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Effect of Priming on Germination Traits and Antioxidant Enzymes of Pumpkin (*Cucurbita pepo L.*) Seeds with Different Vigor under Drought Stress

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ABSTRACT

Drought stress is a critical environmental stress that limits the productivity and sustainability of agriculture in arid and semi-arid regions by reducing the germination rate and delaying the start of germination and seedling establishment. There are various methods to increase the yield of agricultural plants, each of which somehow improves the yield of seeds in terms of germination indicators and seedling establishment. One of the most common methods to increase poor seed germination is priming before planting. To investigate the effects of priming on aspects of germination of aged pumpkin seeds under drought stress, a factorial experiment was conducted based on a completely randomized design with four replications in the laboratory. Treatments that include seed aging (control, 85% and 75% of control germination), drought stress (0, -0.75 and -1.5 MPa), and priming (control, hydro, gibberellin, GR24, benzyl aminopurine and spermidine). The results of a mean comparison between drought stress, seed ageing and priming showed that the lowest germination percentage (GP) of pumpkin (43.6%), protein (0.82 mg.g^{-1}Fw), radical length (RL, 9.24 mm), plumule length (PL, 1.19 mm), relative water content (RWC, 35.5%), carotenoid (1.253 mg.g^{-1}Fw), catalase (CAT, 7.03 µmol.gmin^{-1}.mgprotein^{-1}), peroxidase (POX, 47.72 µmol.gmin^{-1}.mgprotein^{-1}) and superoxide dismutase activity (SOD, 53.48 mmol.gmin^{-1}.mgprotein^{-1}) were associated with drought treatment at -1.5 MPa and with 75% aging in the absence of priming. In addition, the lowest content of malone dialdehyde (MDA, 3.75 mmol.g^{-1}Fw) and proline (1.573 µg.g^{-1}Fw) were obtained from the absence of drought and aging with priming using spermidine. The highest GP (98.03%) and protein (9.54 mg.g^{-1}Fw) were obtained from gibberellin treatment, RL (15.73 mm) and PL (4.26 mm) in priming using GR24 hormone, RWC (83.64%), carotenoid (6.277 mg.g^{-1}Fw), CAT (40.44 µmol.gmin^{-1}.mg protein^{-1}), POX (95.71 µmol.gmin^{-1}.mgprotein^{-1}) and SOD activity (131.68 µmol.gmin^{-1}.mg protein^{-1}) in priming with spermidine with non-drought stress and aging conditions. The highest MDA (32.42 mmol.g^{-1}Fw) and proline (0.55 µg.g^{-1}Fw) were observed in drought conditions with an intensity of -1.5 MPa and 75% aging in the absence of priming.

Keywords: Abiotic stress, Catalase, Deterioration, Seed vigor, Water potential, Strigolactone

1. Introduction

Seed germination as the first stage of plant growth is one of the most sensitive stages of crop life. So that successfully passing this period will play a very important role in the subsequent optimal growth of the plant and the damage at this stage cannot be rectified in any way. In many fields, seeds are exposed to environmental stresses during germination. Drought is one of the most important abiotic stresses, limiting plant growth and crop yields (Khan et al. 2010).

Another important factor influencing the yield of crops is the agronomic quality of seeds or seed bulks. Factors such as temperature, relative humidity, and seed moisture during storage lead to seed deterioration, which ultimately causes oxidative stress (Sharma et al. 2007). Catalase is an antioxidant enzyme that detoxifies free radicals. In fact, catalase uses hydrogen peroxide as a substrate. In undeteriorated seeds, these radicals are at steady state levels due to the action of superoxide dismutase (SOD), catalase and peroxidase, which use these electron donors, and the activity of these enzymes increases during germination. For this reason, seed aging may depend on the effectiveness of the seed to maintain a sufficient level of the enzyme system to protect against oxidative stress (Zhan et al. 2014).

There are a number of methods employed to increase crop yield, each of which in some wayt improves the seed yield in terms of germination characteristics and seedling establishment. One of the most common methods of improving poor germination of seeds pre-sowing treatment (priming) before planting. The purpose of priming is to increase the germination percentage (GP) and reduce the average germination time and improve the growth and germination power of seeds in both favorable and unfavorable environmental conditions (Sedghi et al. 2010). The treatment of seeds with GR24 (a synthetic strigolactone) can strengthen a plant's tolerance to drought and salinity stress (Kapulnik & Koltai 2014). The exogenous use of spermidine reduces the adverse effects of drought stress (Hu et al. 2012). In addition to eliminating free radicals and stabilizing membranes, spermidine can increase the activity of antioxidant enzymes (catalase, peroxidase, superoxide and dismutase) as well as proline (Ndayiragije & Lutts 2006). Hydropriming treatment increases in seedling germination index, can be due to the effects of increasing in seed imbibition time. Gibberellin is a known hormone in the germination process (Graeber et al. 2012; Nee et al. 2017; Shu et al. 2016). Gibberellins can minimize water loss during drought stress by maintaining membrane stability and reducing permeability. Thereby, the activity of lipase and protease enzymes increases, releasing storage substances and converting them into transportable substances of sucrose and glucose, improving vigor and germination index. As the level of drought stress increased, the germination index decreased, but the germination index of the treated seeds decreased only slightly.

Pumpkin (*Cucurbita pepo L.*) is a new variety of that Cucurbitinae first appeared in the mid-nineteenth century in the Austrian state of Ashtria due to a natural mutation. This plant belongs to the family Cucurbitaceae, subfamily Cucurbitinae, genus *Cucurbiteae* and subfamily Cucurbitinae (Jellin et al. 2000). One of the most noticeable characteristics of this plant is its skinless seeds. Mutations in this plant led to thinning of the seed coat, which facilitated the extraction of green oil (Fruhwirth & Hermetter 2008). The oil obtained contains unsaturated fatty acids, vitamin A, vitamin E, minerals, phytosterols, carotenoids and protochlorophyll. Linoleic, oleic and palmitic acids account for 90% of the seed oil content. The content of linoleic acid (50%) is higher than other fatty acids. (Fruhwirth & Hermetter 2008). Geravandi et al. (2010) noted that there is a significant correlation between the GP, germination rate and seed vigor in wheat seeds. Ghiyasi & Tajbakhsh (2013) showed that the application of priming reduced the negative effect of drought stress on the germination and growth of soybean seedlings and improved the yield under stressful conditions in Urmia of Iran. Study of Ansari and Sharifzadeh (2012) has been performed on the effects of seed aging on germination. Basra et al. (2003a) showed that the GP of cotton seeds decreases with the acceleration of aging. Antioxidant enzymes reduce the rate of membrane lipid peroxidation during germination, thereby increasing in the germination rate. For this reason, it is expected that seed priming can be an effective way to improve the germination traits of pumpkin seeds in adverse environmental conditions.

One problem in developing countries is soil heterogeneity and the lack of suitable soil conditions, which causes problems such as reduced germination rate and lack of uniform crop growth, uneven growth of germinated plants and their Unequal competition in the use of sources such as light, food and water leads to differing performance among species of plants. To address these problems, seed priming is a potential solution. We expect that the use of these mechanisms in Iran will have a significant impact on performance and production and will improve the country's share of the global trade of this profitable plant. This study evaluates the seedlings obtained from the deteriorated seeds of pumpkins under drought stress are evaluated and different pretreatment materials are analyzed to reduce their adverse effects or improve their properties under stress conditions.

2. Material and Methods

The effect of priming on the germination of aged pumpkin seeds was studied under low osmotic potential conditions at the University of Mohaghegh Ardabili, Ardabil, Iran, in 2018-2019. A factorial experiment conducted on a completely randomized design with four replications and treatments included: 1- different levels of aging (control, 85% and 75% of control germination), 2- water potential (0, -0.75 and -1.5 MPa) and 3- priming (control, hydro, gibberellin, GR24 (a strigolactone), benzyl aminopurine and spermidine). In order to apply priming, the seeds were soaked for 24 hours at 25 °C and at a concentration of 100 μM for each hormone. Control group was without priming and distilled water was used for hydropriming. The seed bulks reached different germination degrees of 85% and 75% by being placed in an oven at 40 °C and 100% humidity for 5 and 10 days using a rapid aging procedure. For control treatment, seeds were prepared with 95% viability.

In order to apply priming, a hydropriming method with distilled water and hormone priming including gibberellin, GR24, benzyl aminopurine and spermidine were used. The seeds were then soaked in hormones for 16 hours in a germinator at 20 °C and after the priming period, the seeds were washed with distilled water and dried. Polyethylene glycol 6000 was used to prepare the osmotic solutions. The Michel and Kaufmann relationship (Michel & Kaufmann 1973) was used to create the desired osmotic potential for drought stress as below:

$\Psi s= -(1.18 \times 10^{-2})\,C - (1.18 \times 10^{-4})\,C^2 + (2.67 \times 10^{-4})\,CT + (8.39 \times 10^{-7})\,C^{2T}$

Which, Ψs is osmotic pressure (Bar), C is the concentration of polyethylene glycol 6000 (g kg^{-1} H_2O) and also T is the temperature (°C). By substituting the optimum germination temperature (25 °C) and the desired drought concentrations (7.5 and 15 bar), the amount of polyethylene glycol obtained was 127 g and 185 g, respectively (1 MPa= 10 bar).

To perform a standard germination test, 25 seeds from each treatment were planted between paper (BP) in disposable containers. The dishes were then transferred to an IKH.RI model germinator with a temperature of 25 °C and in dark. The germinated seeds were counted daily for 7 days. The criterion for the germination of a seed was radicle (RL) growth and its emergence at a rate of 2 mm from the seed coat.

2.1. Germination percentage

The GP was obtained from the following equation (ISTA 2002):
$GP= 100\times(ni_{ti}/s)$

Which, GP is the GP, ni germinated seeds at considered time ti and S is the total number of seeds.

2.2. Radicle and plumule length

At the end of the seventh day of planting and after the complete opening of the cotyledon leaves, the normal seedlings from each treatment were selected and the radical length (RL) and plumule length (PL) were measured in millimeters.

2.3. Relative water content of leaves

To measure the relative water content (RWC), 0.5 g of isolated leaf samples were immediately weighed to determine the fresh weight using an accurate scale (0.001 g). The leaf samples were then immersed in closed test tubes containing 100 mL of distilled water and kept in a dry and light-free environment for 6 hours. After this period, the leaves were removed from the test tubes and quickly dried with a paper towel and re-weighed. Then, the leaf samples were transferred into an oven for 72 hours at 70 °C, after which the dry weight (DW) of the leaves was determined. The relative moisture content of the leaves was calculated using the formula of Qasim et al. (2003) as below:

$RWC (\%)= (FW-DW/TW-DW) \times 100$

Which, FW is leaf fresh weight, DW is the dry weight and TW is the leaf weight in the full turgor state.

2.4. Extraction and measurement of catalase activity

Catalase activity (CAT) was measured using the Aebi method (Aebi 1984). A 3 mL of the reaction complex consisted of 0.5 mL of 7.5 mM hydrogen peroxide, 1.5 mL of 100 mM potassium phosphate buffer (pH=7) and 50 µl of enzyme extract. The reaction was started by adding hydrogen peroxide. The specific activity of the enzyme was measured at a wavelength of 240 nm by spectrophotometer and expressed in micromoles of hydrogen peroxide decomposed per minute per milligram of protein.

2.5. Peroxidase enzyme assay

Peroxidase enzyme activity (POX) was measured according to the Kato and Shimizu method (Kato & Shimizu 1985). The reaction mixture contained 100 mM sodium phosphate buffer (pH=5.8) and 7.2 mM guaiacol, 11.8 mM H_2O_2 and 0.1 mL of enzyme extract. The reaction was started by adding hydrogen peroxide and changing the optical density. Changes in absorption were recorded using a spectrophotometer at 470 nm. The buffers required to measure the peroxidase were: 45 mM guaiacol, and 225 mM oxygenated water (H_2O_2). Enzymatic activity was calculated using Lambert-Beer's law and the extinction coefficient of the product of guaiacol peroxidase reaction (13.3 mM^{-1} cm^{-1}) as below:

$$(\text{Unit mg}^{-1}) = \frac{\text{POX/min}}{13.3}$$

2.6. Assessment of superoxide dismutase enzyme activity

The activity of SOD was measured using the Giannopolitis and Ries method (Giannopolitis & Ries 1977). The buffers used in the SOD enzyme assay were: (1) 50 mM K-phosphate buffer containing 0.1 mM EDTA, 13 mM methionine and 75 µM nitroblutetrazolium at pH=7 and (2) 0.12 mM Riboflavin solution. The control sample was a combination of 885 µL of buffer 1+15 µL of buffer 2+100 µL of phosphate buffer and the blank sample was a combination of 885 µL of buffer 1+15 µL of buffer 2+100 µ of phosphate buffer. In addition, an enzyme sample was obtained from a combination of 885 µL of first buffer + 15 µL of second buffer and 100 µL of enzyme extract. The absorbance was read at 560 nm with a spectrophotometer and SOD activity was calculated as below:

2.7. Measurement of malondialdehyde content

The amount of malondialdehyde (MDA) was measured according to McCue and Shetty (McCue & Shetty 2002). In the test tubes, 200 mL of homogeneous tissue was mixed with 800 mL of distilled water. 500 mL of 20% trichloroacetic acid was mixed with 1 mL of 10 mM thiobarbituric acid. The test tubes were incubated at 100 °C for 30 min. Then, they were centrifuged at 13000 g for 10 min. The resulting supernatant was used to measure the amount of MDA. The amount of light absorption was read at 532 nm and the concentration of MDA was expressed in terms of mM g^{-1}.

2.8. Measuring the amount of protein

Seed protein was measured using the Kjeldahl method (Kjeldahl 1883). In this method, first the organic nitrogen of the seeds was calculated by adding concentrated sulfuric acid and catalyst to 0.5 g of the sample and then multiplied by 6.25 to obtain the protein content of the sample in mg g^{-1}.

2.9. Measurement of proline content

Proline as an osmotic regulator and stress reagent was measured using the method of Bates et al. (1973). First, 0.02 g of fresh tissue was homogenized in 100 mL of 3% sulfosalicylic acid solution. Then, 2 mL of the filtered solution was combined with 2 mL of ninhydrin reagent and 2 mL of pure acetic acid and then 64 mL of toluene was added. The solution was kept constant until two layers formed. Then, a certain amount of supernatant was obtained and its adsorption was determined at 520 nm in the spectrophotometer and reported as µM per gram of fresh weight.

2.10. Carotenoid measurement

To measure the amount of carotenoid pigments, 1 g of the leaf was separated and homogenized with 15 mL of acetone. The light absorption of carotenoids was read at 480, 645 and 663 nm and was expressed as mg g^{-1} fresh weight according to Bruisma (1963) using the formula below:

$$\text{Carotenoid content} = A_{480} + [(0.114)\ (A_{663}) - (0.638)\ (A_{645})]$$

2.11. Statistical analysis

Statistical calculations of data and their analysis were performed using SAS 9.4 software. Before performing the statistical calculations, the normality of the experimental error variance was evaluated using SAS software for each trait. Analyses of variance performed based on factorial experiment in which three treatments (priming, aging and water potential) and their interactions were included. To compare the means of the treatments, the least significant difference test was used at the probability level of $p=0.05$ and the least squares means and pdiff were used for interactions.

3. Results and Discussion

3.1. Germination percentage

The effect of different levels of drought, aging and priming as well as their interactions on GP was significant) $p \leq 0.05$) (Table 1). The results of the mean comparison table (Table 2) indicated that the lowest GP (43.6%) was related to drought treatment with an intensity of -1.5 MPa and with 75% aging. This was while seed priming significantly compensated for the damage caused by stresses and led to an increase in GP compared to the control, so that the highest GP (98.03%) were obtained from gibberellin treatment with non-drought stress and aging conditions. The results showed that with aging seed in a drought-stress situation, the GP decreased that the effect of drought stress on reducing the GP was greater than the effect of aging. Different priming compensated for this decrease, making the role of gibberellins more affected.

Table 1- Analysis of variance for the effect of water potential and seed deterioration on some physiological and morphological traits of primed pumpkin seeds

SOV	*Df*	*MS*							*F Value*							*Pr > F*
		GP	*RL*	*PL*	*RWC*	*Pro*	*Cart*	*PR*	*GP*	*RL*	*PL*	*RWC*	*Pro*	*Cart*	*PR*	
D	2	8124.8**	133.4**	6.3**	7146.7**	52.67**	190.43**	2.71**	64562.7	54070	11144	21195	3348	29564	2134	<0.0001
P	5	202.06**	0.47**	0. 1**	312.1**	4.92**	0.37**	0.51**	1605.72	192	174	928	313	58	405	<0.0001
A	2	6483.3**	116.5**	111.1**	3132.8**	257.4**	31.43**	1.2**	51519.3	47240	196553	9291	16360	4881	949	<0.0001
D*P	10	6.43**	0.03^{ns}	0.012**	4.43**	0.82**	0.05**	0.008**	51.14	12	21	13	52	8	6	<0.0001
D*A	4	84.9**	6.33**	2.7**	255.1**	13.02**	1.53**	0.0006^{ns}	674.61	2569	4730	756	827	237	0.5	<0.0001
P*A	10	3.31**	0.01**	0.005**	1.28**	0.68**	0.03**	0.0004^{ns}	26.38	4	10	3	43	4	0.3	<0.0001
D*P*A	20	2.24**	0.01**	0.006**	2.11**	0.7**	0.03**	0.002*	17.86	4	12	6	44	5	1.8	<0.0001
Error	108	0.12	0.26	0.0005	0.58	0.01	0.006	0.001								
%CV		0.47	1.3	0.8	0.93	3.8	2.05	3.35								

^{*,**,ns}Statistically significant at $p \leq 0.05$, $p \leq 0.01$, respectively and non-significant.
SOV: Source of variation, Df: Degrees of freedom, MS: Mean square, D: water potential, P: Priming, A: Ageing, GP: Germination Percentage, RL: Radical Length, PL: Plumule Length, RWC: Relative Water Content, Pro: Protein, Cart: Carotenoid, PR: Proline

Low water potential reduces the GP and average germination rate as the reduced water uptake leads to slow metabolism in embryonic tissues resulting in reduced seedling emergence and vigor (Larcher 2001). Moreover, Ghaderi-Far et al. (2020) in their a study of pumpkin seeds found that higher temperatures and humidity reduced the germination persentage.

Numerous biochemical and metabolic reasons have been suggested for the reduced germination capacity of aged seeds, including lipid peroxidation, damage to cell membranes, damage to the RNA synthesis process, DNA degradation, as well as the deposition and

Table 2- Means of physiological traits of primed Pumpkin seeds affected by water potential and aging

Drought	*Priming*	*Ageing*	*GP (%)*	*RL (mm)*	*PL (mm)*	*RWC (%)*	*Pro (mg. g-1Fw)*	*Cart (mg. g-1Fw)*	*PR (µg. g-1Fw)*
Non-D	**Non-P**	0	92.3 f	15.11 f	4.15 c	72.48 ef	5.24 f	6.27 a	0.55 i
		85%	83.4 l	14.03 k	3.76 e	68.2 h	2.66 j	5.87 g	0.72 ef
		75%	71.53 r	13.54 q	1.33 h	65.9 i	1.76 m	4.93 m	0.84 d
	Hydro-P	0	94.46 e	15.35 d	4.21 b	75.5 d	6.21 e	6.27 a	0.59 hi
		85%	84.4 k	14.15 i	3.81 d	71.91 fg	2.76 j	5.98 e	0.753 e
		75%	75.03 q	13.72 o	1.38 fg	66.3 i	1.96 l	5.06 k	0.87 cd
	GA3	0	98.03 a	15.22 e	4.19 bc	77.64 c	9.55 a	6.27 a	0.63 gh
		85%	89.5 g	14.08 j	3.77 e	73.4 e	3.58 g	6.04 d	0.76 e
		75%	78.33 m	13.64 p	1.36 f-h	68.02 h	2.67 j	5.12 j	0.907 c
	GR24	0	95.93 c	15.73 a	4.26 a	79.32 b	8.45 c	6.27 a	0.68 fg
		85%	87.6 h	14.12 ij	3.83 d	75.32 d	2.97 hi	5.92 f	0.83 d
		75%	77.26 n	13.78 n	1.39 f	69.2 h	2.23 k	5.01 l	0.97 b
	BAP	0	95.03 d	15.52 c	4.18 bc	83.01 a	7.26 d	6.27 a	0.75 e
		85%	85.13 j	14.34 g	3.77 e	78.04 c	2.83 ij	6.09 c	0.89 cd
		75%	75.76 p	13.87 l	1.35 gh	70.91 g	1.94 l	5.18 i	1.12 a
	SP	0	97.3 b	15.62 b	4.22 b	83.64 a	9.22 b	6.27 a	0.82 d
		85%	86.5 i	14.27 h	3.84 d	80.47 b	3.08 h	6.15 b	0.97 b
		75%	76.43 o	13.8 m	1.4 f	72.85 ef	2.28 k	5.26 h	1.16 a

Table 2. Continued

Drought	***Priming***	***Ageing***	***GP (%)***	***RL (mm)***	***PL (mm)***	***RWC (%)***	***Pro (mg. g-1Fw)***	***Cart (mg. g-1Fw)***	***PR (µg. g-1Fw)***
-0.75 MP	**Non-P**	0	80.63 e	14.46 c	4.02 de	65.08 e	5.56 e	4.61 b	0.76 k
		85%	74.36 j	12.97 f	3.84 f	57.01 l	2.42 j	3.81 e	0.87 ij
		75%	60.56 p	11.34 h	1.3 i	51.93 p	1.22 n	3.04 f	0.98 g
	Hydro-P	0	82.36 d	14.5 b	4.17 b	65.3 e	5.62 de	4.73 ab	0.83 k
		85%	75.36 i	13.08 ef	3.98 e	58.4 k	2.53 ij	3.9 c-e	0.95 gh
		75%	61.23 o	11.4 h	1.36 gh	53.32 o	1.26 n	2.85 gf	1.09 ef
	GA3	0	86.13 a	14.67 b	4.15 bc	67.47 d	6.23 a	4.81 ab	0.91 hi
		85%	79.9 f	13.17 e	3.97 e	59.46 j	3.17 f	4.01 c-e	1.04 f
		75%	71.3 k	11.47 h	1.34 hi	55.12 n	1.84 k	2.94 gf	1.2 ef
	GR24	0	85.13 b	14.85 a	4.23 a	69.55 c	5.91 bc	4.67 ab	0.98 g
		85%	78.26 g	13.41 d	4.06 d	60.58 h	2.76 h	3.87 de	1.13 e
		75%	64.9 m	11.6 g	1.41 g	56.37 m	1.44 m	2.77 g	1.31 c
	BAP	0	83.53 c	14.8 a	4.12 c	70.48 b	5.77 cd	4.86 a	1.06 f
		85%	76.9 h	13.41 d	3.87 f	62.18 g	2.68 hi	4.06 cd	1.21 d
		75%	63.13 n	11.71 g	1.31 i	58.2 k	1.35 mn	3.01 gf	1.38 b
	SP	0	85.5 ab	14.82 a	4.18 b	71.27 a	6.06 b	4.91 a	1.13 e
		85%	79.56 f	13.3 d	4.04 d	63.14 f	2.96 g	4.15 c	1.29 c
		75%	67.1 l	11.68 g	1.38 gh	60.07 i	1.61 l	3.06 f	1.45 a
-1.5 MP	**Non-P**	0	70.8 e	13.2 d	3.8 d	58.88 f	3.44 d	2.29 e	0.91 i
		85%	58.6 j	11.18 h	2.43 j	44.54 l	1.65 g	1.54 kl	1.05 g
		75%	43.6 n	9.24 k	1.19 m	35.5 p	0.82 j	1.25 n	1.24 e
	Hydro-P	0	71.5 e	13.26 c	3.96 a	60.78 e	3.55 cd	3.07 c	0.98 h
		85%	60 i	11.28 g	2.55 h	45.95 k	1.77 fg	1.67 i	1.15 f
		75%	44.07 n	9.34 j	1.26 l	36.57 o	0.93 ij	1.38 m	1.32 d
	GA3	0	78.1 a	13.2 d	3.89 c	62.81 d	4.16 a	3.14 b	1.06 g
		85%	68.43 f	11.2 h	2.53 h	48.02 j	2.34 e	1.75 h	1.23 e
		75%	53.9 k	9.28 k	1.22 m	38.72 n	1.35 h	1.43 m	1.39 c
	GR24	0	74.4 c	13.33 b	3.92 bc	64.78 c	3.78 bc	2.9 d	1.15 f
		85%	62.8 h	11.31 g	2.61 g	49.89 i	1.96 f	1.61 j	1.31 d
		75%	47.13 m	9.34 j	1.31 k	40.44 m	1.14 hi	1.3 n	1.46 b
	BAP	0	72.5 d	13.4 a	3.54 e	66.53 b	3.64 b-d	3.23 a	1.25 e
		85%	60.4 i	11.48 e	2.46 i	53.51 h	1.83 fg	1.82 g	1.4 c
		75%	46.7 m	9.44 i	1.21 m	43.67 l	1.07 h-j	1.5 l	1.5 b
	SP	0	76.6 b	13.3 ab	3.95 ab	67.53 a	3.87 b	3.2 a	1.3 cd
		85%	64.7 g	11.4 f	2.65 f	54.9 g	2.57 e	1.9 f	1.5 c
		75%	49.5 l	9.4 i	1.34 k	45.8 k	1.27 h	1.5 jk	1.57 a
LSD			0.5	0.07	0.2	0.78	0.13	0.13	0.3

Non-P: non-Priming, Hydro-P: Hydro Priming, GA3: Gibberellin, BAP: Benzyl Amino Purine, SP: Spermidine, GP: Germination Percentage, RL: Radical Length, PL: Plumule Length, RWC: Relative Water Content, Pro: Protein, Cart: Carotenoid, PR: Proline, LSD: Least Significant Difference. In each column the comparison of means have been performed with SAS software using SLICE procedure so, letters in every level of water potential have begun from a

inactivation of enzymes (Basra et al. 2003b). Gibberellin is a known hormone in the germination process (Nee et al. 2017; Shu et al. 2016). Gibberellin may minimize water loss during drought stress by maintaining membrane integrity and reducing its permeability, thereby increasing in the activity of lipase and protease enzymes, leading to the release of stored materials and their conversion into transferable materials such as sucrose and glucose and improvement of vigor and germination index (Mohamed et al. 2010). TavakolAfshari et al. (2009) reported that the decrease in seed vigor is due to decreased synthesis and degradation of cellular proteins and increased the electrical conductivity and cell permeability during aging, which priming repairs seeds and increases its vigor. The effect of different seed pretreatments on increasing enzyme activiy has been reported by other researchers studying other plants and it has been shown that increasing antioxidant enzymes is associated with increasing in germination indices under stress and the reason for improvement in the germination index in treated seeds has been attributed to the increase in consumption of seed storage materials and the activity of antioxidant enzymes in treated seeds (Ansari et al. 2013).

3.2. Radical and plumule length

The effect of different levels of drought, aging and priming as well as their interactions was significant on RL, but the results obtained from the combined effect of drought and priming on RL were not significantly different (Table 1). The lowest RL (9.24 mm) was observed in drought with an intensity of -1.5 MPa, 75% aging and control priming (Table 2). The highest RL (15.73 mm) was observed in the absence of drought and aging as well as priming with the GR24 hormone (Table 2). The results of this experiment showed that aging and drought stress had a negative effect on RL. The reduction in RL due to aging was more significtant than drought stress. In addition, each priming increased in the RL compared to the control and stress conditions, which had the least effect on hydropriming and the greatest effect on GR24 hormone. The effect of different levels of drought, aging and priming as well as their interactions was significant on PL (Table 1). The results of the mean comparison table (Table 2) showed that the lowest PL (1.19 mm) was observed in drought with an intensity of -1.5 MPa and 75% aging in the absence of priming. The results of the data showed that the effect of drought stress on PL was more than the effect of aging. The highest PL (4.26 mm) was obtained from the absence of drought and aging as well as priming with the GR24 hormone. It seems that the application of water potential and consequent drought stress (-1.5 MPa) reduces water uptake by seeds, which disrupts the process of hormone secretion and the activity of enzymes that affect seed germination and growth and RL eventually decreases. Usually, RL decreases significantly with in the increase in PEG concentration (Almaghrabi 2012). Mortazavi et al. (2008) showed that RL and PL decreased in aging seeds. One way to reduce the adverse effects of oxidative stress is through seed priming (Krishna et al. 2021). The use of certain polyamines and hormonal priming may be associated with increased ability to reduce oxidation. GR24 as a type of strigolactones plays an important role in plant development through various processes (Dun et al. 2009). Strigolactones act as positive regulators in plant response to abiotic stresses such as drought (Ha et al. 2014). One of the effects of Gr24 is to increase soluble sugars, including glucose, as well as to increase the expression of protein kinase regulation (Modi et al. 2017). Protein kinase (Price et al. 2012) and soluble sugars (Hao et al. 2021) have a major effect on the tolerance of different stresses. Therefore, it is possible that Gr24 improved drought stress tolerance in pumpkin seeds by increasing soluble sugars and protein kinase expression.The results of the present study were in line with the findings of Ahmadi et al. (2016) in investigating the effect of seed priming on salinity and drought stress on the growth characteristics of *Dracocephalum* Spp. seedlings. Their results showed that seed ageing and drought stress had a detrimental effect on RL and PL. The reduction in seedling length due to ageing was greater than that of drought stress. The reason for the reduced RL and PL of aged seeds is the decreased quality of storage materials during the period of aging (Mortazavi et al. 2008).

PL is one of the traits that indicates seed vigor. Seeds with low vigor may germinate, but due to reduced PL they cannot emerge, thus reducing the percentage of green establishment in the field. However, short stems have less emergence power due to lower DW compared to long stems (Matthews & KhajeHosseini 2006). The reduction of water uptake by seeds under stress conditions reduces the secretion of hormones and the activity of enzymes and thus impairs seedling growth, including RL and plumule (Masoumi et al. 2010). Zamani et al. (2010) in a study of lipid peroxidation and enzyme activity in aged safflower seeds noted that the relationship between lipid peroxidation and an increase in the leakage of electrolyte solutions due to degradation of cytoplasmic membrane structure reduces RL and PL. Masoumi et al. (2010) found that priming increases RL and PL by increasing the rate of use of seed storage materials. An increase in the PL of aged canola seeds has been reported with priming (Najafi et al. 2016). Therefore, it seems that the increase of such enzymes may be due to the increase in RL and PL of primed seeds.

3.3. Relative water content

The effect of different levels of drought, aging and priming as well as their interactions was significant on RWC (Table 1). The mean values showed that the lowest RWC (35.5%) was observed in drought with an intensity of -1.5 MPa and 75% aging in the absence of priming. The highest RWC (83.64%) was in the absence of drought and aging and in priming with spermidine (Table 2). The reduction of RWC under drought stress has also been reported in wheat (Liheng et al. 2011) and barley (Bandurska et al. 2012). Plants under drought stress minimize the intercellular space and the amount of water in their organs by increasing the osmotic content within the tissues so that ambient water enters them with greater force. This reduces the RWC under drought stress (Bayoumi et al. 2008). Chadordooz et al. (2015) stated that the reason for the decrease in RWC was the delay in seedling emergence and the reduction of rooting in aged lentil seeds that had been subjected to drought stress. It appears that due to the delay in seedling emergence and reduced root growth in aged seeds, the amount of RWC decreases. Considering that drought indirectly leads to an increase in plant water transpiration therefore, the intensity of its effect on RWC was greater than aging. The RWC in Seedlings of barley (*Hurdeum vulgare*) that were exposed to drought stress, decreased compared to control plants, but exogenously applied spermidine increased in RWC (Kubis, 2003). In rice, the use of spermidine also compensated for the decrease in RWC due to drought stress (Farooq et al. 2009). The results show that priming can help reduce water loss in dry conditions, which may be largely due to the reduction of transpiration and help the root grow more effectively to absorb sufficient water. Since polyamines (spermidine) are involved in the protection of plants against environmental stresses (Bouchereau et al. 1999), they can maintain the required moisture in the plant in stressful environmental conditions.

3.4. Protein content

The effect of different levels of drought, aging and priming as well as their interactions was significant on the rate of protein changes (Table 1). A comparison of the means shows that the lowest protein content (0.82 mg.g^{-1}Fw) was measured in drought with an intensity of -1.5 MPa and 75% aging in the absence of priming and the highest content (9.54 mg.g^{-1}Fw) was observed in the absence of drought and aging and in priming with gibberellin (Table 2). One of the important biochemical changes that occur as a result of the reduction of water in plants is the change in plant protein production. To break down after stress or interfere with the synthesis (Sharma et al. 2019). A reduction in protein content during aging was also reported by Murthy et al. (2002). In addition, the decrease in primary amino acids due to ROS attack is another reason for the decrease in protein levels during the ageing process (Jacoby et al. 2012). The effect of different seed pretreatments on increasing the activity of enzymes has been reported by other researchers in different seeds (Ansari et al. 2012; Bailly 2004). In other words, seed pretreatment by increasing in the activity of antioxidant enzymes in the seed, causes resistance under stress conditions and leads to an increase in germination index (Ansari et al. 2013). According to the data obtained, it was found that in the first level of drought stress, the content of protein increased and decreased with the increasing severity of drought stress. It seems that seedlings in the first stage to combat stress reduce metabolites such as proteins to reduce osmotic pressure, but as the severity of stress increases, this ability is lost. During seed ageing, due to the high affinity of reactive oxygen species and other aldehydes produced by vital biomolecules such as proteins, they are denatured (Kapoor et al. 2010). For this reason, it seems that with the increase of ageing, the proteolysis by protease enzymes increases and leads to a decrease in its quantity. In addition, an increase in the germination index by gibberellin can be associated with an increase in the activity of enzymes and proteins.

3.5. Carotenoid content

The effect of different levels of drought, aging and priming as well as their interactions was significant on the rate of carotenoid changes (Table 1). A comparison of means shows that the lowest carotenoid content (1.253 mg.g^{-1}Fw) was measured in drought with an intensity of -1.5 MPa and 75% aging in the absence of priming and the highest content (6.277 mg.g^{-1}Fw) was observed in the absence of drought and aging and in priming with spermidine (Table 2). Drought stress led to a decrease in carotenoids. In addition, placing the seeds in an environment with both high temperature and high humidity reduced the amount of pigments. Moreover, the effect of drought stress in reducing the carotenoid content was greater than that of the aging. Chlorophylls are sensitive to oxidation and light inhibition, while carotenoids play an antioxidant and protective role for chlorophylls (Ramadan & Omran 2005). Similar results were obtained on olives (Ben Ahamed et al. 2007), sugarcane (Suriyan & Chalermpol 2009) and melons (Korkmaz et al. 2007), indicating a decrease in carotenoid content due to drought stress. Seed aging during improper storage disrupts the enzyme system that suppresses reactive oxygen species and leads to damage to the pigments (Masoumi et al. 2010). A reduction in carotenoids during aging have been reported in tomatoes (Najafi et al. 2009) and maize. Compensation for decreases and increases in chlorophyll a, b and carotenoids in aged seeds has been reported by priming in eggplants (Sardoei et al. 2014) and wheat (Shaddad et al. 2013). It has been reported that priming by plant hormones can affect carotenoid synthesis by affecting the genes encoding the biosynthesis pathway of geranyl pyrophosphate (Shaddad et al. 2013). Increasing in carotenoid content during priming can increase resistance to drought stress by maintaining essential photosynthetic activities (Abid et al. 2017). It seems that the activity of ROS following stress causes discoloration or loss of pigments such as chlorophylls and other pigment compounds. Application of polyamine pretreatment prevents damage to the dye by inhibiting lipid oxidation.

3.6. Proline content

The effect of different levels of drought, aging and priming as well as the interaction effects of priming and drought, as well as the interaction effects of drought, aging and priming were significant on proline content (Table 1). The lowest amount of proline (0.55 µmol.g^{-1} Fw) was observed in the control seeds and the highest amount (1.573 µmol.g^{-1} Fw) was observed in drought with an intensity of -1.5 MPa and 75% aging in priming with spermidine (Table 2). Proline is known as a key amino acid in osmotic regulation to increase salinity and drought resistance, the rate of increase of which varies between different varieties (Woodward & Bennet 2005). An increase and accumulation of proline under stress conditions in many plant species is associated with stress resistance and its concentration in stress-resistant plants is greater than that of sensitive plants (Zebarjadi et al. 2010). Increased proline levels under drought stress in primed safflower seeds have also been reported (Ashrafi & Razmjoo 2010). The results of our study are consistent with studies on chickpea cultivars (Alexieva et al. 2001), European borage (Zahed-Chekovary & Gasemov 2015) and wheat (Pireivatlou et al. 2010). In general, proline accumulation is believed to play an adaptive role in plant stress tolerance (Verbruggen & Hermans 2008). It was found that with increasing the rate of seed aging in wheat, proline content increases (Shaaban 2016). The intensity of proline increase resulting from drought stress was greater than seed aging stress. The findings of Ashrafi and Razmjou (2010) show that the proline content increased in safflower vegetative parts in hydro primed seeds under both stress and non-stress conditions. Polyamines can protect membranes and large molecules from oxidative stress and provide biological membrane stability under stress conditions (Farooq et al. 2009). For this reason, it can be concluded that spermidine, as a type of polyamine, facilitates the accumulation of phenolic compounds and free proline to protect against oxidative damage.

3.7. Catalase activity

The effect of different levels of drought, aging and priming as well as their interactions was significant on CAT activity (Table 3). The lowest CAT activity (7.03 µmol.g min^{-1}.mg protein^{-1}) observed in drought with an intensity of -1.5 MPa and 75% aging in the absence of priming. The highest rate (40.44 µmol.g min^{-1}.mg protein^{-1}) was achieved in drought with an intensity of -7.5 MPa in the conditions of non-aged and in priming with spermidine (Table 4). The mean comparison Table 4 indicates that the activity of CAT decreased in aging seed. However, with the application of drought, an increase in the activity of this antioxidant was observed and its activity decreased with the intensification of drought stress. An increase in CAT has been observed in olive leaves under water stress (Sofo et al. 2008). Plants increase in the amount of antioxidant enzymes during drought stress to reduce damages from reactive oxygen species (Farooq et al. 2009). Jyoti and Malik (2013) reported a decrease in CAT during the seed aging process. Researchers have also shown that priming can compensate damages through the formation of catalase subunits in the cytoplasm and complete its synthesis and reduce the severity of oxidative stress, in part by improving the activity of antioxidant enzymes (Xia et al. 2015). Catalase enzyme is one of the most important components of the antioxidant system, which increases in drought stress conditions. However, by using the seed priming technique, the quantity of this enzyme can be increased in plants under stress (Khan et al. 2020). Increased activity of the catalase enzyme with the help of polyamines under water stress has been reported by Amraiyatbar et al. (2016). Polyamines, as signaling molecules, are likely to trigger a chain of defense reactions that result in the increased activity of antioxidant enzymes (Toumi

Table 3- Analysis of variance for the effect of water potential and seed deterioration on some physiological and biochemical traits of primed pumpkin seeds

SOV	*Df*	*MS*				*F Value*				*Pr > F*
		CAT	*POX*	*SOD*	*MDA*	*CAT*	*POX*	*SOD*	*MDA*	
D	2	4883.1^{**}	3562.9^{**}	9719.8^{**}	2119.9^{**}	65906	72574	70456	47204	<.0001
P	5	123.33^{**}	658.8^{**}	734.8^{**}	61.62^{**}	1664	13419	5326	1372	<.0001
A	2	550.45^{**}	5329.3^{**}	21580.4^{**}	2092.8^{**}	7429	108555	156430	46601	<.0001
D*P	10	23^{**}	13.48^{**}	1.72^{**}	1.16^{**}	310	274	12	25	<.0001
D*A	4	9.95^{**}	22.52^{**}	45.22^{**}	62.9^{**}	134	458	327	1401	<.0001
P*A	10	0.49^{**}	3.4^{**}	2.15^{**}	13.84^{**}	6	70	15	308	<.0001
D*P*A	20	1.48^{**}	2.7^{**}	2.9^{**}	1.05^{**}	20	55	20	23	<.0001
Error	108	0.07	0.04	0.13	0.04					
%CV		1.13	0.29	0.39	1.5					

$^{*, **, ns}$Statistically significant at p≤0.05, p≤0.01, respectively and non-significant.
SOV: Source of variation, Df: Degrees of freedom, MS: Mean square, D: water potential; P: Priming, A: Ageing, CAT: Catalase, POX: Peroxidase, SOD: Superoxide dismutase, MDA: Malone dialdehyde

et al. 2010). Seed antioxidant enzymes may increase in the first days of aging, but as the aging process increases, they lose their ability to defend and their quantity decreases (Amirjani 2010). Therefore, its presence as a pre-treatment agent can be a reason to increase the amount of catalase in these conditions.

3.8. Peroxidase activity

The effect of different levels of drought, aging and priming as well as their interactions were significant in POX activity (Table 3). According to the mean comparison table, the lowest POX activity (47.72 µmol.g min^{-1}.mg protein^{-1}) observed in drought with an intensity of -1.5 MPa and 75% aging in the absence of priming and the highest rate (95.71 µmol.g min^{-1}.mg protein^{-1}) was obtained from the absence of drought and aging in seed priming by spermidine (Table 4). Drought stress and aging led to the reduction of POX and the intensity of aging was greater than that of drought. Decreased peroxidase activity under drought stress has been shown in other studies (Csiszár et al. 2012). Murthy et al. (2002) showed that by increasing storage time and seed aging, the amount of MDA in the seed increases and in contrast, a decrease in the activity of catalase, glutathione reductase and ascorbate peroxidase is also observed, which has a positive correlation with seed vigor. Decreased activity of antioxidant enzymes in aged seeds has been reported due to an increase in free radicals in corn (Siadat et al. 2012) and rye (Ansari & Sharifzadeh 2012). TavakolAfshari et al. (2009) also reported in their study that the activity of peroxidase enzyme in canola seedlings is reduced due to seed aging. Decreased activity of antioxidant enzymes due to seed aging will ultimately reduce protein production for various reasons such as damage to RNA synthesis. The addition of reduced sugars to proteins, which is non-enzymatic and is known as the Maillard reaction, also inactivates antioxidant enzymes under seed aging conditions (Murthy et al. 2003). The application of polyamine in rice under drought conditions activated hydrogen peroxide purification enzymes, thereby increasing water tolerance while preventing protein degradation and membrane peroxidation (Farooq et al. 2009). In spermidine-treated barley grains, the activity of catalase and guaiacol peroxidase enzymes increased in under dehydration, and thus polyamines were able to activate H_2O_2-purifying enzymes and tolerance to water depletion (Kubis 2003). Increased in peroxidase activity during drought stress is associated with plant tolerance to dehydration (Xiong et al. 2002) and due to the sensitivity of pumpkin to drought stress, the increase of peroxidase enzyme can be justified.

Table 4- Means of antioxidant enzyme and malondialdehyde content of primed Pumpkin seeds affected by water potential and aging

Drought	***Priming***	***Ageing***	***CAT (µmol.min^{-1}.mg^{-1}protein)***	***POX (µmol.min^{-1}.mg^{-1}protein)***	***SOD (µmol.min^{-1}.mg^{-1}protein)***	***MDA (µmol.min^{-1}.mg^{-1}protein)***
Non-D	**Non-P**	0	26.6 g	85.17 h	118.67 f	4.4 m
		85%	24 k	78.9 k	103.12 j	9.31 g
		75%	21.51 m	66.13 p	76.37 p	15.71 a
	Hydro-P	0	28.46 d	89.35 d	122.4 d	4.31 m
		85%	26.64 g	83.19 i	105.2 i	8.44 i
		75%	24.7 j	71.3 n	81.8 n	13.9 c
	GA3	0	30.7 b	92.02 c	125.6 c	4.24 m
		85%	27.3 f	85.7 g	108.8 h	7.85 j
		75%	25.19 ij	73.93 m	85.62 m	12.56 d
	GR24	0	27.7 e	88.06 e	121.4 e	4.41 m
		85%	25.67 h	81.8 j	103.53 j	8.75 h
		75%	23.1 l	69.36 o	79.63 o	14.3 b
	BAP	0	29.02 c	93.2 b	128.1 b	3.94 n
		85%	25.34 hi	87.19 f	113.21 g	5.53 k
		75%	22.95 l	76.2 l	89.5 l	10.78 e
	SP	0	32.26 a	95.71 a	131.6 a	3.75 n
		85%	29.37 c	88.01 e	119.14 f	4.93 l
		75%	27.09 fg	76.34 l	92.18 k	9.95 f

Table 4. Continued

Drought	***Priming***	***Ageing***	***CAT (μmol.min^{-1}.mg^{-1}protein)***	***POX (μmol.min^{-1}.mg^{-1}protein)***	***SOD (μmol.min^{-1}.mg^{-1}protein)***	***MDA (μmol.min^{-1}.mg^{-1}protein)***
-0.75 MP	**Non-P**	0	29.3 hi	81.18 h	110.8 f	7.73 m
		85%	25.48 l	73.06 m	93.2 l	16.57 g
		75%	23.68 m	61.38 q	72.2 r	23.13 a
	Hydro-P	0	34.5 d	85.05 f	113.67 d	7.72 m
		85%	31.23 g	79.28 i	97.67 j	14.42 i
		75%	28.27 j	67.48 q	76.28 p	20.53 c
	GA3	0	35.07 d	88.95 d	115.85 c	7.59 m
		85%	33.18 f	81.26 h	100.7 i	12.03 j
		75%	31.46 g	70.23 o	79.83 o	19.25 d
	GR24	0	32.9 f	83.36 g	112.7 e	7.64 m
		85%	29.8 h	76.47 k	95.84 k	15.58 h
		75%	26.06 k	64.6 p	74.35 q	21.1 b
	BAP	0	36.73 c	92.46 b	119.03 b	7.54 m
		85%	32.65 f	86.4 e	104.24 h	10.22 k
		75%	28.93 i	74.05 k	83.74 n	18.46 e
	SP	0	40.44 a	93.95 a	121.89 a	7.55 m
		85%	37.99 b	89.96 c	107.08 g	8.64 l
		75%	33.8 e	77.57 j	87.21 m	17.44 f
-1.5 MP	**Non-P**	0	16.17 d	72.4 h	94.31 f	13.23 m
		85%	11.95 h	61.18 m	75.5 l	21.71 g
		75%	7.03 m	47.72 r	53.4 q	32.42 a
	Hydro-P	0	17.42 c	75.5 e	97.59 d	13.33 m
		85%	12.74 g	67.37 j	78.9 j	21.09 h
		75%	8.75 l	53.84 p	55.2 p	31.26 b
	GA3	0	17.56 c	76.06 d	101.06 c	13.33 m
		85%	12.83 g	71.5 i	82.5 i	18.63 j
		75%	9.24 k	55.3 o	57.7 o	28.22 d
	GR24	0	17.42 c	73.1 g	95.7 e	13.34 m
		85%	12.88 g	63.9 k	76.3 k	20.32 i
		75%	10.33 j	50.17 q	54.6 p	30.37 c
	BAP	0	17.98 b	80.01 b	104.8 b	13.32 m
		85%	13.34 f	74.66 f	85.27 h	16.32 l
		75%	10.87 i	58.02 n	61.8 n	27.22 e
	SP	0	18.36 a	83.04 a	107.7 a	13.37 m
		85%	13.7 e	78.4 c	88.62 g	17.76 k
		75%	11.12 i	63.15 l	64.6 m	26.6 f
LSD			0.34	0.25	0.49	0.23

Non-P: non-Priming, Hydro-P: Hydro priming, GA3: Gibberellin, BAP: Benzyl amino purine, SP: Spermidine, CAT: Catalase, POX: Peroxidase, SOD: Superoxide dismutase, MDA: Malone dialdehyde, LSD: Least significant difference

3.9. Superoxide dismutase activity

The effect of different levels of drought, aging and priming as well as their interactions were significant on SOD activity (Table 3). The lowest activity of SOD (53.48 μmol.g min^{-1}.mg protein^{-1}) was measured in drought with an intensity of -1.5 MPa and 75% aging in the absence of priming and the highest amount (131.68 μmol.g min^{-1}.mg protein^{-1}) was obtained from the absence of drought and aging with seed priming by spermidine (Table 4). Drought stress and seed deterioration decreased the activity of SOD enzyme. Decreased

SOD activity under drought stress has also been reported in pumpkins (Yasar et al. 2014). Cakmak et al. (2010) observed that in the long-term aging of alfalfa seeds, the amount of hydrogen peroxide and the activity of CAT and SOD enzymes decreased, citing a decrease in the germination capacity of aged alfalfa seeds. Mehravar et al. (2014) also noted the decrease in SOD activity due to seed aging. Polyamines can increase in stress resistance by stimulating the activity of various antioxidant systems (Yiu et al. 2009). The maximum activity of SOD enzyme in rice was obtained through the use of priming under drought stress (Farooq et al. 2010). According to Kubi (2005), the antioxidant system, can be changed with the help of polyamines, this system is able to modify the system of radical correction under oxidative stress. In addition, an increase in the amount of superoxidase dismutase and peroxidase due to priming in aged onion seeds has been proven (Yalamalle et al. 2019). Priming enhances antioxidant enzymes, a process that appears to occur through the optimization of defense mechanisms during germination. Seed priming reduces the activity of antioxidant enzymes. This has been proven in experiments on sesame seeds (Somasundaram et al. 2009).

3.10. Malone dialdehyde content

The effects of different levels of drought, aging and priming as well as their interactions were significant on the amount of MDA (Table 3). The highest amount of MDA (32.42 mmol. g^{-1}Fw) was observed in drought with an intensity of -1.5 MPa and 75% aging in the absence of priming and the lowest amount of MDA (3.75 mmol. g^{-1}Fw) was obtained from the absence of drought and aging in priming with spermidine (Table 4). The effect of drought on lipid peroxidation and increase in MDA, which is one of the products of lipid peroxidation in *Populus euphratica* has been reported (Bogeat-Triboulot et al. 2007). In drought-sensitive species, the MDA content is much higher than in resistant species (Bogeat-Triboulot et al. 2007), which is an internal physiological regulation in response to environmental stimuli (Salazar-Parra et al. 2012). MDA is an indicator of cell membrane peroxidation and is positively correlated with the leakage of electrolytes from the membrane. The higher the value of this index, the greater the peroxidation of the membrane and the leakage of electrolytes from the membrane, resulting in more potential damage (Jyoti & Malik 2013). MDA is the product of linoleic acid peroxidation and has the ability to damage membrane proteins through cross-linking (Varier et al. 2010). Ghahremani et al. (2017) in their study of pumpkin seeds found that with increasing temperature and aging, the number of free radicals increases and the accumulation of these harmful compounds leads to the lipid peroxidation of cell membranes and organelles, which increased the amount of MDA. Lipid peroxidation and cell membrane damage are reduced during stress by seed priming (Meng et al. 2014). The reduction of lipid peroxidation through a reduction of MDA by GR24 has also been reported in stress conditions (Ma et al. 2017). Li et al. (2015) reported that resistance to oxidants in spermidine-treated plants resulted in a lower production of O^-_2, H_2O_2, and MDA content to improve cell membrane stability. Bakheet et al. (2017) in a study on flax seeds aging concluded that priming can reduce the production of MDA by reducing the rate of peroxidation through increasing the activity of antioxidant enzymes. In general, it can be said that MDA is an indicator of the degree of damage caused by various oxidants during stress. In addition, it may be that spermidine can impact upon the ability of the activities of inhibitory systems to affect the severity of oxidative stress.

4. Conclusions

Pumpkin is classified as a plant sensitive to drought stress, however, its oily seeds are more sensitive to seed ageing conditions. In this study, germination characteristics were affected by drought stress more than seed deterioration. In the case of morphological characteristics, the decrease in burnout stress exceeded that of drought stress. While the reduction of morphological characteristics, due to seed deterioration was greater than that of drought stress. The activity of antioxidant enzymes under drought stress increased and decreased in aging seed. The carotenoid content decreased during stress and the decrease due to drought stress was more noticeable than seed ageing. Accumulated MDA and proline content make seedlings more resistant to stress, the effect of seed deterioration on these compounds was greater than that of drought stress. In general, in the case of seed pretreatment, gibberellin had the greatest effect on germination traits. Seedlings obtained from the Gr24 hormone priming had significant differences in weight and size with other treatments. In addition, seed pretreatment with spermidine reduced all the negative effects of stress, particularly that of drought stress.

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