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RESEARCH PAPERS

Evaluation of Germination and Antioxidant Activity in GA_3-Primed Deteriorated Wheat Seed

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Abstract—Seed aging or deterioration is considered as one of the seed capacity reducing factors and limiting seed germination, hence, realizing the effective physiological factors on seed deterioration is very important. In order to study the germination indices and enzymatic and non-enzymatic antioxidants in wheat (*Triticum aestivum* L. cv. Pishtaz) seed as affected by accelerated seed aging and gibberellic acid (GA_3) priming, a laboratory experiment was conducted in Ferdowsi University of Mashhad, Mashhad, Iran, during 2017–2018. For this purpose, different levels of seed aging (100% relative humidity at 40°C for 4, 6 and 7 days, respectively) and GA_3-primed seeds (0, 25, 50 and 100 mg/L) were the first and second experimental factors, respectively. The results indicated that seed germination percentage and indices, superoxide dismutase and ascorbate peroxidase activities, vitamin E, glutathione and ascorbic acid contents were reduced with severe deterioration, compared with low deterioration, whereas, the mentioned traits were improved by increasing the levels of GA_3 seed priming. Overall, the results of this experiment suggest that the GA_3-primed seed can be an effective approach to improve the seed germination and seedling growth in wheat under aging conditions.

Keywords: Triticum aestivum, antioxidant assay, ascorbate peroxidase, seed deterioration, superoxide dismutase

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INTRODUCTION

Seeds are often stored for a few days, weeks, months or even years close to the time of their deteriorations as they often are not planted immediately after harvest [1]. Long term storage imposes the reduction or loss of the seed vigor and low temperature and moisture result in commercial and genetic losses. Due to the role of temperature and moisture in accelerating seed deterioration, they are considered as determinative factors in the storage longevity of seeds [2].

To consider the main influencing factors in seed storage capacity, it can be referred to high temperature, ambient relative humidity and seed moisture content [3]. Accordingly, the vigor index as the first component of seed quality would be lost during deterioration and followed by the loss of germination capacity and viability.

Seed aging mechanisms at the time of storage were used to manipulate the rate of aging when exposing seeds to age-accelerating conditions. Genetic degradation, reduced respiration, loss of membrane integrity and enzyme degradation, are considered as the main physiological changes of seed deterioration [4]. In fact, what occurred at the time of aging is considered as seed deterioration and plentiful cellular and biochemical changes such as loss of membrane integrity, impairment of RNA, protein syntheses and DNA degradation [1]. On the other hand, due to the long process of natural aging in the seeds, artificially accelerated aging is considered as an alternative method to predict biochemical mechanisms. In this regard, disruption in anti-oxidative systems is known as the main results of aged seeds in higher plants [5].

Generally, seed priming with plant hormones such as gibberellic acid (GA_3) is considered as a practical approach for better seedling establishment, especially under stressful conditions [3]. However, GA_3 function has not been well understood in terms of its effects on enzymatic reactions, especially during the accelerated seed aging process. Therefore, the current study was aimed to investigate the effect of seed deterioration on germination indices and enzymatic and non-enzy-

Table 1. Analysis of variance on studied traits of wheat seed

Source of variation	GP	GI	CAT	APX	SOD	AA	GSH	α-tocopherol
Seed deterioration (D)	**	**	**	**	**	**	**	**
GA_3 priming (G)	**	**	**	**	**	**	**	**
D × G	**	**	**	**	**	**	**	**
CV, %	6.19	6.75	6.52	5.23	3.62	0.85	3.94	1.98

GP—germination percentage; GI—germination index; CAT—catalase; APX—ascorbate peroxidase; SOD—superoxide dismutase; AA—ascorbic acid; GSH—glutathione. ** Significant at $P < 0.01$.

matic antioxidants activities in wheat. Moreover, the effect of GA_3-primed seeds on the mentioned traits and determining the best level were studied.

MATERIALS AND METHODS

Experiments layout and seed material. The study was conducted as a laboratory experiment based on a completely randomized design arranged in factorial with four replications at Faculty of Agriculture, Ferdowsi University of Mashhad, Iran during 2017–2018. Different levels of seed aging (4, 6 and 7 days) and GA_3-primed seeds (0, 25, 50 and 100 mg/L) were the first and the second experimental factors, respectively.

The wheat seeds (*Triticum aestivum* L. cv. Pishtaz) were acquired in the Research Center for Plant Sciences, Ferdowsi University of Mashhad, Iran. The seeds were produced in 2016, with the germination of 98%.

Accelerated aging and standard germination tests. First, wheat seeds were sterilized for 1 min with 1% sodium hypochlorite and then washed with distilled water. In order to evaluate seed vigor, an accelerated aging test was set according to Bai and Tang [6]. Wheat seeds were placed in some plastic boxes with 100% humidity and 40°C for 4 (low deterioration), 6 (medium deterioration) and 7 days (severe deterioration). After aging treatments, the samples were placed in a laboratory temperature (25 ± 1°C for 24 h) to reach the initial moisture content (about 15%). Due to the high initial germination percentage in the provided seeds, the non-deterioration treatment was ignored. In the next step, treated seeds were primed with GA_3 at 25 ± 1°C for 24 h and then dried under same conditions.

After performing the accelerated aging test, 50 seeds from each treatment were germinated (on Whatman filter paper no. 1 in 9 cm Petri dishes) in a germinator at 20°C for 7 days. The germinated seeds (with 2 mm radicle growth) were counted daily. Then, weighted germination index (WGI) was calculated according to following equation [7]:

$$\mathrm{WGI} = (7n_1 + 6n_2 + \ldots + n_7)/(7 \times N),$$

where n_1–n_7—number of germinated seeds from the first to seventh day; N—the total number of seeds per Petri dish.

Antioxidant enzymes activity. In order to extract antioxidative enzymes, 0.5 g of deteriorated seeds were homogenized with 10 mL of 1 mM K_2HPO_4-NaH_2PO_4 buffer (pH 7.4). The homogenate was centrifuged at 12000 *g* for 24 min and enzyme activities were determined by using the resulted supernatant. The whole extraction procedure was carried out at 4°C [8]. Evaluation of SOD activity was done by measuring its ability to prevent photochemical reduction of nitroblue tetrazolium based on Stewart and Bewley [9]. In accordance with Chandlee and Scandalios [10] method, due to the reduction of H_2O_2 extinction, the activity of CAT was measured at 240 nm as the reduction of absorbance. As ascorbate was oxidized, APX activity was measured at 290 nm by reduction of absorbance [11].

Non-enzymatic antioxidants determination. Vitamin E (tocopherol), reduced glutathione (GSH), and ascorbate (vitamin C) were determined according to Desai [12], Ellman [13], and Himesh et al. [14], respectively. The content of non-enzymatic antioxidants was calculated using the standard curve.

Statistical analysis. All data were subjected to analysis of variance (ANOVA) using Minitab software (Minitab Inc., State College, PA, United States). Means were compared with Tukey test at 5% probability level ($P < 0.05$).

RESULTS

Wheat Seed Vigor due to Artificial Aging

According to the results, the individual effects of seed deterioration and GA_3 priming were significant on all seed indices. In addition, interactions between experimental factors were significant on these variables (Table 1).

As can be seen from Fig. 1, the germination percentage and germination index were significantly reduced when the accelerated aging test was performed. For instance, germination percentage decreased by 93.9% with severely deteriorated seeds, compared with low deteriorated seeds. Nonetheless, the application of GA_3 at 25, 50 or 100 mg/L significantly increased germination percentage at the low and medium deterioration levels (Fig. 1a). Moreover, the same trend was also observed for germination index. The highest value was obtained from 100 mg/L GA_3 (Fig. 1b).

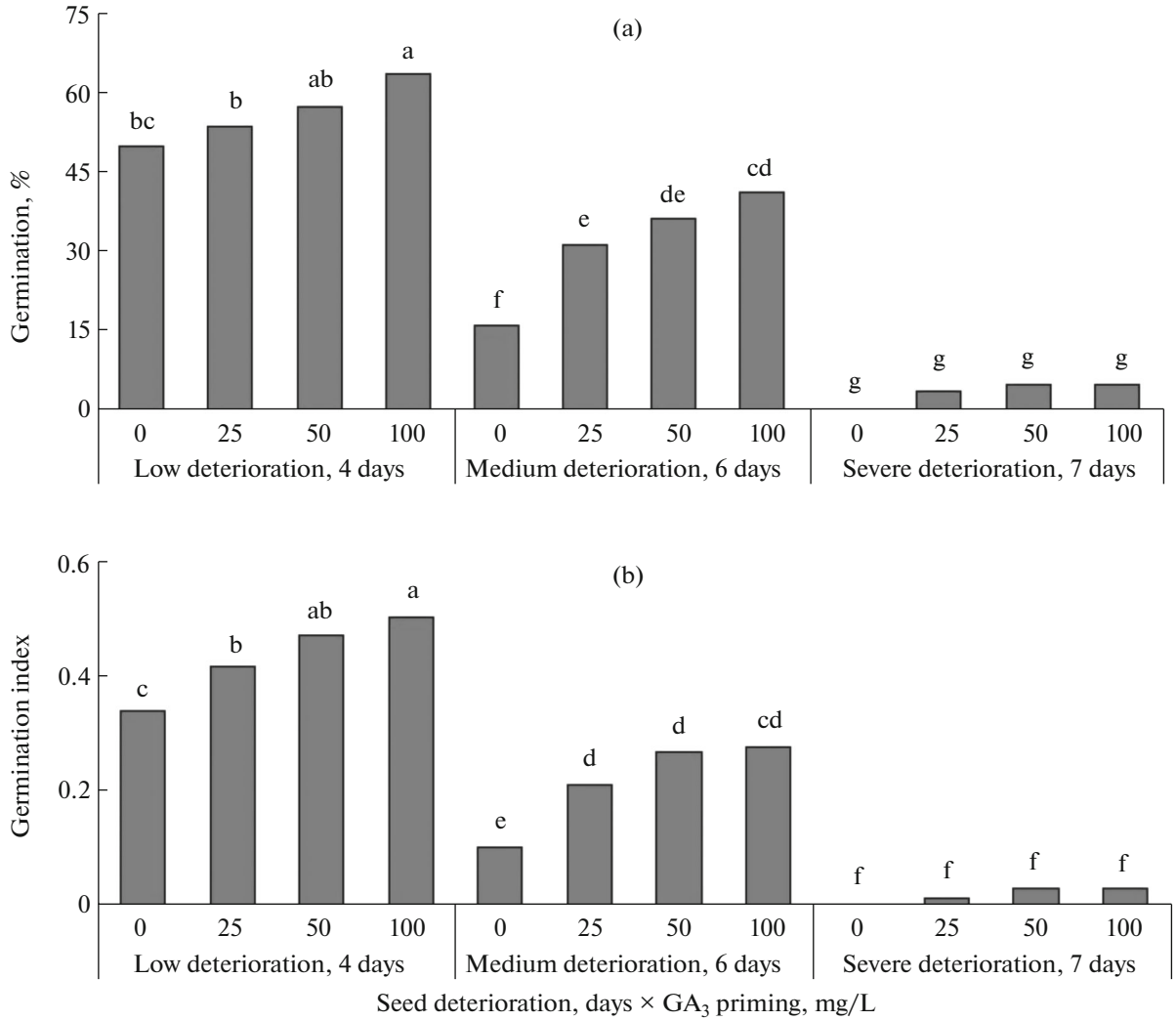

Fig. 1. Interaction effects of seed deterioration and GA_3 priming on germination percentage (a) and germination index (b). The same letters are not significantly different at $P \leq 0.05$ (Tukey test). The non-deterioration of seed is ignored.

Assay of Antioxidant Enzymes Activity in Wheat Seeds after Artificial Aging

Increase in seed aging severity significantly decreased CAT, APX and SOD activity (Figs. 2a–2c). For instance, under severe seed deterioration, in comparison with low seed deterioration, CAT and APX activity decreased by 59.8 and 56.1%, respectively. By contrast, GA_3 application mitigated the adverse effect of seed deterioration on CAT, APX and SOD activity. For example, under severe seed deterioration, application of 100 mg/L GA_3 increased CAT, APX and SOD activity by 61.5, 32.6 and 6.4%, respectively, compared with control (no GA_3 application) (Figs. 2a–2c).

Non-enzymatic Antioxidants in Wheat Seeds after Artificial Aging

According to the results, the increase in seed deterioration severity significantly decreased AA, GSH and α-tocopherol content. However, GA_3 seed priming alleviated the negative effect of seed deterioration on mentioned indices (Figs. 3a–3c). For instance, when GA_3 at 100 mg/L was applied on severely deteriorated seeds, AA, GSH and α-tocopherol content increased by 5.7, 44.3 and 21.6%, respectively, compared with control (no priming).

At the low, medium and severe seed aging levels, CAT, APX, and SOD activity showed a strong positive correlation with germination index (Figs. 4a–4c). Furthermore, there was a similar correlation between AA, GSH and α-tocopherol content with germination index (Figs. 4d–4f). For instance, under severe seed deterioration, R^2-values for AA, GSH and α-tocopherol content were recorded as 99, 91 and 97%, respectively. Nonetheless, the severity of seed deterioration caused a slight impact on R^2-values, with a variation range between 87–99% in all correlations (Figs. 4a–4f).

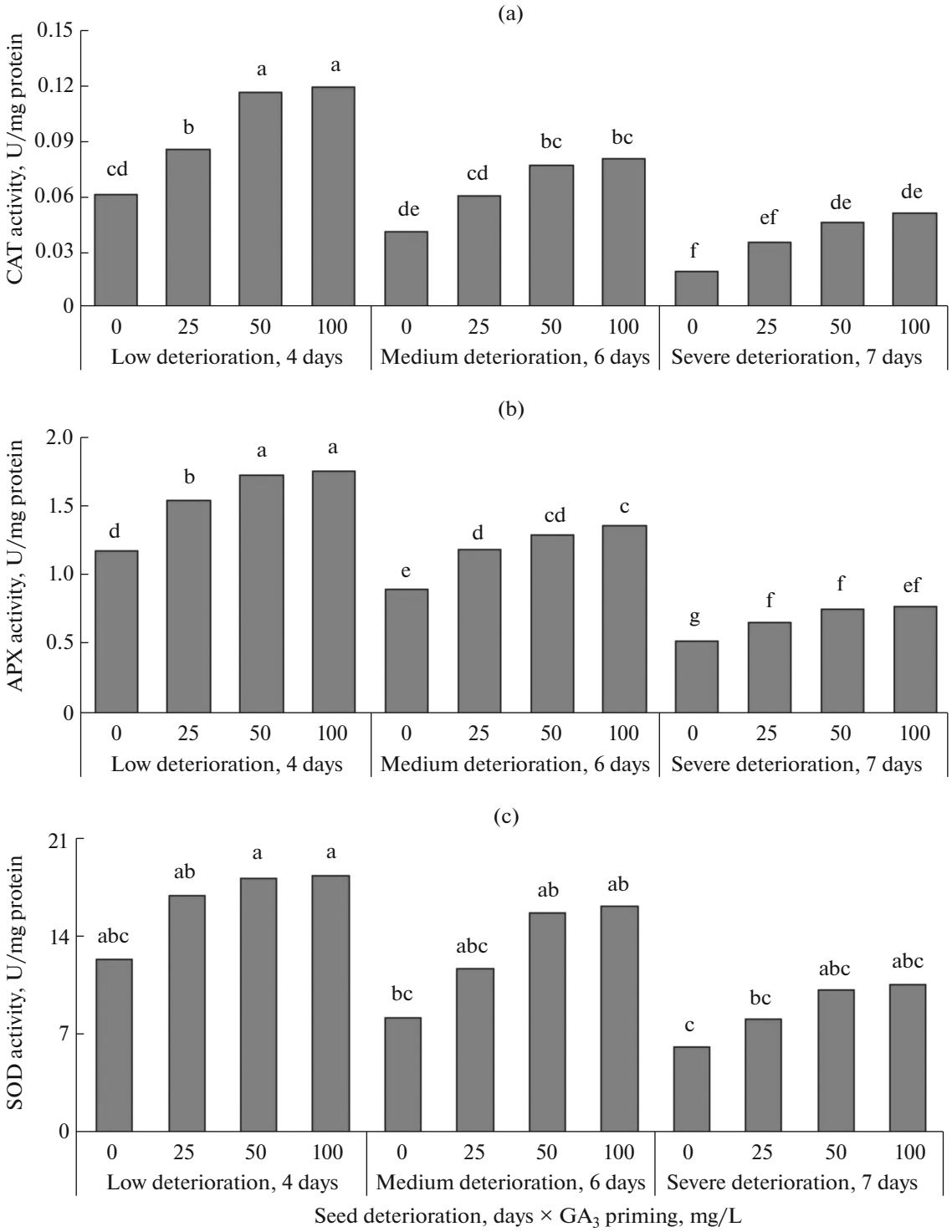

Fig. 2. Interaction effects of seed deterioration and GA_3 priming on CAT (a), APX (b) and SOD (c) activity. The same letters are not significantly different at $P \leq 0.05$ (Tukey test). The non-deterioration of seed is ignored. CAT—catalase, APX—ascorbate peroxidase, SOD—superoxide dismutase.

DISCUSSION

The present study demonstrated that deterioration reduced germination index and germination percentage in comparison with not-deteriorated seeds, however, GA_3 priming in deteriorated seeds increased germination index and germination percentage. Losing

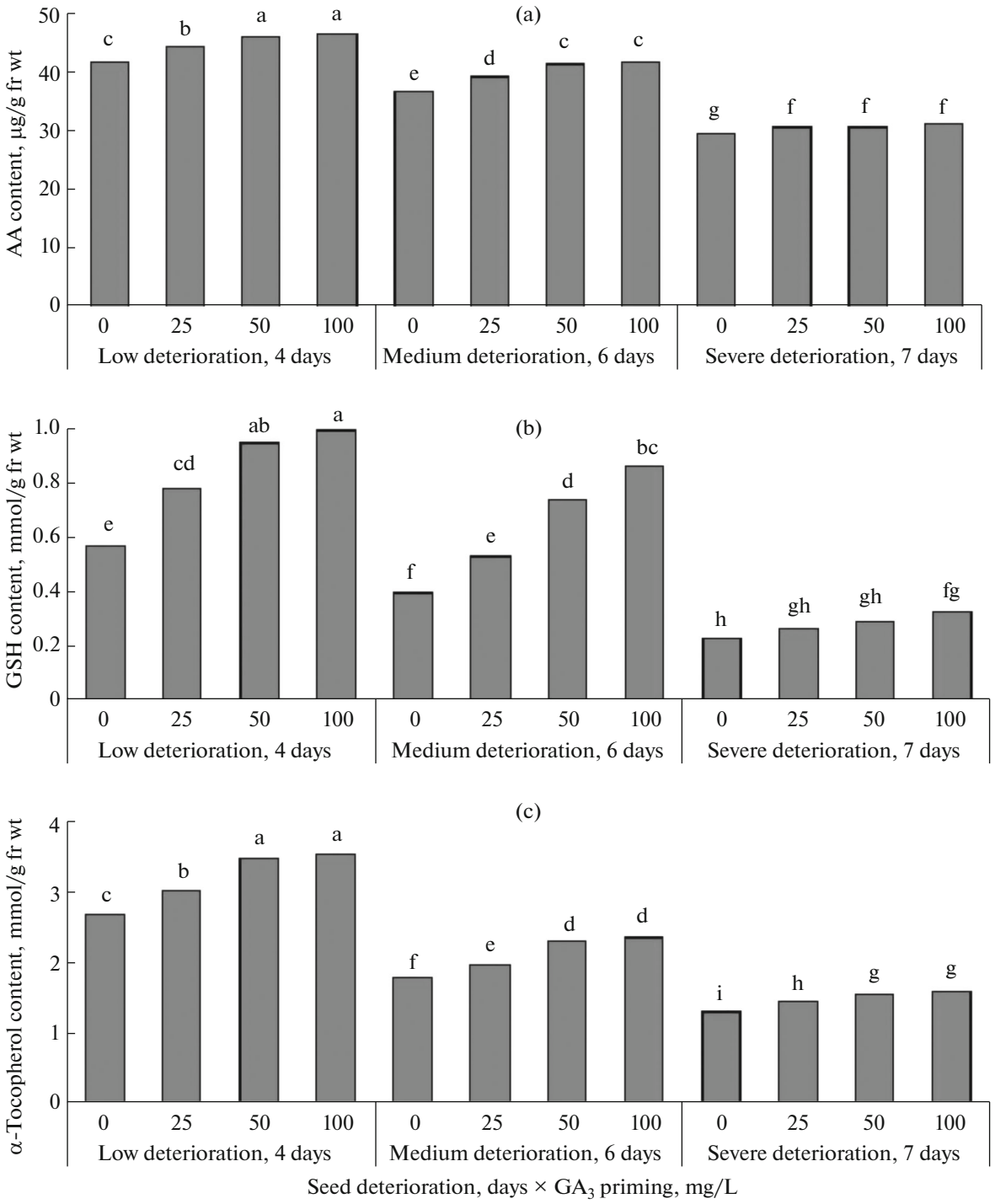

Fig. 3. Interaction effects of seed deterioration and GA_3 priming on AA (a), GSH (b) and α-tocopherol (c) content. The same letters are not significantly different at $P \leq 0.05$ (Tukey test). The non-deterioration of seed is ignored. AA—ascorbic acid; GSH—glutathione.

seed viability is considered as a reason for reducing germination index and germination percentage under high temperature and moisture [15].

One of the important factors in seed deterioration is considered as damage to cell membrane structures at the time of seed aging [16]. High temperature and moisture reduced germination percentage and germination speed and the higher impact was recorded on reducing vigor and moisture of the seeds [17].

Different storage conditions make significant differences in enabling the germination of seeds with moisture content [18]. Increasing germination time is

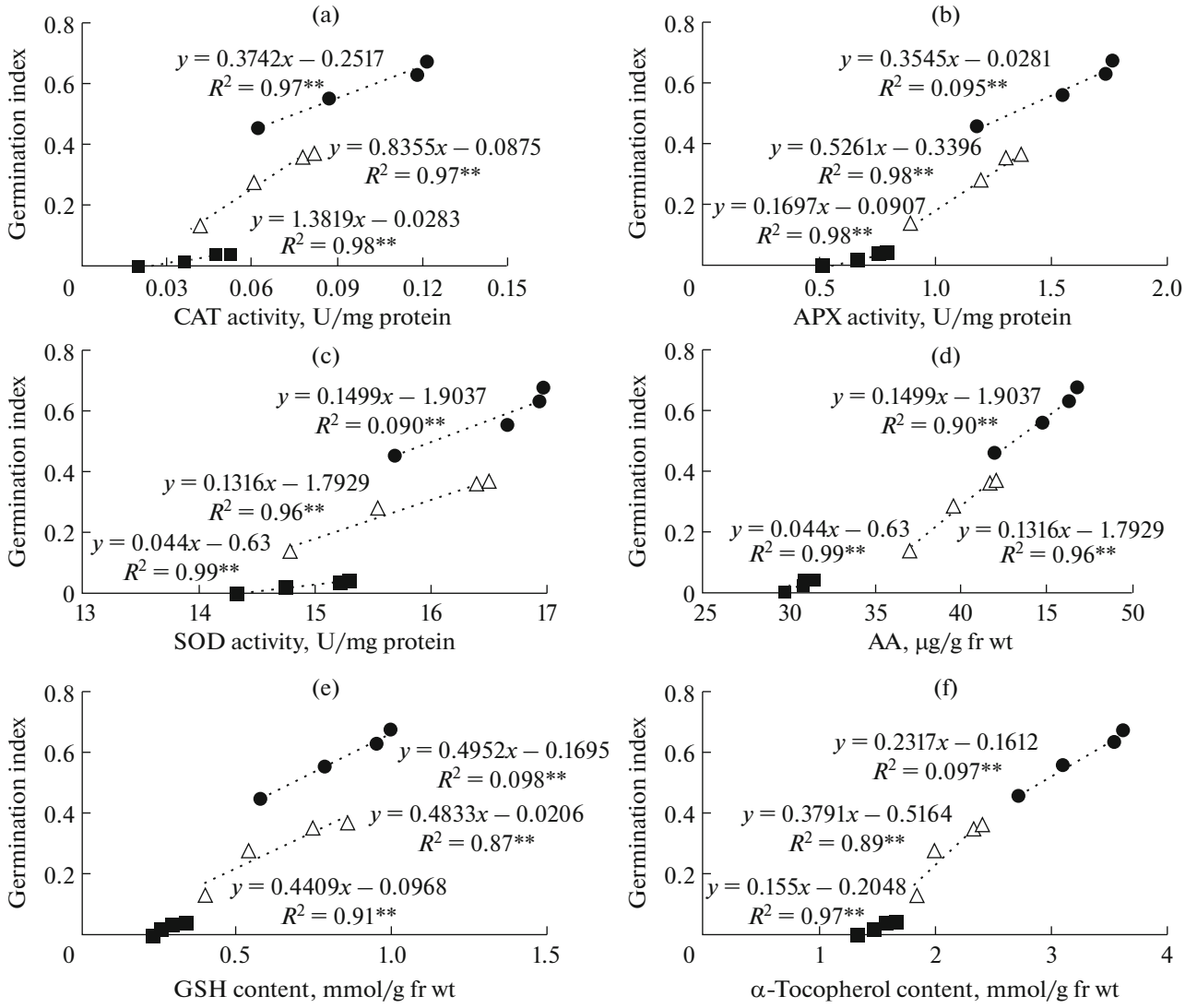

Fig. 4. Correlation between experimental traits with germination index. ** Significant at $P < 0.01$. The non-deterioration of seed is ignored. CAT—catalase; APX—ascorbate peroxidase; SOD—superoxide dismutase; AA—ascorbic acid; GSH—glutathione. Low deterioration (circle), medium deterioration (triangle), and severe deterioration (square): 4, 6 and 7 days, respectively.

possible as a result of interruption at the time of germination in deteriorated seeds. Possible cause of the interruption is that seeds need time to recover damages to the membrane and other parts of the cell and also to restart the antioxidant system and prevent oxidative stress, considering that recovering of all mentioned damages is possible only after priming. So, time duration to achieve complete germination increases in damaged seeds, therefore, it reduces speed germination [19]. In this context, it has been reported that ascorbic acid and α-tocopherol application increased the germination index of deteriorated sesame seeds [20].

Seeds treatment with gibberellic acid and salicylic acid after deterioration caused an increase in sesame germination index [20]. In other studies, GA_3 priming improved the vigor of deteriorated seeds in wheat [21].

Reactive oxygen species (ROS) play an important role in the progression of aging and various age-related disorders due to the "free radical theory of aging." Enzymatic and non-enzymatic antioxidant systems such as SOD, APX, CAT, ASA, and GSH regulate the "redox homeostasis" [22].

Antioxidant enzyme activity including CAT, APX and SOD are used to clarify the role of ROS scavenging system in seed aging progress. As mentioned before, there was a significant reduction in the content of CAT, APX and SOD activity in severely deteriorated seeds in comparison with low deteriorated seeds. However; the content of enzymes has increased by applying GA_3. CAT is known as one of the primary enzymatic defense of plants against oxidative stress induced by aging, freezing, dehydration, osmotic stress and heavy metals [23]. About 30% reduction was

observed in CAT activity after 7 days of aging compared to the activity of non-aged sunflower seeds. Catalase activity of 7 days aged seeds was considerably restored by priming treatment. CAT transcripts decreased to an indiscoverable level at 7 days of aging, so it was occurred because of the degradation of oxidized RNA after aging-induced ROS storage. Indeed, the content of total extracted RNA from aged seeds was 2.6-fold lower than that of non-aged seeds [24].

H_2O_2 was mainly enzymatically scavenged by CAT and APX. The mentioned antioxidant enzymes may act together to deactivate H_2O_2 produced under oxidative environments. It has been reported that the ability of rice seeds to scavenge H_2O_2 may reduce after artificial aging of seeds [25].

SOD catalyzes the dismutation of O_2^- to H_2O_2 and O_2, it also protects cells from oxidative stress. However, the aging process of rice seeds did not affect SOD activity levels [26]. SOD does not play a major role in seed aging as already observed by Stewart and Bewley [9] in soybean. Lack of sunflower seed viability at the time of incubation at 45°C in water or at 100% RH reduced the activities of SOD and CAT. In addition, catalase was more sensitive to high temperature than SOD [27].

In the present study, the content of non-enzymatic antioxidants was significantly reduced in aged seeds, whereas the seed priming with GA_3 increased the content of non-enzymatic antioxidants. Since vitamin C regenerates other antioxidants such as α-tocopherol (vitamin E) and glutathione peroxidase, its role in improving seed vigor is very important [28].

GSH may be involved in reducing H_2O_2. However, amount of total GSH was reduced in aged rice seeds [26]. Radical scavenging enhances GSH concentration and primary rate of lipid peroxidation along with the protection and secondary antioxidants (i.e. α-tocopherol) works by decreasing the chain propagation and amplification of lipid peroxidation [29].

Generally, tocopherols are needed to protect polyunsaturated fatty acids (PUFAs) from damaging effects of ROS at the time of germination and early seedling growth. It is demonstrated that PUFAs in seed lipids are protected from oxidation by tocopherols [30].

According to the results of this study, controlled aging in wheat seed led to a significant reduction of the features of germination, and enzymatic and non-enzymatic antioxidants in comparison with control and it were significant compared to not-deteriorated seeds. The highest reduction belonged to the severe deterioration. Seed priming with GA_3 increased measured factors and the maximum amount was related to 100 mg/L GA_3 in severe deterioration.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

REFERENCES

1. Hu, D., Ma, G., Wang, Q., Yao, J., Wang, Y., Pritchard, H.W., and Wang, X.F., Spatial and temporal nature of reactive oxygen species production and programmed cell death in elm (*Ulmus pumila* L.) seeds during controlled deterioration, *Plant Cell Environ.*, 2012, vol. 35, pp. 2045–2059. https://doi.org/10.1111/j.1365-3040.2012.02535.x
2. Seyyedi, S.M., Tavakkol Afshari, R., and Daneshmandi, M.S., The relationships between fatty acids and heterotrophic seedling growth in winter canola cultivars during accelerated seed aging process, *S. Afr. J. Bot.*, 2018, vol. 119, pp. 353–361. https://doi.org/10.1016/j.sajb.2018.09.034
3. Shahi-Gharahlar, A., Yavari, A.R., Khayyat, M., Jalali, N., and Farhoudi, R., Effects of soaking temperature, stratification, potassium nitrate and gibberellic acid on seed germination of loquat trees, *J. Plant Nutr.*, 2012, vol. 35, pp. 1735–1746. https://doi.org/10.1080/01904167.2012.698353
4. Dell'Aquila, A., Wheat seed ageing and embryo protein degradation, *Seed Sci. Res.*, 1994, vol. 4, pp. 293–298. https://doi.org/10.1017/S0960258500002324
5. Bijanzadeh, E., Naderi, R., Nosrati, K., and Egan, T.P., Effects of accelerated ageing on germination and biochemistry of eight rice cultivars, *J. Plant Nutr.*, 2017, vol. 40, pp. 156–164. https://doi.org/10.1080/01904167.2016.1201502
6. Bai, B.Z. and Tang, X.J., *Plant Physiology Testing Technology,* Beijing: China Sci. Press, 1993.
7. Walker-Simmons, M.K. and Sesing, J., Temperature effects on embryonic abscisic acid levels during development of wheat grain dormancy, *J. Plant Growth Regul.*, 1990, vol. 9: 51. https://doi.org/10.1007/BF02041941
8. Sairam, R.K., Rao, K.V., and Srivastava, G., Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration, *Plant Sci.*, 2002, vol. 163, pp. 1037–1046. https://doi.org/10.1016/S0168-9452(02)00278-9
9. Stewart, R.C. and Bewley, J.D., Lipid peroxidation associated with accelerated aging of soybean axes, *Plant Physiol.*, 1980, vol. 65, pp. 245–248. https://doi.org/10.1104/pp.65.2.245
10. Chandlee, J.M. and Scandalios, J.G., Analysis of variants affecting the catalase development program in

maize scutellum, *Theor. Appl. Genet.*, 1984, vol. 69, pp. 71–77.
https://doi.org/10.1007/BF00262543

11. Nakano, Y. and Asada, K., Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplast, *Plant Cell Physiol.*, 1981, vol. 22, pp. 867–880.
https://doi.org/10.1093/oxfordjournals.pcp.a076232
12. Desai, I.D., Vitamin E methods for animal tissues, *Methods Enzymol.*, 1984, vol. 105, pp. 138–147.
https://doi.org/10.1016/S0076-6879(84)05019-9
13. Ellman, G.C., Tissue sulfhydryl groups, *Arch. Biochem. Biophys.*, 1959, vol. 82, pp. 70–77.
14. Himesh, S., Singhai, A.K., and Sarvesh, S., Quantification of ascorbic acid in leaves of *Annona squamosa, Int. J. Pharm. Pharm. Sci.*, 2012, vol. 4, pp. 144–147.
15. Gheidary, S., Akhzari, D., and Pessarakli, M., Effects of salinity, drought, and priming treatments on seed germination and growth parameters of *Lathyrus sativus* L., *J. Plant Nutr.*, 2017, vol. 40, pp. 1507–1514.
https://doi.org/10.1080/01904167.2016.1269349
16. Veselovsky, V.A. and Veselova, T.V., Lipid peroxidation, carbohydrate hydrolysis, and Amadori–Maillard reaction at early stages of dry seed aging, *Russ. J. Plant Physiol.*, 2012, vol. 59, pp. 811–817.
https://doi.org/10.1134/S1021443712030181
17. Li, S., Li, F., Wang, J., Zhang, W., Meng, Q., Chen, T.H.H., Murata, N., and Yang, X., Glycinebetaine enhances the tolerance of tomato plants to high temperature during germination of seeds and growth of seedlings, *Plant Cell Environ.*, 2011, vol. 34, pp. 1931–1943.
https://doi.org/10.1111/j.1365-3040.2011.02389.x
18. Marshal, A. and Lewis, D.N., Influence of seed storage conditions on seedling emergence, seedling growth and dry matter production of temperate forage grasses, *Seed Sci. Technol.*, 2004, vol. 32, pp. 493–501.
https://doi.org/10.15258/sst.2004.32.2.19
19. Bailly, C., Benamar, A., Corbineau, F., and Come, D., Antioxidant systems in sunflower (*Helianthus annuus* L.) seeds as affected by priming, *Seed Sci. Res.*, 2000, vol. 10, pp. 35–42.
https://doi.org/10.1017/S0960258500000040
20. Hoseinikhah, F.S., Parsa, S., Tavakkol Afshari, R., and Esmaeili, A., Effect of ascorbic acid (vitamin C) and alpha-tocopherol (vitamin E) on seed deterioration process of two sesame (*Sesamum indicum* L.) cultivars, *Seed Plant Prod. J.*, 2013, vol. 2, pp. 83–100.
21. Eisvand, H.R., Tavakkol Afshari, R., Sharifzadeh, F., Maddah Arefi, H., and Hesamzadeh Hejazi, S.M., Effects of hormonal priming and drought stress on activity and isozyme profiles of antioxidant enzymes in deteriorated seed of tall wheatgrass (*Agropyron elongatum* Host.), *Seed Sci. Technol.*, 2010, vol. 38, pp. 280–297.
https://doi.org/10.15258/sst.2010.38.2.02
22. Ahmad, P., Sarwat, M., and Sharma, S., Reactive oxygen species, antioxidants and signaling in plants, *J. Plant Biol.*, 2008, vol. 51, pp. 167–173.
https://doi.org/10.1007/BF03030694
23. Park, S.J., Huang, Y., and Ayoubi, P., Identification of expression profiles of sorghum genes in response to greenbug phloem-feeding using cDNA subtraction and microarray analysis, *Planta*, 2006, vol. 223, pp. 932–947.
https://doi.org/10.1007/s00425-005-0148-1
24. Kibinza, S., Bazin, J., Bailly, C., Farrant, J., and Corbineaua, F., Catalase is a key enzyme in seed recovery from ageing during priming, *Plant Sci.*, 2011, vol. 181, pp. 309–315.
https://doi.org/10.1016/j.plantsci.2011.06.003
25. Teixeira, F.K., Menezes-Benzvente, L., Galváo, V.C., Margis, R., and Margis-Pinheiro, M., Rice ascorbate peroxidase gene family encodes functionally diverse isoforms localized in different subcellular compartments, *Planta*, 2006, vol. 224, pp. 300–314.
https://doi.org/10.1007/s00425-005-0214-8
26. Yin, G., Xin, X., Song, C., Chen, X., Zhang, J., Wu, S., Li, R., Liu, X., and Lu, X., Activity levels and expression of antioxidant enzymes in the ascorbate–glutathione cycle in artificially aged rice seed, *Plant Physiol. Biochem.*, 2014, vol. 80, pp. 1–9.
https://doi.org/10.1016/j.plaphy.2014.03.006
27. Bailly, C., Benamar, A., Corbineau, F., Corbineau, O., and Come, D., Changes in malondialdehyde content and in superoxide dismutase, catalase and glutathione reductase activities in sunflower seeds as related to deterioration during accelerated aging, *Physiol. Plant.*, 1996, vol. 97, pp. 104–110.
https://doi.org/10.1111/j.1399-3054.1996.tb00485.x
28. Maeda, H., Sakuragi, Y., Bryant, D.A., and Penna, D.D., Tocopherols protect *Synechocystis* sp. strain PCC 6803 from lipid peroxidation, *Plant Physiol.*, 2005, vol. 138, pp. 1422–1435.
https://doi.org/10.1104/pp.105.061135
29. Ghiselli, A., Serafini, M., Maiani, G., Azzini, E., and Ferro-Luzzi, A., A fluorescence-based method for measuring total plasma antioxidant capability, *Free Radic. Biol. Med.*, 1995, vol. 18, pp. 29–36.
https://doi.org/10.1016/0891-5849(94)00102-P
30. Sattler, S., Gilliland, L., Lundback, M., Pollard, M., and DellaPenna, D., Vitamin E is essential for seed longevity and for preventing lipid peroxidation during germination, *Plant Cell*, 2004, vol. 16, pp. 1419–1432.
https://doi.org/10.1105/tpc.021360