Chapter

Pumpkin Seeds Germination and Seedling Growth under Abiotic Stress

Masoumeh Asadi Aghbolaghi, Mohammad Sedghi, Ghasem Parmoon and Beata Dedicova

Abstract

An increase in heavy metals (HMs) pollution due to agricultural and industrial activities has become a serious environmental problem. Heavy metal poisoning and its accumulation in food chains are one of the major environmental and health problems of modern societies. Heavy metal poisoning and its accumulation in food chains are major environmental and health risks. Among these metals, lead and cadmium are the metals with the most concern due to their toxicity potential for animals and plants even for humans. The effect of seed aging on the germination and growth of *Cucurbita pepo* L. seedlings has been evaluated in an experiment with a completely random design where seeds were exposed to deterioration at 45°C and 95% humidity for 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 days. The measured characteristics included germination percentage and speed, germination uniformity, and days to reach 10 and 50% of germination. Changes in seed viability were evaluated by using the tetrazolium test. The results of the analysis of variance showed that aging had a significant effect on all studied characteristics at the level of 1%. It was also observed that during aging, the amount of color accepted by the seed tissue is decreasing, which indicated a decrease in the vigor and viability of seeds.

Keywords: *C. pepo*, accelerating aging, tetrazolium test, heavy metals, cadmium

1. Introduction

An increase in heavy metals (HMs) pollution due to agricultural and industrial activities has become a serious environmental problem in the current world [1–3]. Heavy metal poisoning and its accumulation in food chains are major environmental and health problems in modern societies [3–5]. Among these metals, lead and cadmium are the metals with the most concern due to their toxicity potential for animals and plants [6, 7]. The most significant effects of heavy metal toxicity are oxidative stress, pigment dysfunction, and change in protein activity [8].

Plants are equipped with antioxidant defense systems to eliminate or reduce oxidative damage [9]. The plant antioxidant system is composed of antioxidant

enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and catalase (CAT) [10]. It has been shown that high cadmium concentrations disrupt most physiological processes in plants [11]. As cadmium concentration increased, there is a significant increase in catalase and peroxidase. As it was shown in our previous study There have been reports that pumpkin tends to collect small amounts of heavy metals [12] and also showed that concomitant use of humic acid reduced the destructive effects of heavy metals on the *C. pepo* L. by reducing cadmium uptake by seeds.

C. pepo L. is one of the most important crops that belong to the *Cucurbitaceae* family, an herbaceous, perennial, and polymorphic vegetable that grows in tropical conditions [13, 14]. Pumpkin fruit contains large amounts of vitamins, minerals, and biologically active substances [15]. The pumpkin seed oil contains high amounts of unsaturated fatty acids and is effective in treating intestinal worms, prostate hypertrophy, stomach, intestinal inflammation, and atherosclerosis lowers LDL levels, prevents irregular heart contractions, reduces common blood clots, and reduces the risk of bladder and kidney stone formation [16, 17].

Medicinal plants are the main reservoirs of many medicinal compounds and substances, which are influenced by environmental factors in addition to genetic factors. *C. pepo* L. is one of the valuable medicinal plants in the pharmaceutical industry and its oil is used in medicine. The most important components of the oil of this plant are linoleic and oleic fatty acids, sterols, micronutrients, vitamins, and carotenoids [18].

Seedling germination and growth are one of the most important stages of plant growth, which determines the degree of success of agricultural systems [19]. These stages are strongly influenced by seed quality (viability and seed vigor) [20]. Temperature, relative humidity of the environment, and seed humidity are the main factors in the preservation and viability of seeds during storage [21]. Increasing the amount of seed moisture causes an increase in the aging speed [22] therefore if the temperature and relative humidity of the environment are high, the seeds will deteriorate sooner and they will be closer to death while reducing the quality. The reduction of plasma membrane integrity, molecular changes in the structure of nucleic acids and stimulation of lipid peroxidation, and reduction of the activity of hydrolytic enzymes are among the most important changes that occur in the seed during deterioration. These changes can lead to a decrease in seed quality, percentage, and speed of germination, and slower growth of seedlings [23].

In the deteriorated seeds due to disturbances in cellular organelles such as mitochondria and glyoxysomes, the production rate of reactive oxygen species and superoxide radicals increases, which causes damage to cells [24]. The tetrazolium test is used to estimate the viability of seeds. In this test, dead and alive tissues are distinguished based on their relative respiration rate in the condition that they have absorbed water.

In the tetrazolium test, seed viability is determined based on the activity level of dehydrogenase enzymes. The dehydrogenase enzyme reacts with the precursors during respiration and causes the release of hydrogen ions into the oxidized and colorless salt solution. These ions are combined with tetrazolium and turn this colorless salt into red formazan salt. The continuation of the viability of the seeds is determined based on the staining pattern of the embryo and the intensity of its staining [25]. Therefore, we use the tetrazolium test to study the viability and aging process of pumpkin seeds as a complementary test to our previous study with accumulations of cadmium in pumpkin seeds and the effect of humic acid.

2. Materials and methods

2.1 Seeds germination and tetrazolium test

Pumpkin seeds for this experiment were obtained from Pakan Bazr Isfahan Company, Isfahan, Iran (32°38′41"N51°40′03″ E).

This experiment was carried out in the form of a completely random design with three repetitions. In order to perform aging, an accelerated aging test was used. In this method, the seeds were kept in a mesh container in a seeds germinator (RICO, Scientific Instruments, Germany) with a relative humidity of 95% and with a temperature of 45°C for 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 days. To carry out the germination test, the seeds were disinfected with 1% sodium hypochlorite for 3 minutes and then 25 seeds were placed in a 9 cm Petri dish with two layers of Whatman paper and were transferred to the germinator with a temperature of 25°C. Counting of germinating was done daily for 14 days and the criterion of germinating was 2 mm of the root.

The Germinator software was used to calculate the trait characteristics related to seed germination. This software calculates Gmax (maximum germination), T50 (time required to reach 50% germination) and T10 (time required to reach 10% germination), CU (uniformity of germination = the time interval for germination rate to reach from 25–75%) and AUC (germination speed). Regarding germination uniformity, the lower the number obtained, the more uniform the germination of the seeds is [26].

The tetrazolium test was used to evaluate the seed viability and vigor. For this purpose, seeds were first soaked in distilled water for 24 hours to increase their respiratory activity. Then, the seeds were kept for 2 hours in 5 ml of 1% tetrazolium solution (2,3,5triphenyl tetrazolium chloride (TTC)) with 0.05 M phosphate buffer (pH 3.7) and then their viability and dyeability were evaluated [27].

Analysis of data was done using SAS 9.2 software and a comparison of means was done using an LSD test at a 5% probability level. The graphs were drawn with Excel.

3. Results and discussion

Germination ($P \leq 0.1$), (**Table 1**). Aging caused a decrease in the percentage of germination. So that in the conditions of nonaging and up to 2 days after aging, pumpkin seeds germination was 92%. However, with the increase in seed, the results

		MS				
SOV	**df**	**Gmax**	**T50**	**T10**	**CU**	**AUC**
Accelerated aging	10	0/17**	2568/57**	4327/9**	939/28**	521/39**
Error	22	0/0005	13/96	35/47	19/73	1/95
CV (%)	—	13/7	13/2	7/37	9/72	9/66

***Significant at the 1% level p < 0.01 probability level. Gmax: max of germination, T10 and T50 days: 10 and 50% of germination; CU: germination uniformity, AUC: speed of germination. SOV: the source of variation; MS: the mean square; CV: the coefficient of variation.*

Table 1.
Analysis of the variance of pumpkin seeds aging.

of the analysis of variances showed that accelerated aging had an effect on the maximum aging time, germination decreased and reached its minimum value of 29% in 20 days of aging (**Figure 1**). Gholami and Benny [15] also reported a reduction of germination during seed deterioration. During the deterioration, the production of reactive oxygen species, causing lipid peroxidation and damage to the cell membrane, and the increase of released fatty created a disturbance in the process of seed imbibition, which causes an increase in the leakage of seed reserves and a decrease in germination [28]. Govender et al. [29] also showed that corn seed storage for 1 year under natural conditions caused a decrease in germination percentage. They stated that the reason could be the presence of disease-causing fungi in natural storage conditions. It has been shown in research that the activity of enzymes, especially hydrolytic enzymes, decreases during seed aging, which can cause a decrease in germination [30], and also reported a reduction of germination during seed deterioration. During the deterioration, the production of reactive oxygen species, causing lipid peroxidation and damage to the cell membrane, and the increase of released fatty created a disturbance in the process of seed imbibition, which causes an increase in the leakage of seed reserves and a decrease in germination [31]. Govender et al. [32] showed that corn seed storage for 1 year under natural conditions caused a decrease in germination percentage. They stated that the reason could be the presence of disease-causing fungi in natural storage conditions. It has been shown in research that the activity of enzymes, especially hydrolytic enzymes, decreases during seed aging, which can cause a decrease in germination [19]. It has also been reported that deterioration causes a decrease in seed vigor and can disrupt the activity of antioxidant enzymes in pumpkin seeds [15].

The germination period was also influenced by seed aging, so aging at the level of 1 percent was significant in the time required to reach 10 and 50 percent germination (**Table 1**). The comparison of the means showed that accelerated aging for 20 days caused an increase in the time of germination (**Figures 2** and **3**). The highest rate of germination about time was obtained in the control treatment, and its value reached its lowest value from thy 16 of deterioration (**Figure 4**). Being high in this quality, in addition to showing high germination, is also an expression of high germination

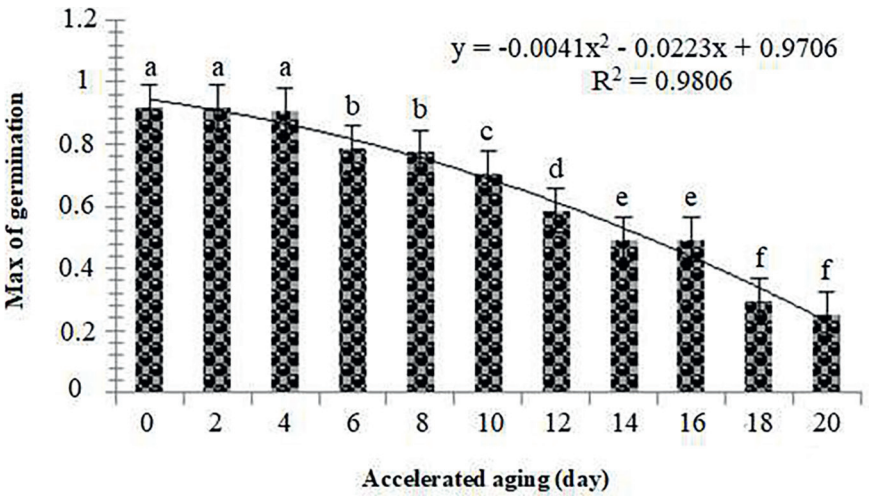

Figure 1.
Effect of accelerated aging on the maximum germination of pumpkin seed.

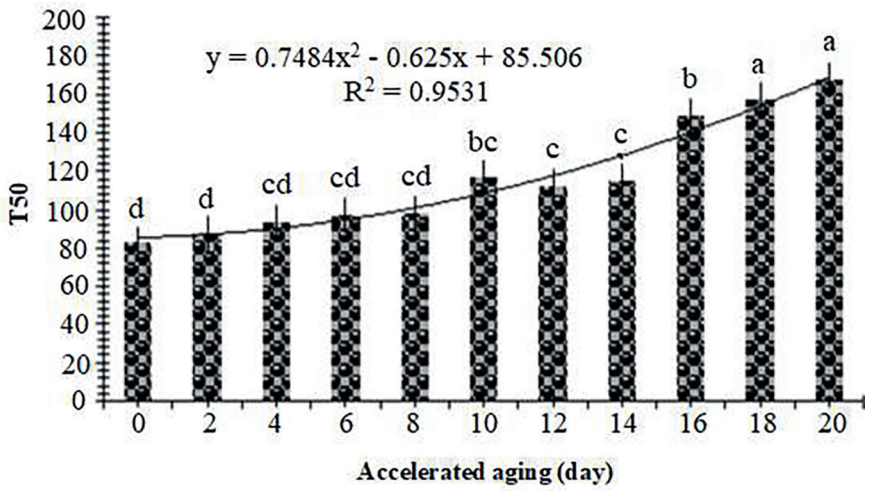

Figure 2.
Effect of accelerated aging on T50 (day to 50% germination) of pumpkin seed.

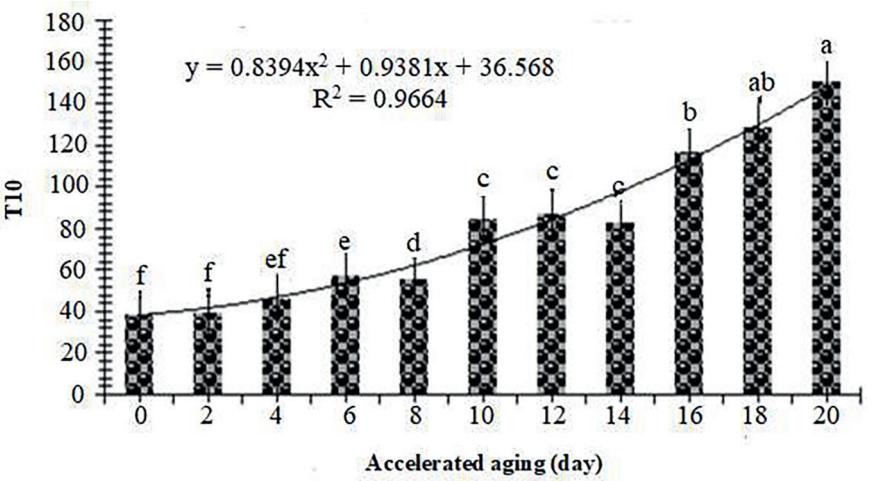

Figure 3.
Effect of accelerated aging on T10 (day to 10% germination) of pumpkin seed.

speed. Accelerated aging at the level of 1 percent also had a significant difference in the uniformity of germination (**Table 1**). In general, it can be said that accelerated aging caused a decrease in the uniformity of germination (**Figure 5**).

A high germination time indicates a lower germination speed, which indicates low seed quality. The faster emergence of crops on the farm, the better the establishment of the seedlings. High germination speed gives agricultural plants the possibility of better competition against weeds, reduces their damage, and increases the yield of agricultural products. Soltani et al. [33] showed that increasing the storage time of wheat seeds at a temperature of 40°C caused an increase in the average germination time. It has been said that the reason for the decrease in seed germination speed under high temperatures and temperatures is the loss of seed viability due to the loss of membrane health [34]. Veselova and Veslovesky [35] noted increasing the storage

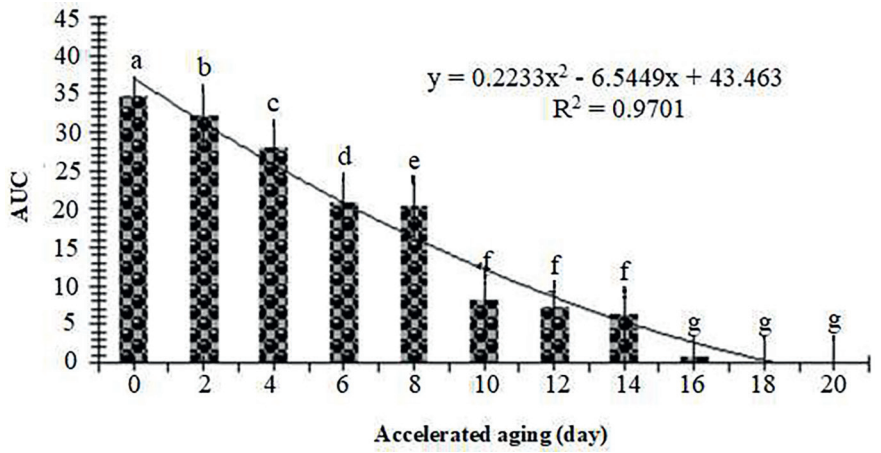

Figure 4.
The effect of accelerated aging on the germination speed of pumpkin seed.

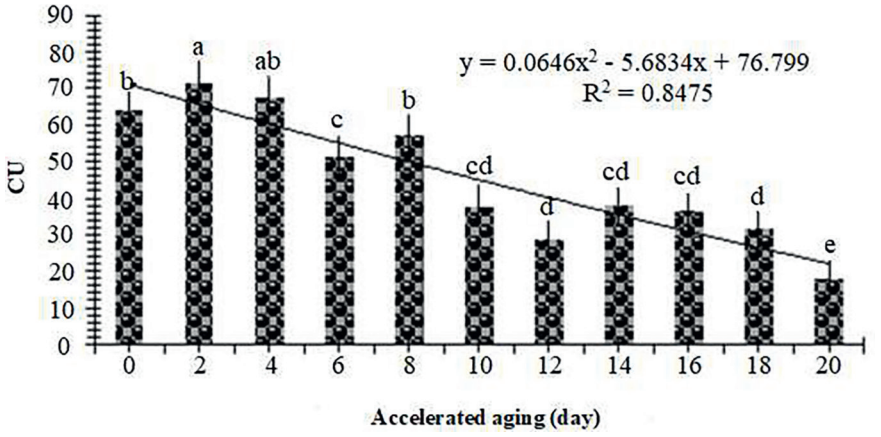

Figure 5.
The effect of accelerated aging on the germination uniformity of pumpkin seed.

time of seeds under the conditions of accelerated aging causes an increase in seed deterioration and a decrease in the percentage and speed of germination.

According to the results of biochemical tests, as a result of aging, the dyeability of the seed tissues is reduced, which indicates the reduction of seed viability and vigor. The results show that 2 days of aging did not affect seedling axis tissues in the embryo, but the differences with the increased aging time. At the lower level of aging, the first part whose dye ability decreases is part of the root and shoot. The increase in the aging time causes the expansion of the area of noncoloring to the center of the seed and cotyledons. The results showed that the dyeability of the seed tissues is the same as the results of the germination test, no significant frequencies were observed between the low levels of aging in terms of dyeability and germination.

At the beginning of germination, the rate of seed respiration increases by absorbing water during the imbibition process. An increase in respiration causes an increase

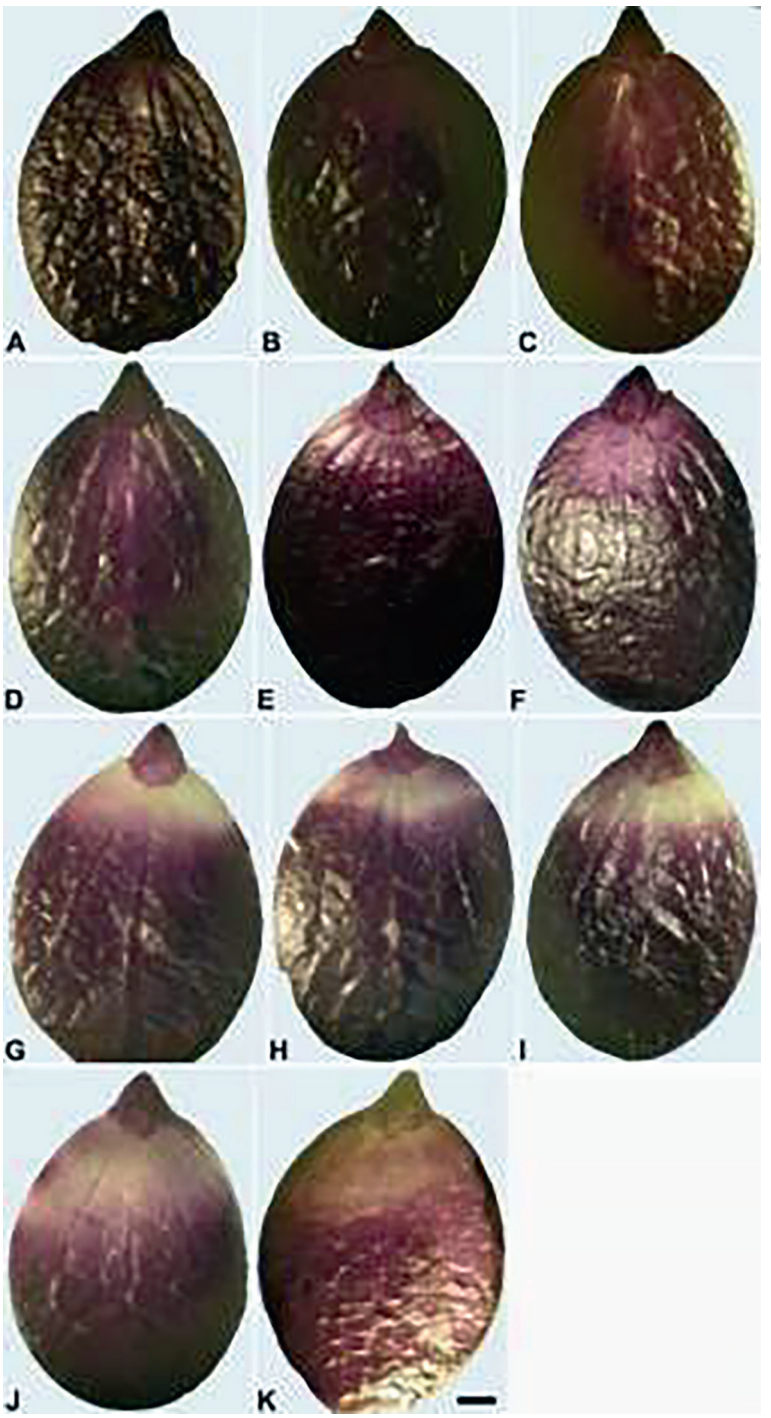

Figure 6.
Effect of accelerated aging on the viability of pumpkin seed using Tetrazolium test. The size bar is 1 mm. A No aging, B is 2 days C is 4 days. D is 6 days, E 8 days, F 10 days, G is 12 days, H is 14 days, I 16 days, F 18 days, and K is 20 days of aging.



Figure 7.
Effect of accelerated aging on the growth of pumpkin seedlings (a: Non-aging, B: 2 days aging, C: 4 days aging, D: 6 days aging, E: 8 days aging, F: 10 days aging, G: 12 days aging, H: 14 days aging, I: 16 days aging, J: 18 days aging, K: 20 days aging).

in the reaction of the hydrogen produced during respiration with the hydrogenase enzyme, which causes the tetrazolium salt to create a colored compound that causes different parts of the seed to be colored. The higher the level of coloring, the higher the respiration in the seed and the higher the vigor and viability. Other studies have shown that during aging, the activity of hydrolytic enzymes decreases, which can cause a decrease in respiration [31]. As a result, the increase of H_2O_2 and free radicals in the cytoplasm of aging cells causes photosynthetic activities to become inactive. Protein binding decreases, and the sensitivity of proteins to proteolytic enzymes increases, which ultimately leads to a decrease in the viability of seeds [24].

Aging, in addition to the decrease in germination ability, caused a decrease in seed vigor. In fact, seed vigor is the first factor that decreases during aging [36]. The staining of the tissues during the tetrazolium test can be an indicator of seed vigor. The higher the coloring of the seed tissues, the higher the enzyme activity and the higher seed's vigor.

According to the results in **Figure 6** it can be seen that during the aging, the coloring of the seed tissues was reduced, which can be an indicator of the reduction of the vigor of this seed. The results of seedlings' growth also showed that their growth rate of them decreased due to aging. The decrease in the growth rate of seedlings can be caused by the decrease in seed vigor during aging. The growth of the seedlings showed that there is not much difference between the low levels of aging as well as the germination ability and seed survival and the length of their roots and stems was also the same, but the increase in the aging time caused a decrease in the growth rate of the seedlings (**Figure 7**). The length of the stems is one of the attributes that indicate a seed vigor. The seeds with low vigor may germinate, but due to the reduction in the length of the stem, they cannot emerge, and in this way, the percentage of emergence in the field is reduced. On the other hand, short stems have less emerging power due to their lower dry weight compared to long stems [23]. Soltani et al. [33] reported that the dry weight of seedlings decreased with increasing storage periods. The decrease

in the dry weight of seedlings can be due to the decrease in the seed reserve or the decrease in the conversion efficiency of the dynamic reserve due to the decrease in the activity of hydrolytic enzymes during aging.

4. Conclusions

In conclusion, it can be confirmed that *C. pepo* L. in the tetrazolium test showed that aging causes a decreased percentage of germination, the uniformity of germination, and an increase in the average time required for germination, which ultimately causes a decrease in the viability of seeds. The decrease in the uniformity of germination caused the decrease in seed vigor due to aging, which ultimately causes a decrease in the growth of seedlings. Seed aging with the production of active oxygen species causes damage to the cell membrane and the activity of seed enzymes especially hydrolytic enzymes, which reduces the vigor of seeds and their viability.

Conflict of interest

The authors declare that they have no conflict of interest.

Author details

Masoumeh Asadi Aghbolaghi[1], Mohammad Sedghi[2], Ghasem Parmoon[3] and Beata Dedicova[4*]

1 Department of Agronomy and Plant Breeding, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

2 Department of Agronomy and Plant Breeding, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran

3 Sugar Beet Research Department, Kermanshah, Agricultural and Natural Resources Research and Education Center, AREEO, Kermanshah, Iran

4 Department of Plant Breeding, Swedish University of Agricultural Sciences (SLU), Sweden

*Address all correspondence to: beata.dedicova@slu.se

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References

[1] Imran MA, Sajid ZA, Chaudhry MN. Arsenic (As) toxicity to germination and vegetative growth of sunflower (*Helianthus annuus* L.). Polish Journal of Environmental Studies. 2015;**24**:1993-2002

[2] Khan MU, Shahbaz N, Waheed S, Mahmood A, Shinwari ZK, Malik RN. Comparative health risk surveillance of heavy metals via dietary foodstuff consumption in different land-use types of Pakistan. Human and Ecological Risk Assessment: An International Journal. 2016;**22**:168-186

[3] Rizwan M, Ali S, Qayyum MF, Ibrahim M, Zia-ur-Rehman M, Abbas T, et al. Mechanisms of biochar-mediated alleviation of toxicity of trace elements in plants: A critical review. Environmental Science and Pollution Research International. 2016;**23**:2230-2248

[4] Khan MJ, Jones DL. Effect of composts, lime and diammonium phosphate on the phytoavailability of heavy metals in a copper mine tailing soil. Pedosphere. 2009;**19**:631-641

[5] Gupta SK, Scott C, Mitra A. Advances in Land Resource Management for 21st Century. New Delhi, India: Soil Conservation Society of India; 2011. pp. 446-46

[6] Salazar MJ, Rodriguez JH, Nieto GL, Pignata ML. Effects of heavy metal concentrations (Cd, Zn, and Pb) in agricultural soils near different emission sources on quality, accumulation and food safety in soybean [*Glycine max* (L.) Merrill]. Journal of Hazardous Materials. 2012;**233**:244-253

[7] Zhao Y, Fang X, Mu Y, Cheng Y, Ma Q, Nian H, et al. Metal pollution (Cd, Pb, Zn, and As) in agricultural soils and soybean, glycine max, in southern China. Bulletin of Environmental Contamination and Toxicology. 2014;**92**:427-432

[8] Rastagoo L, Alemzadeh A, Tale AM, Tazangi SE, Eslamzadeh T. Effects of copper, nickel, and zinc on biochemical parameters and metal accumulation in gouan,' Aeluropus littoralis'. Plant Knowl. J. 2014;**3**:42-49

[9] Petrov VD, Van Breusegem F. Hydrogen peroxide–a central hub for information flow in plant cells. AoB Plants. 2012;**14**:1-13

[10] Michalak A. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Polish. Journal of Environmental Studies. 2006;**15**:523-530

[11] Tito GA, Chaves LHG, Fernandes JD, Monteiro DR, de Vasconcelos ACF. Effect of copper, zinc, cadmium, and chromium in the growth of crambe. Agricultural Sciences. 2014;**5**:975-983

[12] AghbolaghiM A, Sedghi M, Seyed Sharifi R. Dedicova B: Germination and the biochemical response of pumpkin seeds to different concentrations of humic acid under cadmium stress. Agriculture. 2022;**12**:1-15

[13] Sharma OP. Plant Taxonomy. New York City, United States: Tata McGraw-Hill Education. Pvt. Ltd.; 2010. p 553

[14] Tartoura EAA, El-Gamily EI, El-Waraky YBA, Kamel MKY. Effect of phosphorus fertilization and fruit thinning on seed production of summer squash plants 1-vegetative traits and leaves chlorophyll constituent. Journal of Plant Production. 2014;**5**:1807-1816

[15] Asadi, Aghbolaghi M, Razavi F. Improving germination and biochemical aspects in pumpkin (*Cucurbita pepo*) seeds deterioration under priming by salicylic acid and ascorbic acid. Iranian Journal of Seed Science and Technology. 2021;**10**:75-88

[16] Fruhwirth GO, Hermetter A. Production technology and characteristics of Styrian pumpkin seed oil. European Journal of Lipid Science and Technology. 2008;**110**:637-644

[17] Danilcenko H, Jariene E, Gajewski M, Cerniauskiene J, Kulaitiene J, Sawicka B, et al. Accumulation of elements in some organicallygrownalternativehorticultural crops in Lithuania. Acta Scientiarum Polonorum Hortorum Cultus. 2011;**10**:23-31

[18] Yadav M, Jain S, Tomar R, Prasad GBKS, Yadav H. Medicinal and biological potential of pumpkin: An updated review. Nutrition Research Reviews. 2010;**23**:184-190

[19] Soltani A, Robertson MJ, Torabi B, Yousefi-Daz M, Sarparast R. Modeling seedling emergence in chickpeas as influenced by temperature and sowing depth. Agricultural and Forest Meteorology. 2006;**138**:156-167

[20] De Figueiredo E, Albuquerque MC, De Carvalho NM. Effect of the type of environmental stress on the emergence of sunflower (*Helianthus annum* L.), soybean (*Glycine max* L.) and maize (*Zea mays* L.) seeds with different levels of vigor. Seed Science and Technology. 2003;**31**:465-479

[21] McDonough CM, Floyd CD, Waniska RD, Rooney LW. Effect of accelerated aging on maize, sorghum, and sorghum meal. Journal of Cereal Science. 2004;**39**:351-361

[22] Eisvand HR, Alizadeh MA, Fekri A. How hormonal priming of aged and non-aged seeds of brome grass affects seedling physiological characters. Journal of New Seeds. 2010;**11**:52-64

[23] Khajeh-Hosseini M, Powell AA, Bingham IJ. The interaction between salinity stress and seed vigor during germination of soybean seeds. Seed Science Technology. 2003;**31**:715-725

[24] Kibinza S, Vinel D, Come D, Bailly C, Corbineau F. Sunflower seed deterioration as related to moisture content during aging, energy metabolism, and active oxygen species scavenging. Plant Physiology. 2006;**128**:496-506

[25] Franca-Neto J, Kryzanonwski FC. Tetrazolium: An important test for physiological seed quality evaluation. Journal of Seeds Science. 2019;**41**:359-366

[26] Soltani A, Zeinali E, Galeshi S, Latifi N. Genetic variation for and interrelationships among seed vigor traits in wheat from the Caspian Sea coast of Iran. Seed Science and Technology. 2001;**29**:653-662 (In Persian)

[27] Lester R, Smith AL. The mode of reduction of tetrazolium salt by beef heart mitochondria; the role of coenzyme Q and other lipids. Biochemical Biophysical Act. 1961;**47**:475-496

[28] Fath A, Bethke P, Beligni V, Jones R. Active oxygen and cell death in cereal aleurone cells. Journal of Experimental Botany. 2002;**53**:1273-1282

[29] Sneideris LC, Gavassi MA, Campos ML, D'Amico-Damiao V, Carvalho RF. Effects of hormonal priming on seed germination of pigeon pea under cadmium stress. Anais da Academia Brasileira de Ciências. 2015;**87**:1847-1852

[30] Gholami Tile Benny H, Salehi Balashhry M, Farhady R. Priming effect, and deterioration of seed germination and seedling growth changes of rice (*Oryza sativa* L). Seed Science and Technology. 2013;**1**:1-13 (In Persian)

[31] Basra SMA, Ahmad N, Khan MM, Iqbal N, Cheema MA. Assessment of cotton seeds deterioration during accelerated aging. Seed Science and Technology. 2003;**31**:531-540

[32] Govender V, Aveling TAS, Kritzinger Q. The effect of traditional storage methods on germination and vigor of maize (*Zea mays* L.) from northern Kwa Zulu-Natal and southern Mozambique. South African Journal of Botany. 2007;**74**:190-196

[33] Soltani A, Kamkar B, Galeshi S, Akram Ghaderi F. Effect of seed storage on resource depletion and heterotrophic growth of the wheat seedling. Iranian journal of agriculture. Science. 2008;**15**:229-259

[34] Krishnan P, Nagarajan S, Moharir AV: Thermodynamic characterization of seed deterioration during storage under accelerated aging conditions. Biosystems Engineering. 2004;**89**:425-433

[35] Veselova TV, Veslovsky VA. Investigation of typical germination changes during accelerated aging of pea seeds. Seed Science and Technology. 2003;**31**:517-530

[36] McDonald MB. Seed deterioration. Physiology, repair, and assessment. Seed Science and Technology. 1999;**27**:177-237