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PLANT GROWTH REGULATORS AND ASCORBIC ACID EFFECTS ON PHYSIOLOGICAL QUALITY OF WHEAT SEEDLINGS OBTAINED FROM DETERIORATED SEEDS

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Abstract

This study attempted to examine the effect of seed priming using plant growth regulators and vitamin C on the physiological traits of non-aged and aged seeds of wheat and their obtained seedlings. Accelerated aging (AA) method (40°C, RH=100% for 72h) was used for aging seeds. The seeds were pre-treated by gibberellin (GA), salicylic acid (SA), brassinosteroid (BR), and ascorbic acid (AS). Some seed traits such as germination and electric conductivity (EC) and seedling traits such as malondialdehyde (MDA) content, activity of some antioxidant enzymes, soluble protein content (SP), soluble sugar (SS), and proline were measured seven days after germination. The results showed that accelerated aging of seeds reduces the germination percentage and speed, increases soluble sugar, and reduces soluble protein, activity of catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) in the seedling. Pre-treatment of the aged seed by GA had the maximum positive impact on seed germination and seedling growth. Priming improved germination indices, quality of seedling, and seedling resistance against the oxidative stress caused by AA. It also improved cell membrane integrity and thus reduced seeds' EC. Priming increased the activity of CAT, POD and SOD enzymes in both aged and non-aged seeds. When the deteriorated seeds were primed, proline and SS contents of the seedling increased significantly, but SP and MDA decreased. In general, pre-treatment of the non-aged and aged seeds by gibberellin improved the physiological quality of the seed and seedling.

Key words: Seed aging, PGRs, Physiological characteristics, Priming, Wheat, Vitamin C.

Abbreviations: AAS, accelerated aging seed; CAT, catalase; EC, Electrical conductivity; MDA, malondialdehyde; POD, peroxidase; ROS, reactive oxygen species; SOD, super-oxide dismutase; GA, gibberllin; BR, brassinosteroid; AS, ascorbic acid; SA, salicylic acid; SS, soluble sugar; SP, soluble protein; VI, vigour index; HP, hydro-primed; NP, non-primed; NAS, non-aged seed.

Introduction

Seed aging leads to its deterioration. Seed deterioration leads to genetic and economic losses in agricultural products (McDonald, 1999; Walters, 1998). Due to the disorders in cell organelles such as mitochondria and glyoxysomes, reactive oxygen species (ROS) including H_2O_2, OH-, and O_2^- increase in the deteriorated seeds (Bailly, 2004). A good understanding of seed deterioration mechanism would reveal new methods for preservation and longevity of seeds (Bailly *et al.*, 2008).

If ROS production exceeds their removal, seeds will suffer from oxidative stress and their germination power will reduce. Several tests have proven the adverse effects of accelerated aging on antioxidative factors associated with seed viability and its germination in some crops (Bailly *et al.*, 1998; Chiu *et al.*, 1995; Sung & Jeng, 1994). Several studies showed induction of various species of ROS in different tissues, which were exposed to oxidative stresses (Anderson *et al.*, 1995; Bailly, 2004; Bailly *et al.*, 1998).

Release of active species of oxygen increases lipid and membrane proteins peroxidation and accelerates seed deterioration by destroying membrane structure (Goel *et al.*, 2003). Radicals of (O_2^-) and hydrogen peroxide (H_2O_2), which may cause ROS accumulation, are the major factors of seed aging and deterioration, thus reducing power and vitality of seed (Bailly *et al.*, 2008; Hu *et al.*, 2012; Oracz *et al.*, 2009; Rajjou & Debeaujon, 2008). Antioxidant systems prevent ROS accumulation, which leads to seed deterioration (Bailly, 2004; Pukacka & Ratajczak, 2005).

Accelerated aging has been widely recognized as a suitable test for predicting storage time of seeds and as a criterion for estimating vigor of cereal seeds. The mechanisms involved in seed deterioration vary in different conditions. The share of peroxidation of lipids, hydrolysis of sugars, and the Maillard reaction in seed deterioration is highly dependent on water and temperature content; therefore, seed aging, in the first place, is highly dependent on seed moisture and temperature during storage (Kibinza *et al.*, 2006; Walters, 1998). Seed aging may reduce viability and germination power of seeds (Eisvand *et al.*, 2010a; McDonald, 1999).

Seed priming is one of the applied techniques for improving performance of crop products (Murungu *et al.*, 2004). Seed priming treatments mainly have certain effects on metabolism, biochemistry and enzymatic activities of seed; therefore, they may result in better performance of seed's biological processes such as germination and seed establishment (Demir & Mavi, 2004). So far, some studies have discussed the effects of priming on germination and resistance to adverse conditions such as oxidative stress (Espanany *et al.*, 2015; Goel *et al.*, 2003; Shinwari *et al.*, 2015).

This study discusses the effect of priming of aged seeds of wheat with plant growth regulators and ascorbic acid on germination performance and activity of several enzymes capable of removing free radicals in wheat

seedlings. Research results may give suitable information on the role of plant growth regulators and vitamin C in seed germination and seedling quality obtained from deteriorated and normal seeds. Indeed, we seek to unravel the physiological changes that seed aging induce in seed and seedling. Also, we want to discover the differences between PGRs and vitamin C effects on seedling physiological characteristics when they are applied as seed priming.

Materials and Methods

Plant material, accelerated aging, seed priming, germination, and sampling: Wheat seeds (*Triticum aestivum* L. cv. Pishtaz) were provided from Agriculture Research and Education Center of Kermanshah, Iran. The seeds were surface-sterilized for 5 min in sodium hypochlorite solution (1%) and then in 96% ethanol for 30s. After sterilization, seeds were subjected to accelerated aging condition (40±1°C and 100% RH) for 72h (Delouche & Baskin, 1973). Then they were soaked for 12h (Khan *et al*., 2011) in the five priming media at 21±1°C including distilled water, GA3, L(+) ascorbic acid, 24-epibrassinoloide and acetyl salicylic acid. Each of them at 100 ppm concentration. All chemicals were prepared from Merck Company. Non-primed seeds were considered as the control. Then seeds were removed from the priming solutions and dried by the mild air forced method at 24±2°C for 10 h. Fifty seeds of each treatment were put in Petri dishes using top of paper method and received 8ml distilled water. Three replications were considered for each treatment. The experiment was conducted at 21±1°C with alternative light of 12-12 h and relative moisture of 75%. Numbers of germinated seeds were recorded daily during seven days. Total number of germinated seeds was recorded immediately after incubation was terminated. The percentage of germinated seeds was calculated. The seedlings were frozen in liquid N2 and stored at -80C until biochemical analysis.

Electrical conductivity (EC): Three replications of seeds (2.25 gr) from each lot were soaked in 75 ml deionized water and kept in the incubator at 25±1°C for 24 h and the electrical conductivity of the seed leachates was recorded using a direct reading conductivity meter. The electrical conductivity was expressed as dSm–1 gr–1.

Germination percentage (GP) is calculated using the Eq. (1) (Ikić *et al.*, 2012):

$$GP = \frac{\text{Total seeds germination after day 7} \times 100}{\text{Total number of seeds}}$$

Speed of germination is calculated using the Eq. (2)(Agrawal 1982):

$$\text{Speed of germination} = \sum_{I}^{J} \frac{ni}{Di}$$

Ni= number of germinated seeds in I day
Di= number of day after experiment

Seed vigor index was calculated by the Eq. (3) (Abdul-Baki and Anderson, 1973):

VI= GP (%) × SL (mm)
VI= vigor index.
GP= standard germination (%).
SL= seedling length (mm).

Enzyme extraction and assay: Seedlings (0.5 g) were homogenized using a mortar and pestle with 3 ml ice-cold extraction buffer (25 mM sodium phosphate buffer, pH 7.8). The homogenate was centrifuged at 15000 g for 30 min at 4°C and the supernatant was filtered through filter paper. The supernatant fraction was used as a crude extract for the assay of enzyme activity and protein content.

Catalase activity was estimated by the method of (Cakmak & Horst, 1991). The reaction mixture contained 100 µl crude enzyme extract, 500 µl 10 mM H_2O_2, and 1.4 µl 25 mM sodium phosphate buffer. The decrease in A240 was recorded for 1 min by spectrophotometer, and enzyme activity of the extract was expressed as units per milligram of protein.

Superoxide dismutase activity was determined according to the method of (Giannopolitis & 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 µl 25 mM sodium phosphate buffer (pH 6.8), with 200 µl crude enzyme extract in a final volume of 3 µl. SOD activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of NBT. Glass test tubes that contained the mixture were illuminated with a fluorescent lamp (120 W). Identical tubes that were not illuminated served as controls. After illumination for 15 min, the absorbance was measured at 560 nm.

Peroxidase activity was determined by the oxidation of guaiacol in the presence of H2O2. The increase in A470 was recorded for 1 min (Ghanati *et al.*, 2002). The reaction mixture contained 100 µl crude enzymes, 500 µl 5 mM H_2O_2, 500 µl 28 mM guaiacol, and 1.9 µl 60 mM potassium phosphate buffer (pH 6.1).

Lipid peroxidation: The level of membrane damage was determined by measuring malondialdehyde (MDA) as the end-product of membrane lipid peroxidation (De Vos and Schat, 1991). Samples were homogenized in an aqueous solution of 10% trichloroacetic acid (w/v) and aliquots of the filtrates were heated at 100°C for 30 min in 0.25% thiobarbituric acid. The amount of MDA was determined from A532, followed by correction for nonspecific absorbance at 600 nm. MDA (µmol g-1 Fw) = 6.45 (OD532 − OD600) − 0.56OD450.

Determination of soluble protein: To extract the soluble protein, one gram of fresh tissue of seedling in the presence of extraction buffer (Tris-HCl pH = 7.5) was homogenized. For extraction, the mixture was

transferred to centrifuge tubes and 13,000 rpm for 20 min at 4°C was centrifuged. Then protein concentration was measured according to Bradford. Protein concentrations were determined as described by (Bradford, 1976), using BSA standard.

Determination of soluble sugars: The seedling, sampled as described above, were homogenized in 10.0 ml of 80% (v/v) ethanol in a mortar and the mixture boiled for 10 min. The samples were centrifuged at 3000g for 10 min and the supernatant was removed. One additional extraction using 10.0 ml of hot 80% ethanol was carried out. The supernatant fractions were combined and soluble sugars were then determined by the phenol sulfuric acid method (Dubois *et al.,* 1956) using glucose as standard.

Proline assay: Proline content was measured using the acid ninhydrin by method of (Bates *et al*., 1973). Seedlings were hemogenized with 3% (W/V) sulfosalicylic acid aqueous solutions and centrifuged at 4000 rpm and 4ºC for 10 min. 2 ml of supernatant was taken for the analysis to which 2 ml acid ninhydrin and 2 ml glacial acetic acid were added. The reaction mixture was boiled in a water bath for 1 h. Four milliliter of toluene was added to the reaction mixture and the organic phase was extracted, the absorbance read at 520 nm by UV-visible spectrophotometer.

Statistical analyses: All analyses were performed based on a factorial experiment in completely randomized design. The data was analyzed using ANOVA and mean comparison was performed by LSD. The F-test was considered significant at $p<0.05$ between treatments.

Results

Seed traits

Electrical conductivity: Applying aging treatment had a significant impact on the electrical conductivity of seeds (Table 1). Maximum leakage of electrolytes from both aged and non-aged seeds occurred when they had not been primed. However, when they were primed by GA, metabolites leakage reached its minimum level. Aging significantly increased the electrical conductivity of seeds (Table 2).

Table 1. Analysis of variance (mean square) for the electrical conductivity, germination percentage, speed of germination and vigor index of wheat seeds affected by accelerated ageing and priming.

Source of variance	Degree of freedom	Electrical conductivity	Germination percentage	Speed of germination	Vigor index
Accelerated ageing (AA)	1	0.0098**	7269.83**	369.92**	8928476.16**
Priming(P)	5	0.0187**	255.02**	18.57**	426797.53**
AA*P	5	0.00059*	11.92^{ns}	4.72*	235032.91^{ns}
Error	24	0.00017	9.37	1.38	96494.35
C.V.(%)		6.85	3.93	13.46	16.70

**, * and ns, significant at 0.01 and 0.05 probability level and non-significant, respectively

Table 2. Effects of seed aging and seed pre-treatments on electrical conductivity and speed of germination of wheat seed.

Priming treatment	Seed aging	EC (dS m^{-1} gr^{-1})	Speed of germination (seed day^{-1})
Non Primed	NAS	0.21d*	8.38e
	AA	0.29a	4.02h
Hydropriming	NAS	0.18f	9.80d
	AA	0.24b	4.29h
Ascorbic acid	NAS	0.16g	13.21b
	AA	0.20e	6.35f
Gibberellic acid	NAS	0.13j	14.38a
	AA	0.15h	6.73f
Brassionosteroid	NAS	0.19f	11.40c
	AA	0.22c	6.37f
Salicylic acid	NAS	0.14i	14.61a
	AA	0.19f	5.54g

*Means with the same letter in each column are not significantly different ($p\leq0.05$) based on LSD test.

Germination percentage, germination speed and vigor index: Results of Table 1 show that priming had significant effect on seed germination. Seed aging reduced germination percentage considerably (Fig. 1A). Among the priming treatments, the GA induces greatest percentage of germination; however, it had no significant difference with AS, BR and SA treatments. Seed deterioration had a significant effect on germination speed. Interaction of deterioration and type of regulators had a significant effect on germination speed (Table 1). Maximum speed of germination in the non-aged seeds was seen in GA treatment. Nevertheless, it had no significant difference with SA treatment. However, GA, AS and BR almost equally increased germination speed in the aged seeds, while SA comparatively improved germination to a lesser extent (Table 2). Applying accelerated aging treatment significantly reduced vigor index (Table 1 and Fig 2A), but priming improved it. Maximum of VI were seen in priming treatment using GA and SA (Fig. 2B).

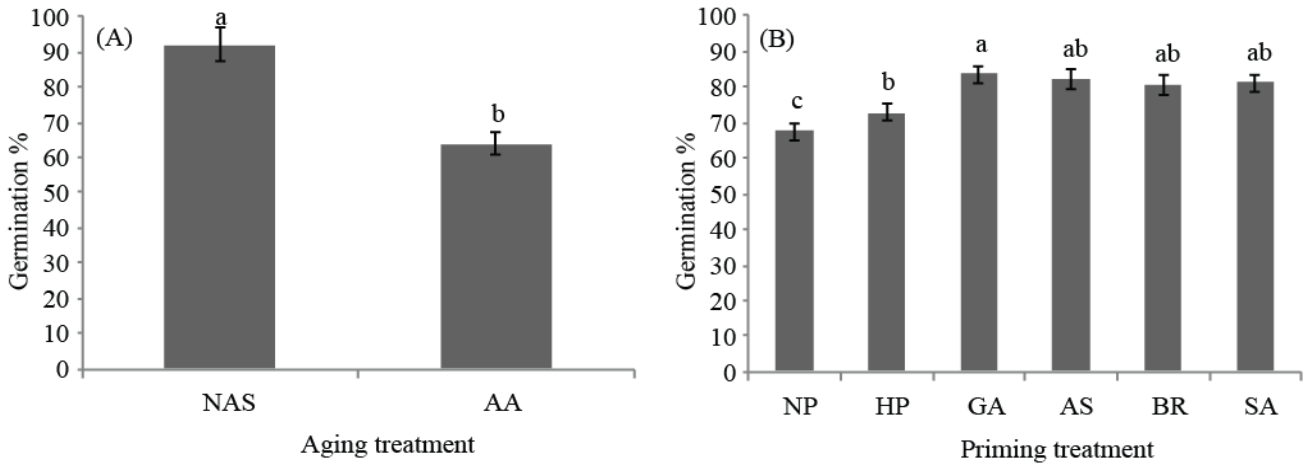

Fig. 1. Effects of accelerated aging (A) and priming n germination percentage. Data are average values from three replicates with standard errors. Means with the same letter in each data series are not significantly different ($p \leq 0.05$) based on LSD test. NAS and AAS, represent non-aged and accelerated aged seeds, respectively. The bars are the standard errors.

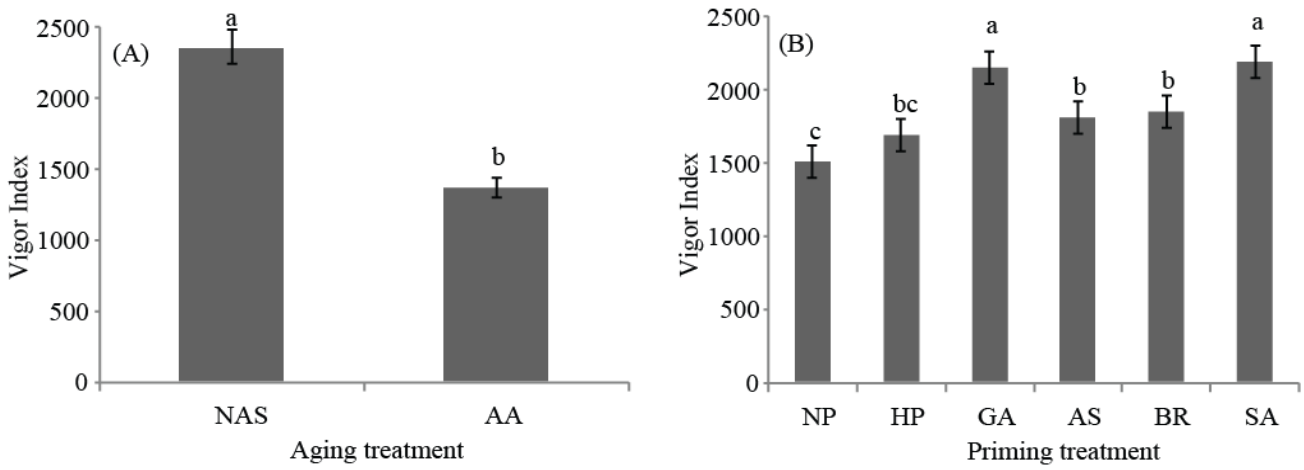

Fig. 2. Effects of accelerated aging (A) and priming treatments (B) on vigor index. Data are average values from three replicates with standard errors. Means with the same letter in each data series are not significantly different ($p \leq 0.05$) based on LSD test. NAS and AAS, represent non-aged and accelerated aged seeds, respectively. . The bars are the standard errors.

Seedling traits

Antioxidant enzymes: Seed ageing, priming and the interaction of ageing in priming have significant effects on the activity of oxidative enzymes (Table 3).Applying accelerated aging on the seed reduced activity of antioxidant enzymes such as CAT, POD, and SOD of the seedling. In general, hormonal priming of the seed increased activities of antioxidant enzymes (Table 4). Maximum of CAT activity was observed in prime treatment by GA. Moreover, POD activity was affected by priming treatment. When the seeds were primed by AS, maximum POD activity was observed in the seedling. Seed deterioration significantly reduced SOD activity. In the seedling obtained from normal seed, the maximum SOD activity was observed in AS treatment. In the seedling obtained from aged seeds, maximum activity was observed in SA treatment (Table 4).

Malondialdehyde, soluble protein, soluble sugar and proline content: Accelerated aging of seed increased MDA content in the seedling. The maximum seedling MDA content obtained when the aged and non-aged seeds were not primed. The lowest content of MDA in seedlings obtained from non-aged seeds ocurred when the seeds were primed with GA; while in seedlings obtained from deteriorated seeds the lowest MDA was seen when the seeds were primed with AS, (or SA).

The results indicated that different treatments of priming can reduce or postpone the oxidative damages caused by deterioration treatment and decrease the MDA production level (Fig. 3A). Accelerated aging of seed reduced SP in the seedling. Maximum SP content was observed in the seedling obtained from normal and deteriorated seeds in GA priming treatment. Control treatment had the minimum SP content under both conditions. Seed deterioration induces reduction of enzymatic activity, followed by reduction of SP contents (Fig. 3B). Seed aging, increased SS in the seedling. The maximum soluble sugar of the seedling was obtained from treating the aged seeds by SA and treating non-aged seeds by AS (Fig. 3C). Seed treatment using SA in the non-aged seeds and seed treatment using GA led to maximum proline content in the seedling (Fig. 3D).

Table 3. Analysis of variance (mean square) for the effects of seed accelerated aging and seed priming on the catalase, peroxidase and superoxide dismutase of wheat seedling.

Source of variance	Degree of freedom	Catalase	Peroxidase	Superoxide dismutase
Accelerated ageing (AA)	1	291.27**	400.00**	724.50**
Priming (P)	5	140.55**	15.97**	81.26**
AA*P	5	2.27*	4.72**	22.76**
Error	24	0.80	1.07	4.41
C.V. (%)		2.46	4.79	3.58

**, * and ns, significant at 0.01 and 0.05 probability level and non-significant, respectively

Table 4. Wheat seedling catalase, peroxidase, and superoxide dismutase affected by seed accelerated aging and seed priming.

Priming treatment	Seed aging	CAT (EU mg^{-1} protein min^{-1})	POD (EU mg^{-1} protein min^{-1})	SOD (EU mg^{-1} protein min^{-1})
NP	NAS	31.10i*	23.13e	61.03d
	AA	25.40j	15.63j	45.20g
HP	NAS	37.03f	24.20d	61.10a
	AA	31.47i	18.90g	51.77f
AS	NAS	38.87e	26.23a	65.50a
	AA	35.37g	22.00f	58.70e
GA	NAS	45.67a	25.53bc	64.00b
	AA	39.53d	19.40g	58.27e
BR	NAS	41.30c	25.83ab	62.30c
	AA	35.33g	18.07h	51.80f
SA	NAS	41.90b	25.03c	64.87ab
	AA	34.63h	15.97i	59.23e

*Means with the same letter in each column are not significantly different (p≤0.05) based on LSD test

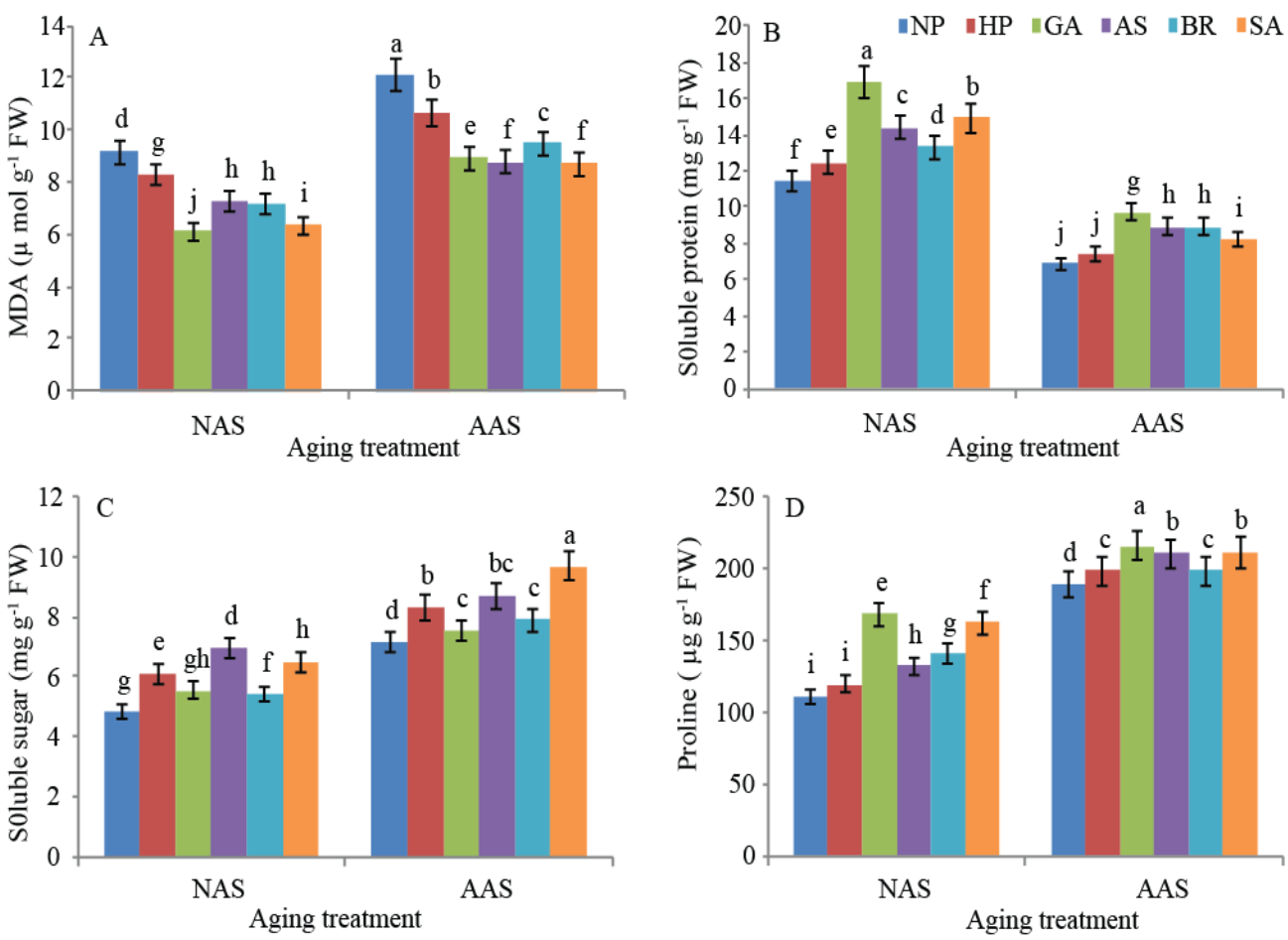

Fig. 3. Malondialdehyde (A) soluble protein (B), soluble sugar (C) and proline (D) content of wheat seedling affected by seed accelerated ageing and seed priming treatments. Data are average values from three replicates with standard errors. Means with the same letter in each data series are not significantly different (p≤0.05) based on LSD test. NAS and AAS, represent non-aged and accelerated aged seeds, respectively. The bars are the standard errors.

Discussion

Increased leakage of electrolytes under deteriorating conditions may denote the inability of cells to integrate cell membrane, which finally reduces germination power and percentage (Chang & Sung, 1998). Free radicals easily damage membranes and other cell contents (Payton *et al.,* 2001). In our research different priming treatments had a significant impact on the electrical conductivity of seeds. It was reported that electrolyte leakage in deteriorated seeds of wild species of the genus Brassica is a good indicator for evaluating the reduction of seed viability under deteriorating conditions (Mira *et al.,* 2011).

Reduction of germination speed occurred in this research probably due to the delay in starting the germination process in the aged seeds (Bailly *et al.,* 2000). The probable reason for the delay is that the seeds need time for repairing the damages to membrane and other parts of cells, resuming antioxidant activity, and preventing oxidative stress. Moreover, the restoration of the damages is only possible after absorbing water by the seed. Prolonged germination duration in aged seeds was also reported in other studies, which resulted in reduced germination speed (Bailly *et al.,* 2000; Basra *et al.,* 2003; Priestley, 1986). Seedling VI reduction is due to reduction of its components, i.e. the germination percentage and seedling length, which reduces under both aging conditions of seeds (Jiang *et al.,* 2016; Sung Jeng, 1994). The mechanism of seed priming is to initiate the repairing system for membrane and the metabolic preparation for germination through controlling the water absorption rate of seed. As a result, the germination capability and resistance to unfavorable conditions of the seed can be promoted obviously.

Percentage and speed of seed germination were improved after hormonal priming as compared to the control treatment, which is probably due to reducing lipid peroxidation and increasing antioxidant activities (Fig. 1; Table 4). Seed priming improves germination and seedling establishment in many plants such as wheat, corn, soybean and sunflowers (Khajeh-Hosseini *et al.,* 2003; Sadeghian & Yavari, 2004). Eisvand *et al.,* (2010b) have reported that hormonal priming of deteriorated seeds of wheatgrass could improve seed germination and consequently result in better seedling establishment.

GA treatment was the most suitable hormonal priming treatment in improving germination of age and non-aged seed (Table 2). Accelerated aging reduces viability and germination percentage of wheat (*Triticum aestivum*), peanuts (*Arachis hypogaea*) and soybean (*Glycine max*) (Lehner *et al.,* 2008; Sung, 1996; Sung & Jeng, 1994). GA is one of the major growth regulators in seed germination. In this process, endosperm or cotyledon is where GA acts (Karssen *et al.,* 1989). This study demonstrated that GA regulator might generally have a positive effect. (Da Silva *et al.,* 2005) proved that GAs are needed for elongating fetal cell and loosening endosperm during germination of coffee beans.

Oxidative stress is the result of increasing levels of intracellular oxygen free radicals. Increasing antioxidant enzymes may enable a plant to tolerate environmental stress (Janda *et al.,* 1999). Another study reported the improving effect of different seed pre-treatments on increasing activity of enzymes (Bailly, 2004). Numerous studies have proved that seed deterioration is closely related to the production of oxygen free radicals (Goel *et al.,* 2003; Rajjou *et al.,* 2008). Seeds are inherently capable of maintaining balance in producing free radicals and eliminating them through a germination process. In these conditions, plants activate digestive systems of oxygen free radicals, which may include antioxidant enzymes and non-enzymatic antioxidant, so as to control damage and protect macromolecules (Sharma *et al.,* 2012).

Our findings also proved that SOD, CAT, and POD activities were increased in wheat seedling by applying the priming treatment (Table 4). Therefore, priming may improve performance of seed and reduce lipid peroxidation throughout the germination. Wheat seed pre-treatment using different growth regulators improved the germination indices. Similar reactions were observed in cucumber (*Cucumis sativus*) seeds (Jennings and Saltveit, 1994). Antioxidant enzymes play a crucial role in seed aging development and changing their activity may reduce seed quality (Bailly & Kranner, 2011; Goel *et al.,* 2003; McDonald, 1999).

The results indicated that AS acted as an effective antioxidant. AS may prevent the risk of oxidative stress in plant due to removing the free radicals produced by stresses, especially radical oxygen, and involving in stimulation, cellular expansion, and absorption of nutrients into cells. One of the reasons for improving physiological traits through AS vitamin is its antioxidant property and limitation of oxygen free radicals. The ability of ascorbate in reducing or donating electron for MDHA production is the basis of biological advantage of its antioxidant capacity (Buettner & Schafer, 2004).

SA increased the activity of antioxidant enzymes such as POD, SOD and CAT. The external use of this regulators improved resistance to oxidative stress in tomatoes (Szepesi *et al.,* 2008). Other studies indicated that external salicylic acid can regulate activity of antioxidant enzymes and increase plant resistance to abiotic stresses (He *et al.,* 2002). SA protects wheat plant against oxidative stress by reducing lipid peroxidation through affecting enzymatic and non-enzymatic mechanisms. Moreover, it reduces oxidative power by affecting H_2O_2 and is effective on activities of ACC synthase and ACC oxidase (Davies, 2004).

In this study, SA reduced MDA content under deteriorating conditions, which was probably due to reducing the damaging effects of free radicals and protecting membrane. This way, it prevents from damaging unsaturated fatty acids and reducing membrane permeability. Consumption of SA for plants reduces ROS production, which enhances resistance. Moreover, SA increases certain plant regulators, including auxins and cytokinins and reduces ion leakage from plant cells (Ghoulam & Fares, 2001; Shakirova *et al.,* 2003). MDA content is an important index, which shows damage to cellular membranes. Therefore, it can be argued that MDA level indicates the degree of damage. Lipid peroxidation is the first indication of oxidative damage. This phenomenon reduces membrane fluidity, increases leakage from cells and membrane, thus damaging membrane proteins, enzymes, and ion channels (Xin *et al.,* 2014).

According to previous studies, degeneration of proteins may be due to the increase of protease activity and/or other catabolic enzymes, which are activated under stress conditions and/or due to fragmentation of proteins caused by the toxic effects of oxygen free radicals, which eventually leads to protein content reduction (Davies, 1987). Protein concentration reduction is an index and a common indicator in oxidative stresses, which was proved by earlier studies (Moran *et al.,* 1994; Seel *et al.*, 1992). SP content is one of the major indices of damage to proteins in the metabolism of plants, denaturation of proteins, and other metabolic processes in plants. Accelerated aging, reduced SP in the seedlings. Control treatment had the Seed deterioration induces reduction of enzymatic activity, followed by reduction of SP contents (Fig. 3B). Accelerated aging may prevent protein synthesis. However, when the aging treatment was carried out, proteins could facilitate refolding processes of the denatured protein level due to the heat shock of hydrophobic areas. In addition, damage to the proteolytic enzyme system leads to increasing the SP content in wheat seeds. Finally, it can be stated that membrane system of seed is damaged severely and osmotic adjustment of solute leakage in seed increases, which finally reduces the SP content (Peng *et al.,* 2011).

As an osmotic regulator, SS functions as the stabilizer of cellular membranes and protector of cell turgor (Slama *et al.*, 2007). Degradation of insoluble carbohydrates can be considered as the major factor in increasing SS under deteriorating conditions. Increasing SS content was also reported in soy and sorghum due to oxidative stress conditions (Newton *et al.,* 1986). Our study indicated that seed aging caused an increase in SS content of seedlings.

It is well known that dissolved organic matters play an important role in mitigating the effects of different stresses (Azooz, 2009). Accumulation of compatible solutes reduces osmotic potential in cytoplasm involved in maintaining water homeostasis in intercellular spaces (Sairam & Tyagi, 2004). It has been proved that soluble sugars have the maximum osmotic activity among organic solutions (Ashraf & Harris, 2004). Increasing SS such as monosaccharides and disaccharides, as osmotic compounds, was reported under stress conditions (Sánchez-Moreno *et al.,* 1998). Hamid *et al.* (2008) proved that wheat pre-treatment using salicylic acid increases SS under salinity conditions.

Proline is one of the osmotic regulators. Identified in most macrophytes, proline amino acid is usually accumulated in large quantities in response to environmental stresses. Rapid degradation of proline amino acid after termination of stress conditions may provide the ATP required for oxidative phosphorylation (OXPHOS) and restoration of stress-caused damages (Bates *et al.,* 1973). Seed deteriorating treatment had a significant effect on the proline content of wheat seedling (Table 5). It was proved that seed priming increased proline and SS under stress conditions as compared with control treatment. Researchers suggested that proline content is a major factor in protective reactions in wheat and response to stresses; it involves in reducing harmful effects of stresses and accelerates repair processes during oxidative stresses (Kuznetsov & Shevyakova, 1999). Proline accumulation in stressful conditions increases intracellular osmotic pressure which is one of the mechanisms of resistance to oxidative stresses in plants (Mauromicale & Cavallaro, 1996). Accumulation of large amounts of osmolytes (such as proline) is an adaptive response in plants exposed to stressful conditions. Proline also acts directly as an antioxidant to protect the cell from free radical damage.

Compatible solutes also prevent destabilization during a drought by replacing the water molecules around the component. As a compatible osmolyte, proline exerts a protective function by regulating osmotic potential and scavenging free radicals. Sugar accumulation in drought stress conditions helps to keep up membrane and protein stability.

Table 5. Analysis of variance (mean square) for the malondialdehyde, soluble protein, soluble sugar, and proline of wheat seedling affected by seed accelerated ageing and priming.

Source of variance	Degree of freedom	Malondialdehyde	Soluble protein	Soluble sugar	Proline
Accelerated ageing (AA)	1	51.12**	279.44**	48.76**	38142.09**
Priming (P)	5	9.25**	12.50**	4.01**	1542.93**
AA*P	5	0.38**	1.94*	0.35**	348.61**
Error	24	0.09	0.52	0.08	49.59
C.V.(%)		3.60	6.54	4.02	4.10

**, * and ns, significant at 0.01 and 0.05 probability level and non-significant, respectively

Conclusion

According to the physiological characteristics in seed and seedling, we confirmed that priming affected the stimulating metabolism in wheat seeds, accelerating germination, and improving quality of seedlings. After priming, wheat seed showed higher abilities of germination and produced seedling with more quality. The mechanism of seed priming is to start the repairing system for membrane and the metabolic preparation for germination through controlling the water absorption rate. As a result, the germination ability and resistance to unfavorable conditions of the seed can be promoted obviously.

Free radical accumulation and decreased antioxidant activities may ultimately lead to reduced seed quality and its resulting seedling. The findings suggested that the seeds

on which hormonal priming was performed may have a better physiological quality. Improvement of seed quality in priming conditions can be attributed to reduced lipid peroxidation and increased antioxidant enzyme activity, which scavenges oxygen free radicals. It appears that seed pre-treatment using GA increased metabolism of sugars and proline and protein contents thereby improved seedling quality. The SA priming had the best effect on reducing MDA content and increasing SS content among other treatments under deteriorating conditions. In general, GA is the most suitable pre-treatment for deteriorated seeds to improve seed and seedling efficiency. Through increasing Xyloglucan endo- transglycosylase (XET) enzyme, which makes expansin proteins penetrate into cell walls, GA provides the desirable conditions for cell growth. Positive effects of priming using GA may be due to membrane structure improvement, germination speed improvement, cell elongation, and cell division in the seedling.

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