought-stressed seed after accelerated ageing (Fig. 2).

As noted above, to neutralize the toxic effects of reactive oxygen species, plants use the antioxidant system including enzymatic and non-enzymatic components. The activity of SOD, CAT, GR, and APX was significantly lower in the seedlings obtained from drought-stressed seeds in comparison with non-drought-stressed ones. However, POD activity was the same in seedlings from both normal and drought stressed seeds. In both drought-stressed and non-drought stressed seeds, accelerated ageing decreases the activity of the antioxidant enzymes such as GR, CAT, APX, and SOD in wheat seedlings, but increased POD (Fig. 3).

Isozyme patterns of APX, CAT, and SOD were not affected by drought stress during seed development (Fig. 4 and 5). Isozyme patterns of CAT and SOD were changed by seed accelerated ageing in seedling obtained from both control and drought stressed plants, but the APX isozyme pattern was not changed by seed accelerated ageing (Fig. 4 and 5).

The accelerated ageing test is commonly used to better understand the mechanism of seed deterioration. The highest power of seed storage is obtained at physiologic maturity (maximum dry weight) or a little after (Bewley, 1997). In large area of wheat production in Iran such as the location of this study (Kermansha province, Iran), rainfall distribution is not suitable (i.e. rainfall occurs in autumn and winter). Therefore, in these areas, water scarcity and drought stress occur in the spring almost simultaneously with pollination and grain filling of wheat. Grain filling rate determines final grain weight and its quality. This process is regulated by genetic and environmental factors. Reduction in seed germination in aged seeds could be due to reduced activity of proteolytic enzymes and carbohydrate contents. Reduced germination of deteriorated seeds of various products such as wheat (Eisvand *et al.*, 2016) and barley has been reported (Anderson and Abdul-Baki, 1971) and peas (Kapoor *et al.*, 2010).

The mitochondrial structure is seriously affected by

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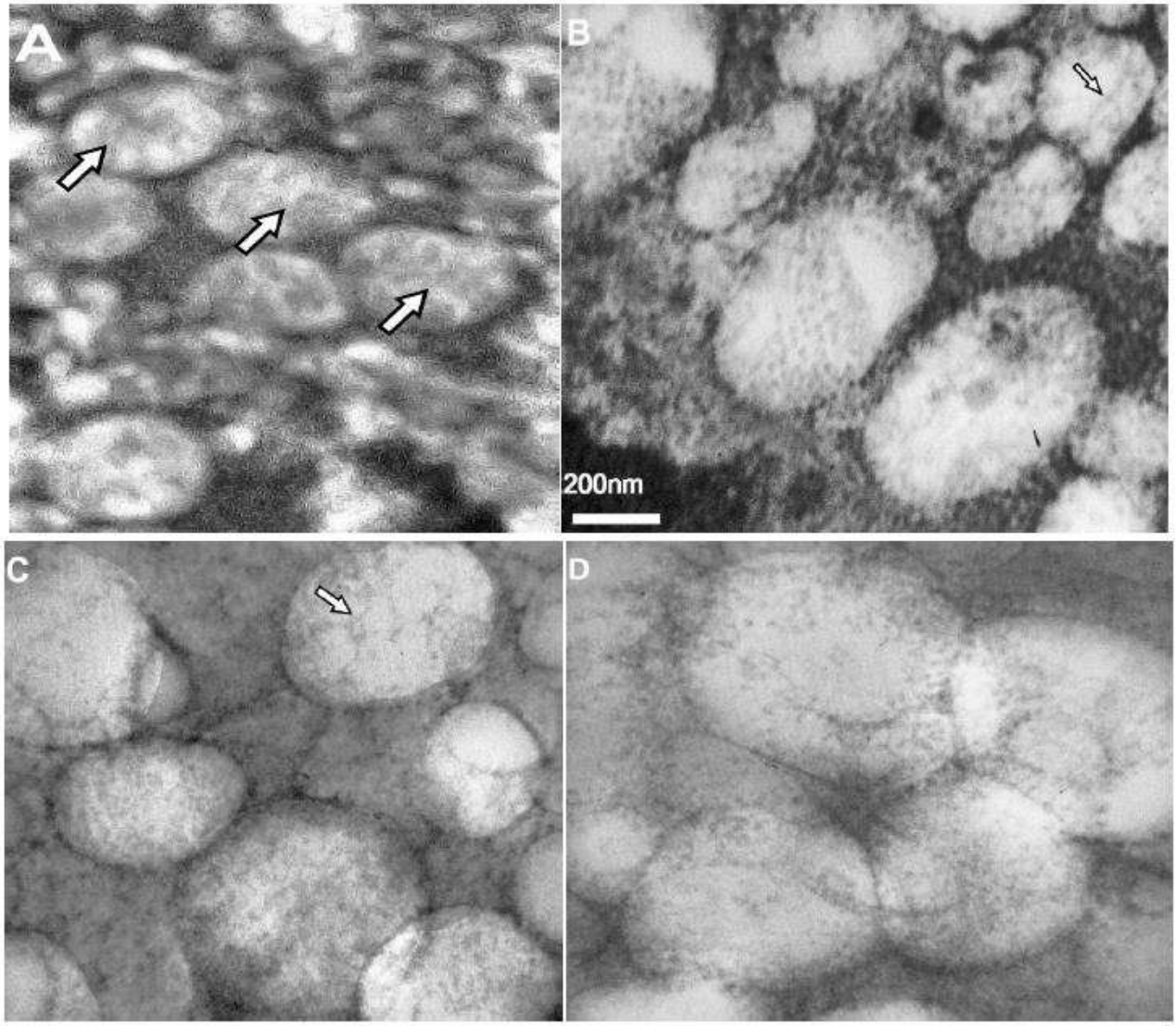

Fig. 1. Transmission electron micrographs (TEM) of the seed mitochondrial ultrastructure. The seeds from control plants (A and B) and drought-stressed plants (C and D). Overview of cells from NAA seeds (A and C) and AA seeds (B and D).

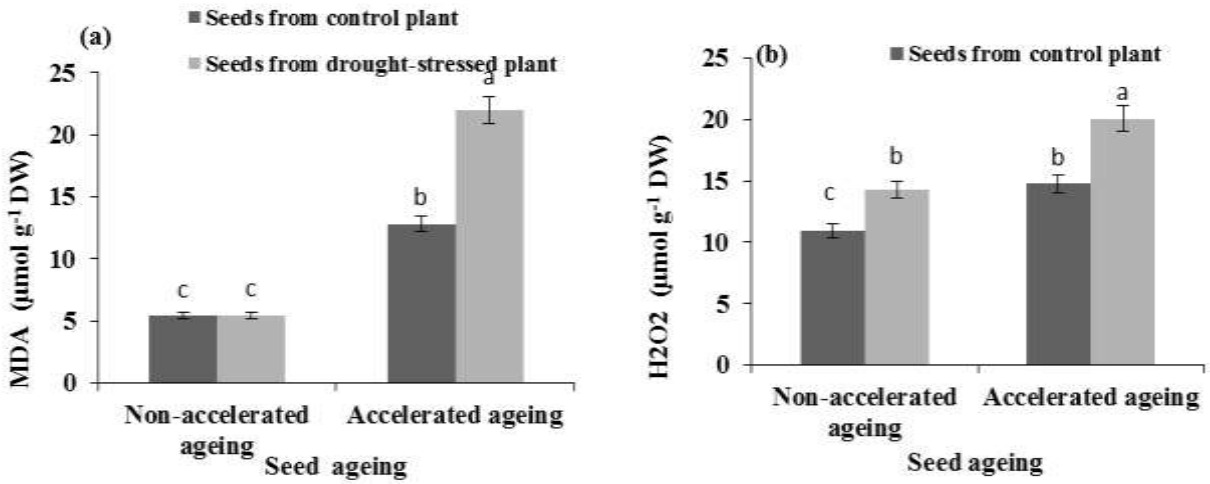

Fig. 2. Change in MDA (a) and H_2O_2 (b) contents in wheat seedlings affected by accelerated ageing and drought stress. Data are means ± standard error (n=3), Means with the same letter in each data series are not significantly different ($P \leq 0.05$) based on the LSD test.

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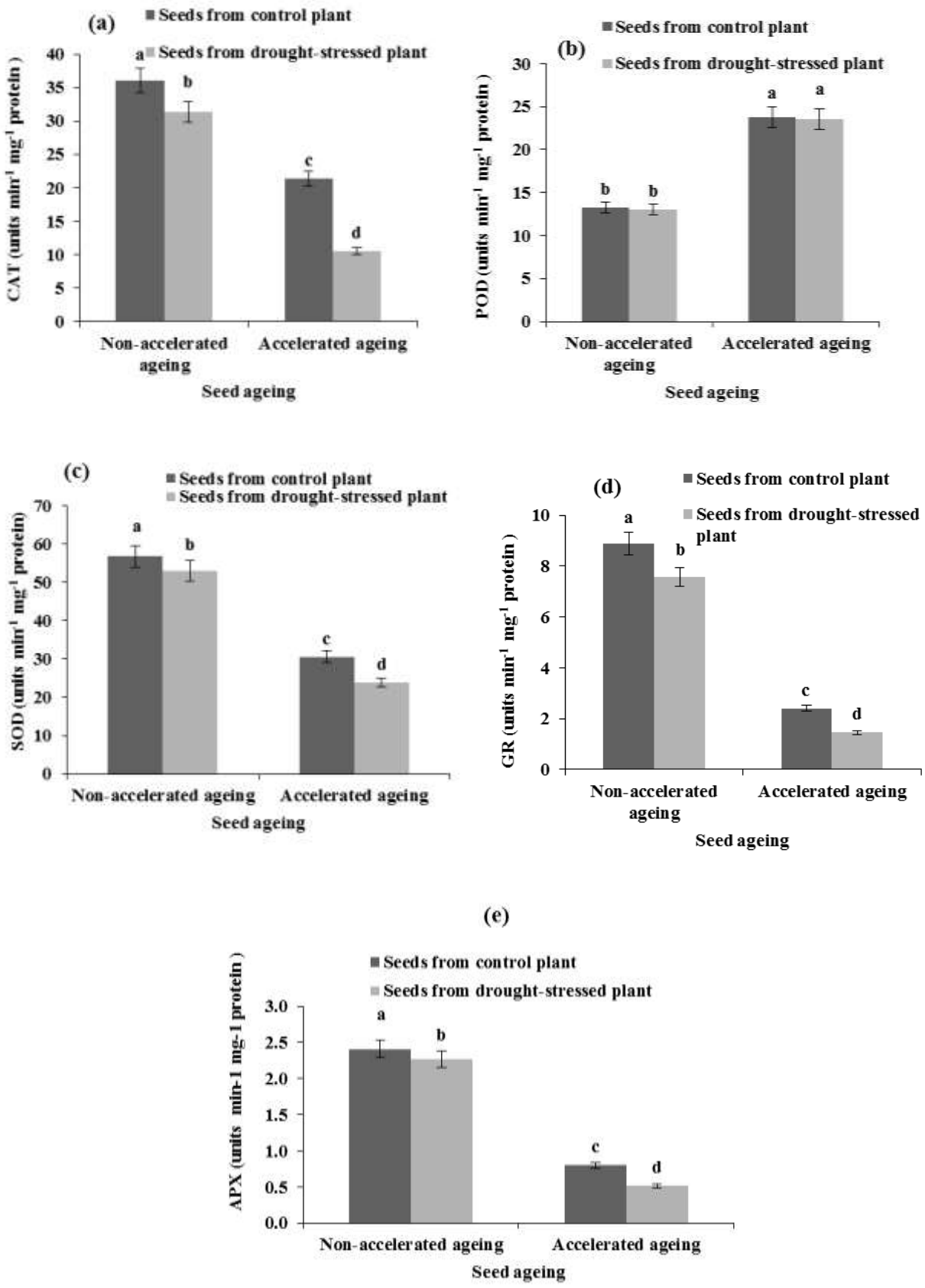

Fig. 3. Change in CAT (a), POD (b), SOD (c), GR (d) and APX (e) activity in wheat seedling produced from seed which affected by drought stress and accelerated ageing. Data are means ± standard error (n=3). Means with the same letter in each data series are not significantly different ($P \leq 0.05$) based on the LSD test.

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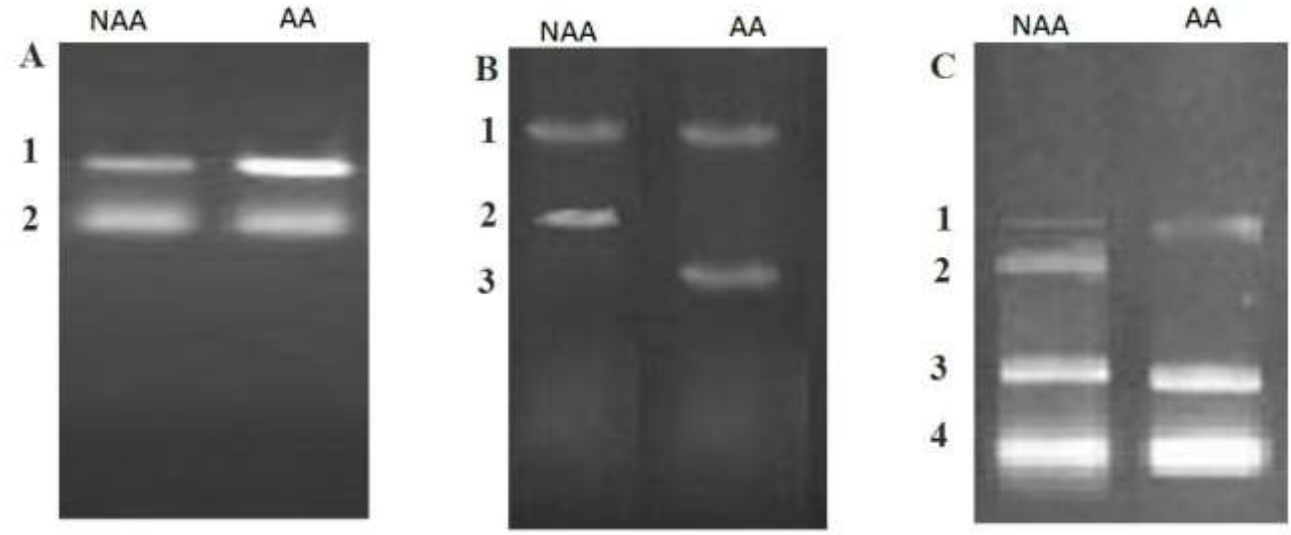

Fig. 4. Isozyme pattern of ascorbate peroxidase (A), catalase (B) and superoxide dismutase (C), in wheat seedling produced from seed which obtained from control plants (abbreviations are NAA: non-accelerated ageing; AA: accelerated ageing).

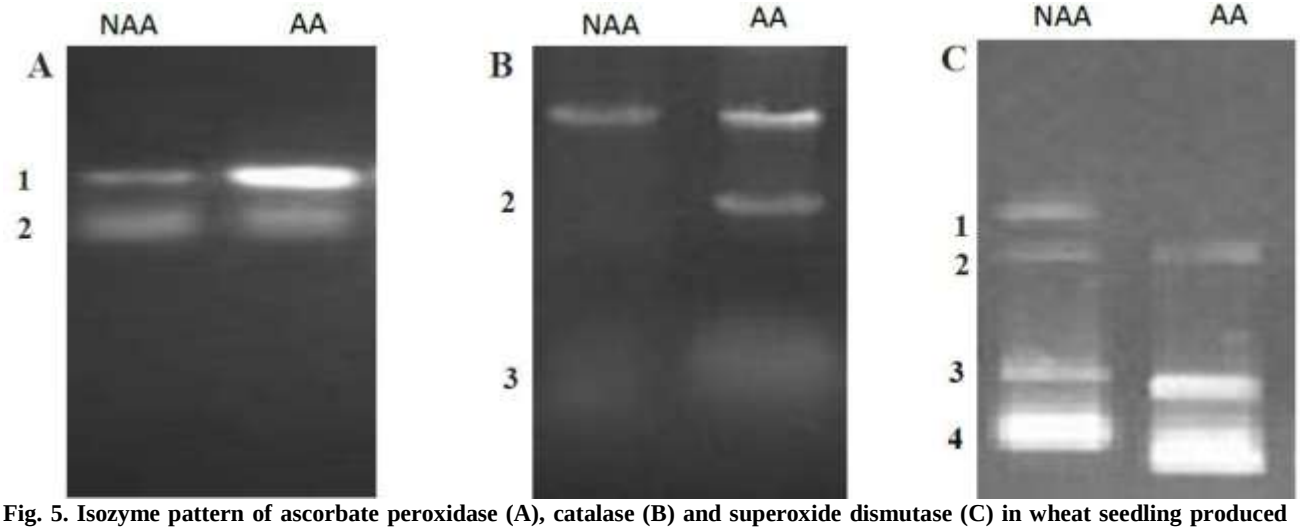

Fig. 5. Isozyme pattern of ascorbate peroxidase (A), catalase (B) and superoxide dismutase (C) in wheat seedling produced from seed which obtained from drought-stressed plants (NAA: non-accelerated ageing; AA: accelerated ageing).

drought stress and accelerated ageing that is due to serious flaws in the mitochondrial function during water attraction period in seeds (Carrie *et al.*, 2013). Observations of mitochondrial ultrastructure in plants in cells that have been exposed to abiotic and oxidative stresses have shown changes in cells compared to the control. Most of the observed changes included mitochondrial swelling, reduction in the number of cristae, and the gradual reduction of resolution of the mitochondrial matrix (Liang *et al.*, 2003).

In the previous study (Eisvand *et al.*, 2016), the MDA content in stressed seed was high; so the high MDA in the seedling obtained from drought-stressed seed may be partly due to its accumulation in the seed. By increasing the content of oxygen free radicals, drought stress increases the malondialdehyde content that is the product of membrane lipid peroxidation (Liang *et al.*, 2003). Hydrogen peroxide is a toxic compound for cells and should quickly be converted to water and oxygen by antioxidant defense system. Otherwise, it can damage cell membrane, protein structure, and DNA through the lipid peroxidation, and prevent photosynthesis and the activity of other enzymes. Several enzymes regulate H_2O_2 levels in the cells in which the most important ones are catalase, ascorbate peroxidase, and peroxidase. H_2O_2 induces lipid peroxidation, so high H_2O_2 can produces more MDA as a product of lipid peroxidation. Increased H_2O_2 under aging conditions may be due to defects in mitochondria, and low activity of CAT.

In addition to damage to the plant cells, the free radicals act as signal molecules and activate defense responses of the cell against the stress (Sharma *et al.*, 2012). In the seedlings obtained from the seeds of the drought-stressed plants, catalase activity significantly decreased (Fig. 3A), and H_2O_2 increased (Fig. 2C). It seems that drought stress during seed development resulted in a weak antioxidant system. As it is shown in the Figures, except the POD, all other studied antioxidants were weaker in the seedling obtained from

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stressed seeds than control. Hydrogen peroxide is generally removed by catalase, which converts H_2O_2 to water and oxygen. Catalase with ascorbate peroxidase can remove produced toxic hydrogen peroxide in the environment where oxidative stress occurs. Catalase isozyme pattern of seedling was affected by the drought that occurred during seed development. Accelerated ageing also changed this pattern. Nevertheless, there were no pattern changes for APX and SOD (Figs. 4 and 5).

Peroxidase in plants is available as multiple isoforms which are regulated exactly and are activated in response to environmental stimuli. It has been reported that peroxidase isozymes have a key role in stress tolerance. In this study, drought during seed development did not affect the seedling peroxidase activity, but seed deterioration treatment increased the activity of this enzyme (Fig. 3). Reduced peroxidase activity due to drought stress in rice has also been reported. Peroxidase enzyme plays role in breaking the hydrogen peroxide and is found in the cell wall, endoplasmic reticulum, golgi, and vacuoles. Superoxide dismutase is the first line of defense in cells against a free radical attack. When drought stressed seeds were aged, a significant decrease in SOD activity was observed in resulted seedlings. Xin *et al.* (Xin *et al.*, 2014) reported that oxidative stress decreases CAT and SOD activity. As seen in Fig. 4, drought stress during seed development and subsequent accelerated ageing decreased SOD and CAT activity. However, the CAT isozyme pattern had the most changes because of aging.

As seen in Fig. 3, the highest activity of APX was observed in non-deteriorated seeds from unstressed plants. One of the H_2O_2 producing sources in plant cells is SOD enzyme that produces H_2O_2 through dismutation of superoxide radicals. The results of this study showed that along with reduced enzyme activity of SOD, APX activity was also reduced. In the event of drought stress during seed filling, APX activity declined. The isozymes of this enzyme have wider bands compared to the control treatments in drought condition. APX enzyme in Mehler and Glutathione-ascorbate cycle plays an important role in scavenging hydrogen peroxide (Xin *et al.*, 2014).

Changes in glutathione reductase activity have been shown in Fig. 3. Glutathione reductase also plays an important role in adaptation to oxidative stress. The highest activity of this enzyme was observed in normal irrigation and non-deterioration condition and the lowest activity was observed in drought stress and seed deterioration treatment. This enzyme is responsible for the conversion of oxidized glutathione (GSSG) to reduce glutathione (GSH) and preserving high GSH to GSSG ratio. GSH can act as an antioxidant and scavenge the ROS. Therefore, the increase in GR is very important due to oxidized glutathione revival. Research has shown that there is a strong correlation between tolerance to oxidative stress that is caused due to environmental stress and increased the concentration of antioxidant enzymes in the photosynthetic plants (Vassileva *et al.*, 2009).

Our previous study concluded that accelerated ageing reduces wheat seed germination. It may be due to membrane damage. This germination reduction was more seen in seeds obtained from late-season drought stress conditions. It may be related to lipid peroxidation during ageing (Eisvand *et al.*, 2016). Also, the current study showed disorders in mitochondrial ultrastructure that would result in the impaired function. In the stress condition, it was observed damages to the mitochondria which are probably one of the main reasons for reduced physiological quality and wheat seed germination. This experiment showed alterations in the seedling catalase isozyme profile because of drought during seed filling and accelerated ageing. Finally, it can be said that drought during seed filling and seed accelerated ageing will result in a decrease of CAT, SOD, GR, and APX in the seedling.

Conclusion

Drought stress during seed development can induce changes in mitochondrial ultra-structure. Also, it decreases antioxidant enzymes activity in the seedlings. Seed accelerated ageing declined antioxidant enzyme activity and changed isozyme patterns of CAT and SOD; however these patterns were not affected by drought stress. Damage to mitochondria may be the main cause for seed ageing. Results of current research show that drought stress during seed development can affect physiological quality of seedling, and seed accelerated aging exaggerates the seedling quality reduction.

References

Anderson, J. D. and Abdul-Baki, A. A. (1971) Glucose metabolism of embryos and endosperms from deteriorating barley and wheat seeds. Plant Physiology 48:270-272

Beauchamp, C. and Fridovich, I. (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry 44:276-287

Bewley, J. D. (1997) Seed germination and dormancy. The Plant Cell 9:1055

Borek, S., Pukacka, S., Michalski, K. and Ratajczak, L. (2009) Lipid and protein accumulation in developing seeds of three lupine species: *Lupinus luteus* L., *Lupinus albus* L., and *Lupinus mutabilis*. Sweet Journal of Experimental Botany 60:3453-3460

Cakmak, I. and Horst, W. J. (1991) Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). Physiologia Plantarum 83:463-468

[DOR: 20.1001.1.23222727.1397.7.28.8.1]

Carrie, C., Murcha, M. W., Giraud, E., Ng, S., Zhang, M. F., Narsai, R. and Whelan, J. (2013) How do plants make mitochondria? Planta 237:429-439

Chen, H., Osuna, D., Colville, L., Lorenzo, O., Graeber, K., Kuster, H., Leubner-Metzger, G. and Kranner, I. (2013) Transcriptome-wide mapping of pea seed ageing reveals a pivotal role for genes related to oxidative stress and programmed cell death. Plos one 8:e78471

Deepa, G., Chetti, M. B., Khetagoudar, M. C. and Adavirao, G. M. (2013) Influence of vacuum packaging on seed quality and mineral contents in chilli (*Capsicum annuum* L.). Journal of Food Science and Technology 50:153-158

Delouche, J. C. and Baskin, C. C. (1973) Accelerated ageing technique for predicting relative storability of seed lots. Seed Science and Technology 1:427-452

Eisvand, H. R., Moori, S., Ismaili, A. and Sasani, S. (2016) Effects of late-season drought stress on physiology of wheat seed deterioration: changes in antioxidant enzymes and compounds. Seed Science and Technology 44:327-341

Foyer, C. H. and Noctor, G. (2003) Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiologia Plantarum 119:355-364

Ghanati, F., Morita, A. and Yokota, H. (2002) Induction of suberin and increase of lignin content by excess boron in tobacco cells. Soil Science and Plant Nutrition 48:357-364

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

Goldberg, D. and Spooner, R. (1983) Glutathione reductase. Methods of Enzymatic Analysis 3:258-265

Heath, R. L. and Packer, L. (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics 125:189-198

ISTA (2012) International rules for seed testing. Bassersdorf, Switzerland

Jacoby, R. P., Li, L., Huang, S., Pong Lee, C., Millar, A. H. and Taylor, N. L. (2012) Mitochondrial composition, function and stress response in plants. Journal of Integrative Plant Biology 54:887-906

Kapoor, N., Arya, A., Siddiqui, M., Amir, A. and Kumar, H. (2010) Seed deterioration in chickpea (*Cicer arietinum* L.) under accelerated ageing. Asian Journal of Plant Sciences 9:158-162

Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685

Liang, Y., Chen, Q., Liu, Q., Zhang, W. and Ding, R. (2003) Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgare* L.). Journal of Plant Physiology 160:1157-1164

Lipiec, J., Doussan, C., Nosalewicz, A. and Kondracka, K. (2013) Effect of drought and heat stresses on plant growth and yield: a review. International Agrophysics 27:463-477

McDonald, M. (1999) Seed deterioration: physiology, repair and assessment. Seed Science and Technology 27:177-237

Najafian, G. (2009) Drought tolerance indices, their relationships and manner of application to wheat breeding programs. Plant Science in Iran Middle Eastern and Russian Journal of Plant Science and Biotechnology 3:25-34

Nakano, Y. and Asada, K. (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology 22:867-880

Patterson, B. D., MacRae, E. A. and Ferguson, I. B. (1984) Estimation of hydrogen peroxide in plant extracts using titanium (IV). Analytical Biochemistry 139:487-492

Rao, M. V., Paliyath, G. and Ormrod, D. P. (1996) Ultraviolet-B-and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. Plant Physiology 110:125-136

Robertson, E. F., Dannelly, H. K., Malloy, P. J. and Reeves, H. C. (1987) Rapid isoelectric focusing in a vertical polyacrylamide minigel system. Analytical Biochemistry 167:290-294

Sharma, P., Gujral , H. S. and Singh, B. (2012) Antioxidant activity of barley as affected by extrusion cooking. Food Chemistry 131:1406-1413

Vassileva, V., Simova-Stoilova, L., Demirevska, K. and Feller, U. (2009) Variety-specific response of wheat (*Triticum aestivum* L.) leaf mitochondria to drought stress. Journal of Plant Research 122:445-454

Xin, X., Tian, Q., Yin, G., Chen, X., Zhang, J., Ng, S. and Lu, X. (2014) Reduced mitochondrial and ascorbate–glutathione activity after artificial ageing in soybean seed. Journal of Plant Physiology 171:140-147

[DOR: 20.1001.1.23222727.1397.7.28.8.1]