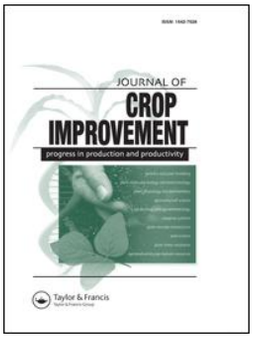

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Salicylic acid priming before and after accelerated aging process increases seedling vigor in aged soybean seed

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ABSTRACT

Seed vigor in soybean (*Glycine max* (L.) Merr.) declines under environmental stresses, and seed shows signs of aging. The aging effects may be alleviated, however, by seed priming with certain chemicals, such as salicylic acid (SA). The objective of this study was to investigate the effects of SA priming on activities of certain enzymes and lipid peroxidation in soybean seedlings under accelerated aging (AA) process. A factorial, completely randomized design with three replicates was used. Different aging durations (48 h and 72 h), SA priming solutions (0, 300 and 600 μM) and priming times (before aging, after aging and both before and after aging, i.e., double priming) were the experimental factors. Increasing aging duration significantly increased mean emergence time (MET) but decreased carotenoid content. Nonetheless, SA solution mitigated the adverse effect of seed deterioration on germination-related traits by increasing enzyme activity and reducing malondialdehyde (MDA) content, especially under double priming. A strong negative correlation ($r = -0.99^{**}$) was found between MDA content and chlorophyll *a* and chlorophyll *b* content. By contrast, the correlation between MDA content and MET ($r = 0.95^{**}$) was found to be positive. The results suggested that double SA priming was an appropriate practical technique to improve seedling establishment from aged soybean seed.

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KEYWORDS
Accelerated aging; enzyme activity; lipid peroxidation; mean emergence time; seed deterioration

Introduction

Soybean (*Glycine max* (L.) Merr.), belonging to Family Fabaceae, is mainly cultivated in semi-arid regions (Gerçek et al. 2009), especially in Iran (Asadi Rahmani et al. 2009). In 2018, Iran's area under soybean cultivation was 53,000 ha, with a production of 139,000 tons. Iran's soybean yield (2.3–2.7 kg ha^{-1}) has been reported to be relatively low, however (Agricultural statistics 2017).

The first step for successful soybean production is to optimize plant density through the establishment of normal seedlings (Muthiah, Longer, and Harris 1994; Cassán et al. 2009). Based on environmental conditions, this

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is essentially determined by selecting the appropriate cultivars (Khajeh-Hosseini, Powell, and Bingham 2002). Nonetheless, even when certified seed is used, unfavorable environmental factors, such as drought, and soil texture can affect soybean seedling vigor and growth (Samarah, Mullen, and Anderson 2009; Arora et al. 2011).

Generally, seed deterioration has been defined as the loss of quality, viability, and vigor during aging (Zheng and Ma 2014). The seed-aging process, as a series of biochemical reactions, is relatively progressive and irreversible and can significantly reduce seedling establishment (Eisvand et al. 2010).

The production of reactive oxygen species, such as superoxide radical, hydrogen peroxide and hydroxyl radical, is one of the known biological consequences of seed deterioration (Parkhey, Naithani, and Keshavkant 2012; Sahu et al. 2017). These metabolites may adversely affect cellular membrane integrity, macromolecule function, and enzymatic activity by inducing oxidative stress (Bailly 2004; de Oliveira et al. 2012). Hence, changes in the MDA content can signal the severity of seed deterioration. In this regard, a decrease in seed viability attributable to hydrogen peroxide and MDA accumulation has been reported in sunflower (Kibinza et al. 2006). Similarly, MDA has been considered an indicator of lipid peroxidation (Parkhey, Naithani, and Keshavkant 2012).

The determination of seed vigor and seedling emergence under stressful conditions is essential for sustainable soybean production (Cho and Scott 2000; Rastegar, Sedghi, and Khomari 2011). To achieve this, accelerated aging (AA) is one of the most important tests to evaluate seed-quality characteristics (Wang et al. 2012). Since the results of the standard germination test cannot usually assess the biological function associated with seedling vigor, especially under stressful conditions (Noli et al. 2008), AA test can serve as an important indicator for predicting seedling growth and establishment (Mavi and Demir 2007; Seyyedi, Tavakkol Afshari, and Daneshmandi 2018).

Basically, the AA test, conducted under a range of environmental conditions, can help determine the seedling-establishment ability (Eisvand et al. 2010). For instance, a significant reduction was in mobilized seed reserve in winter canola after performing the AA test (Seyyedi, Tavakkol Afshari, and Daneshmandi 2018). Other known consequences associated with the seed-aging process are reduced phospholipid concentration (Stewart and Bewley 1980; Ravikumar et al. 2002), impaired cellular respiration (Ferguson, TeKrony, and Egli 1990), increased electrolyte leakage and cellular secretion (Hsu et al. 2003), inhibited enzyme activity (Sung and Jeng 1994), damaged fatty acid and protein structure (Seyyedi, Tavakkol Afshari, and Daneshmandi 2018), and degraded cell membrane (Gidrol et al. 1989).

Previous studies have shown that measuring emergence-related traits, especially MET, on the artificially aged seeds, can simulate seedling growth more precisely than germination test (Chiu, Chuang, and Sung 2006; Mavi

and Demir 2007), because germination and emergence processes are directly influenced by environmental conditions (Hsu et al. 2003; Mavi, Demir, and Matthews 2010). In this regard, it has been reported that seedling establishment ability in cotton (Basra et al. 2003) and canola (Seyyedi, Tavakkol Afshari, and Daneshmandi 2018) can be predicted accurately based on AA.

Based on seedling-related traits, the specific priming approaches may alleviate the adverse effects of stressful conditions (Moosavi et al. 2009; Yadav, Kumari, and Ahmed 2011). In this regard, salicylic acid (SA) priming is recognized as one of the known techniques for improving germination (Farooq et al. 2008), especially under unfavorable environments (Dražić and Mihailović 2009; Pouramir-Dashtmian, Khajeh-Hosseini, and Esfahani 2014). SA is a well-known biologically active compound (Szalai et al. 2016), which plays a role in improving seedling tolerance to environmental stresses by inducing adaptation mechanisms (Janda et al. 2014; Khan et al. 2019). SA has been shown to help adjust osmotic potential (Anaya et al. 2018; Sharma et al. 2018), activate antioxidant defenses (Chavoushi et al. 2019; Zaid et al. 2019), regulate the metabolism of reactive oxygen species (Lee and Park 2010; Yan et al. 2018), and enhance photosynthetic efficiency (Jahan et al. 2019).

The SA-primed seeds have been shown to increase germination rate in common bean (Semida and Rady 2014), maize (Szalai et al. 2016), and rice (Sheteiwy et al. 2019). However, there is still limited information about the time and duration of the SA priming relative to soybean seed germination, especially after the AA process. Hence, the objectives of the present study were to: 1) determine SA priming effects on revitalization of the biological metabolism of aged soybean seed and 2) determine the relationship between time of application and concentration of SA applied as a seed primer.

Materials and methods

Plant materials

The soybean seeds (cv. Williams) were obtained from Seed and Plant Certification and Registration Institute, Karaj, Iran. The seeds were produced in 2017 and had a standard germination of 98%.

Experimental layout

The study was conducted in 2017 in a series of laboratory and greenhouse experiments based on a factorial, completely randomized design with three replicates at College of Agriculture and Natural Resource, University of Tehran, Tehran, Iran. Different durations of inducing AA (48 h and 72 h), SA priming solutions (0, 300 and 600 μM) and priming times (B: before

performing AA; A: after performing AA; and B + A: both before and after performing AA, i.e., double priming) were the experimental factors.

Accelerated seed aging process

To initiate the AA process, soybean seeds were sterilized with 1% sodium hypochlorite for 2 min and then rinsed with distilled water. The washed seeds were placed in plastic mesh boxes (15 × 11 × 6 cm) containing distilled water. During the AA process, a temperature of 40°C and a relative humidity of 100% were maintained (Singh and Ram 1986). Finally, the deteriorated seeds were placed in a laboratory at a temperature of 23–26°C for 24 h to obtain the initial moisture content (about 13%).

Seed priming and germination-related traits

To perform seed priming, seed weight to solution volume ratio was set as 1:5 (g ml^{-1}) (Farooq, Basra, and Hafeez 2006). Fifty g of seeds were primed with 250 ml of respective SA solutions (concentrations of 300 and 600 μM) at three times (B: before performing AA; A: after performing AA, and B + A: both before and after performing AA or double priming). The solutions, kept at 20°C under dark conditions for 8 h (Dallali et al. 2012), were aerated intermittently (Jisha and Puthur 2016). The primed seeds were washed with distilled water, surface dried and placed in a laboratory at a temperature of 23–26°C for 24 h (Dallali et al. 2012).

Four replicates of 25 seeds per treatment were used for the standard germination test. The germination process was performed in 15-cm Petri dishes lined with Whatman filter paper at 25°C in a germinator for 8 d (ISTA 2011). When the radicle length was ≥ 2 mm, germinated seeds were counted daily. Mean germination time (MGT) was calculated according to the following equation (Matthews and Khajeh-Hosseini 2006):

$$\mathrm{MGT} = \Sigma n.t/\Sigma n \tag{1}$$

where n is the number of newly germinated seeds each day, and t is the number of days from the beginning of the germination test.

For dry weight determination, normal seedlings were placed in an oven at 70°C for 48 h and dry weight was expressed as mg $seedling^{-1}$. As defined by Miller (2002), abnormal seedlings were those with incomplete cotyledon emergence from the seed coat, or those in which the radicle failed to emerge, or those that died shortly after radicle emergence.

Enzyme activities and malondialdehyde content

Normal seedlings were used to determine activities of the following enzymes: Catalase (CAT) (Kar and Mishra 1976), peroxidase (POX) (Koroi 1989), ascorbate peroxidase (APX) (Nakano and Asada 1981), glutathione peroxidase (GPX) (Maehly and Chance 1954), glutathione reductase (GR) (Foyer and Halliwell 1976), and superoxide dismutase (SOD) (Beauchamp et al. 1983). Malondialdehyde (MDA) content was measured following Heath and Packer (1968).

Emergence-related traits

To determine the emergence percentage (EP), 10 seeds were sown 2 cm deep in pots (filled with 3.5 kg sandy soil, characterized by organic carbon = 0.91%, pH = 7.10, and electrical conductivity = 1.51 dS m^{-1}); the pots were placed in a greenhouse under controlled conditions (day/night: 25/18°C) with natural light radiation and relative humidity of about 60–70%. During the growing period, regular irrigation with tap water was given to maintain optimal soil field capacity.

Emerged seedlings (based on cotyledon leaf appearance) were counted at 24-h intervals for 14 d. Mean emergence time (MET) was calculated according to the same formula as used for MGT but using counts of seeds emerged.

At the 8-leaf stage (about 28 days after emergence), the plants (with roots) were removed and washed. Freshly developed leaves were used to determine chlorophyll *a*, chlorophyll *b* and carotenoid contents according to Arnon (1967). Four harvested plants were used for fresh tissue measurements and the rest of the plants were oven-dried at 70°C for 48 h to a constant weight.

Statistical analyzes

All experimental data were subjected to analyzes of variance using SAS 9.3 software (SAS 2011). The least significant difference (LSD, 5% level) was used to measure statistical differences between treatment means.

Results and discussion

Seedling-related traits under laboratory conditions (Experiment 1)

Analysis of variance for aging duration, salicylic acid (SA) concentration and priming time for germination-related traits is presented in Table 1. The interaction between aging duration and SA solution was significant for all seed indices, except for seedling dry weight. The interaction between aging duration and priming time, and that between SA solution and priming time were found to be significant for seed traits. The best two-way combination

Table 1. Mean squares attributable to aging duration, salicylic acid (SA) and priming time for germination-related traits† in soybean.

Source of variance	DF	GP	MGT (day)	Seedling dry weight (mg)	CAT	POX	APX	GPX	GR	SOD	MDA content (nmol g^{-1})
					(unit mg $protein^{-1}$)						
Aging duration (*D*)	1	70.6 **	3.2 **	0.01**	1.447 NS	10.06 **	1.73 **	0.0000036 **	0.0000214 **	0.000093 **	45.0 **
SA (*S*)	2	7385.4 **	41.7 **	2.67 **	442.319 **	553.38 **	40.05 **	0.0011496 **	0.0020732 **	0.000890 **	838.3 **
Priming time (*T*)	2	826.7 **	11.9 **	0.58 **	129.070 **	95.91 **	11.32 **	0.0004401 **	0.0003132 **	0.001060 **	150.6 **
$D \times S$	2	168.6 **	3.1 **	0.00001 NS	6.478 **	14.19 **	0.60 **	0.0000125 **	0.0000546 **	0.000063 **	78.1 **
$D \times T$	2	20.4 **	1.2 *	0.006 **	4.785 **	3.39 **	0.05 *	0.0000060 **	0.0000142 **	0.000106 **	3.1 **
$S \times T$	4	200.8**	2.8 **	0.14 **	32.479 **	2365 **	2.69 **	0.0001111 **	0.0000733 **	0.000272 **	36.6 **
$D \times S \times T$	4	48.3**	0.1 NS	0.02 **	7.658 **	6.59 **	0.15 **	0.0000182 **	0.0000171 **	0.000081 **	6.1 **
Error	36	0.3	0.1	0.0001	0.003	0.08	0.01	0.0000005	0.0000005	0.000005	0.1

*, ** Significant at 0.05 and 0.01 probability level, respectively. *NS*: non-significant.

†GP: germination percentage, MGT: mean germination time, CAT: catalase, POX: peroxidase, APX: ascorbate peroxidase, GPX: glutathione peroxidase, GR: glutathione reductase, SOD: superoxide dismutase, and MDA: malondialdehyde.

Table 2. Effects of aging duration, salicylic acid (SA) and priming time for germination-related traits† in soybean.

Experimental treatments	GP	MGT (day)	Seedling dry weight (mg)	CAT	POX	APX	GPX	GR	SOD	MDA content (nmol g^{-1})
				(unit mg $protein^{-1}$)						
Aging duration (h)										
48	73.26	3.92	1.78	16.93	16.98	5.05	0.018	0.037	0.019	10.04
72	69.66	4.42	1.76	16.83	16.11	4.65	0.017	0.035	0.021	11.24
LSD (5% level)	*0.332*	*0.196*	*0.009*	-	*0.167*	*0.061*	*0.0004*	*0.0005*	*0.0012*	*0.254*
SA (μM)										
0	49.81	5.92	1.36	11.54	10.97	3.21	0.008	0.025	0.012	17.45
300	82.17	3.38	1.97	19.39	19.14	5.72	0.022	0.041	0.024	7.51
600	82.39	3.21	1.99	19.72	19.52	5.62	0.022	0.042	0.024	6.96
LSD (5% level)	*0.407*	*0.240*	*0.010*	*0.155*	*0.205*	*0.075*	*0.0005*	*0.0006*	*0.0015*	*0.311*
Priming time‡										
B	63.73	5.02	1.57	13.87	13.91	4.06	0.012	0.031	0.014	14.05
A	71.26	4.23	1.79	16.89	16.45	4.71	0.017	0.036	0.018	10.42
B + A	79.38	3.26	1.96	19.89	19.27	5.78	0.023	0.041	0.029	7.46
LSD (5% level)	*0.407*	*0.240*	*0.010*	*0.155*	*0.205*	*0.075*	*0.0005*	*0.0006*	*0.0015*	*0.311*

†GP: germination percentage, MGT: mean germination time, CAT: catalase, POX: peroxidase, APX: ascorbate peroxidase, GPX: glutathione peroxidase, GR: glutathione reductase, SOD: superoxide dismutase, and MDA: malondialdehyde.

‡B: before performing the AA test, A: after performing the AA test, B + A: double priming (both before and after performing the AA test).

was double priming with 300 μM SA (for GP and seedling dry weight, and for CAT, APX, GPX, GR, and SOD). In addition, the three-way interaction (aging duration × priming time × SA concentration) was significant for all germination-related traits, except for MGT (Table 1). This means that the best three-way combination for germination-related traits (GP, seedling dry weight, CAT, APX, GPX, GR, and SOD), except MGT, was 300 μM SA and double priming on 48-h aged seeds.

Irrespective of SA application, increase in aging duration significantly increased MGT and MDA content, but the germination percentage (GP) decreased (Table 2). For instance, MDA content increased by 10.68% under 72-h aging duration (severely deteriorated seed), compared with 48-h aging duration (mildly deteriorated seed) (Table 3).

As the interaction between aging duration and SA concentration was significant, we determined the best combination of these two factors that alleviated the adverse effect of seed deterioration on seedling-related traits. For example, under 72-h aging duration, SA priming at 600 μM decreased MGT by 47.44% and increased CAT activity by 94.38%, compared with the control (Table 3). Under similar conditions, a considerable reduction in MDA content (from 22.37 to 6.74 nmol g^{-1}) was recorded (Table 3). Overall, the best two-way combination was double priming with 300 μM SA for GP and seedling dry weight, and for CAT, APX, GPX, GR, and SOD (Table 3). For POX, the best two-way combination was double priming and

Table 3. Interaction of aging duration, salicylic acid (SA) and priming time for germination-related traits† in soybean.

Interactions		GP	MGT (day)	Seedling dry weight (mg)	CAT	POX	APX	GPX	GR	SOD	MDA content (nmol g^{-1})
					(unit mg $protein^{-1}$)						
Aging duration (h)	**SA (μM)**										
48	0	51.86	5.41	1.32	11.81	11.16	3.47	0.009	0.025	0.013	15.73
48	300	81.28	3.35	1.98	19.06	18.85	5.75	0.021	0.040	0.022	7.87
48	600	82.05	3.03	2.00	19.71	19.68	5.74	0.022	0.042	0.021	7.18
72	0	42.53	6.45	1.31	10.15	8.31	2.70	0.007	0.020	0.011	22.37
72	300	83.07	3.41	1.97	19.73	19.43	5.69	0.022	0.042	0.027	7.15
72	600	82.72	3.39	1.98	19.73	19.36	5.50	0.022	0.041	0.026	6.74
LSD (5% level)		*0.584*	*0.340*	-	*0.056*	*0.283*	*0.113*	*0.0007*	*0.0007*	*0.0022*	*0.290*
Aging duration (h)	**Priming time‡**										
48	B	66.07	4.69	1.59	14.64	14.75	4.30	0.013	0.032	0.014	12.97
48	A	71.63	3.99	1.78	16.97	16.44	4.89	0.018	0.036	0.017	9.87
48	B + A	77.49	3.11	1.92	18.98	18.50	5.78	0.022	0.039	0.024	7.94
72	B	61.65	5.23	1.55	13.39	12.99	3.89	0.012	0.029	0.013	15.34
72	A	69.34	4.47	1.76	16.44	15.63	4.45	0.017	0.035	0.018	12.10
72	B + A	77.34	3.56	1.95	19.76	18.47	5.55	0.023	0.040	0.033	8.82
LSD (5% level)		*0.584*	*0.340*	*0.012*	*0.056*	*0.283*	*0.113*	*0.0007*	*0.0007*	*0.0022*	*0.290*
SA (μM)	**Priming time**										
0	B	46.88	6.05	1.31	10.95	9.63	3.03	0.008	0.023	0.012	19.15
0	A	47.51	5.81	1.31	11.01	9.83	3.15	0.008	0.023	0.012	18.95
0	B + A	47.20	5.93	1.32	10.97	9.73	3.09	0.008	0.023	0.012	19.05
300	B	72.65	4.40	1.70	15.56	16.05	4.68	0.015	0.035	0.015	12.05
300	A	81.02	3.71	1.98	19.06	18.59	5.52	0.021	0.041	0.019	7.45
300	B + A	92.85	2.04	2.25	23.57	22.79	6.96	0.030	0.048	0.039	3.04
600	B	72.06	4.44	1.70	15.54	15.93	4.58	0.015	0.035	0.015	11.27
600	A	82.91	3.16	2.04	20.06	19.69	5.34	0.023	0.042	0.022	6.56
600	B + A	92.19	2.04	2.23	23.57	22.94	6.95	0.029	0.047	0.035	3.05
LSD (5% level)		*0.715*	*0.417*	*0.015*	*0.069*	*0.347*	*0.139*	*0.0008*	*0.0008*	*0.0026*	*0.355*

†GP: germination percentage, MGT: mean germination time, CAT: catalase, POX: peroxidase, APX: ascorbate peroxidase, GPX: glutathione peroxidase, GR: glutathione reductase, SOD: superoxide dismutase, and MDA: malondialdehyde.

‡B: before performing the AA test, A: after performing the AA test, B + A: double priming (both before and after performing the AA test).

Table 4. Three-way interaction among aging duration, salicylic acid (SA) and priming time for germination-related traits† in soybean.

Aging duration (h)	SA (μM)	Priming time	GP	Seedling dry weight (mg)	CAT	POX	APX	GPX	GR	SOD	MDA content (nmol g^{-1})
					(unit mg $protein^{-1}$)						
48	0	B	51.88	1.32	11.82	11.15	3.40	0.009	0.025	0.012	15.81
48	0	A	51.84	1.32	11.80	11.16	3.55	0.009	0.025	0.013	15.65
48	0	B + A	51.86	1.32	11.81	11.16	3.47	0.009	0.025	0.013	15.73
48	300	B	70.62	1.68	15.06	15.62	4.58	0.014	0.034	0.015	12.58
48	300	A	83.15	2.05	20.06	19.43	5.74	0.023	0.042	0.021	6.42
48	300	B + A	90.06	2.20	22.06	21.51	6.93	0.028	0.045	0.029	4.62
48	600	B	75.71	1.79	17.03	17.48	4.91	0.016	0.038	0.016	10.51
48	600	A	79.89	1.98	19.05	18.74	5.38	0.021	0.041	0.017	7.56
48	600	B + A	90.56	2.22	23.06	22.82	6.94	0.028	0.046	0.031	3.46
72	0	B	41.88	1.30	10.08	8.12	2.65	0.007	0.020	0.011	22.49
72	0	A	43.19	1.31	10.22	8.50	2.76	0.007	0.021	0.011	22.24
72	0	B + A	42.53	1.31	10.14	8.31	2.70	0.007	0.020	0.011	22.37
72	300	B	74.67	1.72	16.05	16.48	4.78	0.015	0.036	0.015	11.51
72	300	A	78.89	1.90	18.05	17.75	5.31	0.019	0.040	0.017	8.49
72	300	B + A	95.64	2.29	25.09	24.06	6.98	0.032	0.050	0.048	1.45
72	600	B	68.41	1.61	14.05	14.38	4.25	0.013	0.032	0.013	12.03
72	600	A	85.93	2.09	21.06	20.64	5.29	0.025	0.043	0.026	5.57
72	600	B + A	93.83	2.25	24.07	23.05	6.95	0.029	0.048	0.039	2.64
LSD (5% level)			*1.011*	*0.021*	*0.098*	*0.490*	*0.196*	*0.0011*	*0.0012*	*0.0037*	*0.503*

†GP: germination percentage, CAT: catalase, POX: peroxidase, APX: ascorbate peroxidase, GPX: glutathione peroxidase, GR: glutathione reductase, SOD: superoxide dismutase, and MDA: malondialdehyde.
‡B: before performing the AA test, A: after performing the AA test, B + A: double priming (both before and after performing the AA test).

600 μM SA. Moreover, the minimum values for MGT and MDA were observed when 300 μM SA was applied during double priming, which was considered the best two-way combination (Table 3).

For GP and seedling dry weight, and for CAT, POX, APX, GPX, GR, and SOD activity, the best three-way combination was the application of 300 μM SA and double priming on 48-h aged seeds (Table 4). Similarly, the minimum MDA content was obtained when 300 μM SA and double priming were applied on 48-h aged seeds, which was found to be the best three-way combination (Table 4).

In general, scavenging of reactive oxygen species and maintenance of cellular function may be induced by antioxidant activity (Pinzino 1999). In this study, SA priming was found to be an effective approach to improve seedling establishment from aged seeds.

Emergence traits under greenhouse conditions (Experiment 2)

All emergence-related traits were affected by the interaction between aging duration and SA concentration, except for plant dry weight (Table 5). The interaction between aging duration and priming time was significant for all

Table 5. Mean squares attributable to aging duration, salicylic acid (SA) and priming time for emergence-related traits† in soybean under greenhouse conditions.

Experimental treatments	DF	EP	MET (day)	Plant dry weight (mg)	Plant leaf area (cm^2)	Carotenoid (μg g^{-1} FW)	Chlorophyll *a* (mg g^{-1} FW)	Chlorophyll *b* (mg g^{-1} FW)
Aging duration (*D*)	1	271 **	2.28 **	0.46 **	65.0 **	41.4 **	1.706 **	1.58 **
SA (*S*)	2	2400 **	27.76 **	4.30 **	730.1 **	4892.4 **	35.916 **	36.80 **
Priming time (*T*)	2	1112 **	9.67 **	3.22 **	181.2 **	1024.3 **	5.807 **	7.76 **
$D \times S$	2	26 **	0.12 **	0.06 NS	5.8 **	226.9 **	1.340 **	1.68 **
$D \times T$	2	7 *	0.08 **	0.32 *	0.5 NS	97.9 **	0.629 **	0.14 **
$S \times T$	4	298 **	2.35 **	0.77 **	47.2 **	268.7 **	1.514 **	2.09 **
$D \times S \times T$	4	2 NS	0.01 NS	0.01 NS	0.4 NS	66.8 **	0.245 **	0.25 **
Error	36	1	0.006	0.02	0.2	0.4	0.002	0.01

*, ** Significant at 0.05 and 0.01 probability level, respectively. *NS*: non-significant.

†EP: emergence percentage, MET: mean emergence time. The plants (with roots) were removed at the 8-leaf stage (28 days after emergence).

traits, except for leaf area. There was a significant interaction between SA concentration and priming time for these traits. Moreover, the three-way interaction was found to be significant on carotenoid, chlorophyll *a*, and chlorophyll *b* contents (Table 5). This means that the best three-way combination for emergence-related traits was double priming with SA at 300 μM on 48-h aged seeds.

Irrespective of SA concentration, EP and leaf area as well as carotenoid content, chlorophyll *a* and chlorophyll *b* contents decreased as a result of the AA process, whereas MET significantly increased. For instance, with increasing aging duration from 48 h to 72 h, EP and chlorophyll *b* content decreased by 7.12% and 7.61%, respectively (Tables 6 and 7).

Although carotenoid content, and chlorophyll *a* and chlorophyll *b* contents decreased with increasing aging duration, SA priming mitigated these adverse effects. For instance, application of SA solution at 300 μM under severe AA increased carotenoid content by 26.43%, compared with the control (Table 7). On the other hand, under the mild and severe seed deterioration, double priming increased EP and plant dry weight as well as carotenoid, chlorophyll *a* and chlorophyll *b* contents, relative to one-time priming (Table 7). For instance, when 72-h aging duration was used, the highest carotenoid, chlorophyll *a*, and chlorophyll *b* contents were recorded with the application of SA at 300 μM under double priming (Table 8). Accordingly, considering the three-way interaction, double priming with SA at 300 μM on 48- or 72-h aged seeds was

Table 6. Effects of aging duration, salicylic acid (SA) and priming time for emergence-related traits † in soybean under greenhouse conditions.

Experimental treatments	EP	MET (day)	Plant dry weight (mg)	Plant leaf area (cm^2)	Carotenoid (μg g^{-1} FW)	Chlorophyll *a* (mg g^{-1} FW)	Chlorophyll *b* (mg g^{-1} FW)
Aging duration (h)							
48	62.96	4.90	2.58	31.69	156.50	7.65	4.73
72	58.48	5.31	2.40	29.50	154.74	7.31	4.37
LSD (5% level)	*0.697*	*0.043*	*0.079*	*0.256*	*0.374*	*0.056*	*0.029*
SA (μM)							
0	47.39	6.54	1.93	23.24	136.59	5.83	2.92
300	67.61	4.35	2.78	34.45	164.57	8.26	5.33
600	67.17	4.43	2.77	34.09	165.69	8.35	5.40
LSD (5% level)	*0.853*	*0.052*	*0.097*	*0.314*	*0.457*	*0.069*	*0.036*
Priming time‡							
B	52.94	5.83	2.06	27.48	148.43	6.77	3.97
A	60.56	5.13	2.50	30.47	154.96	7.61	4.57
B + A	68.67	4.37	2.91	33.83	163.47	8.07	5.11
LSD (5% level)	*0.853*	*0.052*	*0.097*	*0.314*	*0.457*	*0.069*	*0.036*

†EP: emergence percentage, MET: mean emergence time. The plants (with roots) were removed at the 8-leaf stage (28 days after emergence).

‡B: before performing the AA test, A: after performing the AA test, B + A: double priming (both before and after performing the AA test).

Table 7. Interaction among aging duration, salicylic acid (SA) and priming time for emergence-related traits † in soybean under greenhouse conditions.

Interactions		EP	MET (day)	Plant dry weight (mg)	Plant leaf area (cm^2)	Carotenoid (μg g^{-1} FW)	Chlorophyll *a* (mg g^{-1} FW)	Chlorophyll *b* (mg g^{-1} FW)
Aging duration (h)	**SA (μM)**							
48	0	50.89	6.28	1.98	24.85	141.44	6.35	3.41
48	300	68.67	4.24	2.94	34.93	162.59	8.23	5.34
48	600	69.33	4.19	2.83	35.29	165.46	8.37	5.43
72	0	43.89	6.80	1.87	21.63	131.74	5.31	2.42
72	300	66.56	4.46	2.62	33.96	166.56	8.29	5.31
72	600	65.00	4.68	2.71	32.89	165.93	8.33	5.37
LSD (5% level)		*1.207*	*0.074*	-	*0.444*	*0.647*	*0.097*	*0.051*
Aging duration (h)	**Priming time‡**							
48	B	54.56	5.58	2.11	28.40	150.87	7.03	4.21
48	A	62.78	4.89	2.60	31.58	156.96	7.79	4.73
48	B + A	71.56	4.24	3.03	35.09	161.67	8.14	5.23
72	B	51.33	6.08	2.01	26.57	145.99	6.51	3.73
72	A	58.33	5.37	2.41	29.36	152.97	7.43	4.40
72	B + A	65.78	4.50	2.78	32.56	165.28	7.99	4.98
LSD (5% level)		*1.207*	*0.074*	*0.137*	-	*0.647*	*0.097*	*0.051*
SA (μM)	**Priming time**							
0	B	47.67	6.56	1.90	23.17	136.58	5.85	2.93
0	A	47.33	6.54	1.96	23.45	136.85	5.83	2.91
0	B + A	47.17	6.53	1.92	23.11	136.35	5.81	2.91
300	B	56.50	5.33	2.18	30.15	154.22	7.17	4.49
300	A	66.83	4.43	2.71	34.15	162.60	8.39	5.29
300	B + A	79.50	3.30	3.44	39.03	176.90	9.23	6.20
600	B	54.67	5.61	2.11	29.13	154.48	7.29	4.49
600	A	67.50	4.42	2.84	33.81	165.43	8.59	5.50
600	B + A	79.33	3.27	3.36	39.34	177.17	9.17	6.21
LSD (5% level)		*1.478*	*0.091*	*0.167*	*0.543*	*0.792*	*0.119*	*0.062*

†EP: emergence percentage, MET: mean emergence time. The plants (with roots) were removed at the 8-leaf stage (28 days after emergence).
‡B: before performing the AA test, A: after performing the AA test, B + A: double priming (both before and after performing the AA test).

Table 8. Three-way interaction among aging duration, salicylic acid (SA) and priming time for emergence-related traits † in soybean under greenhouse conditions.

Aging duration (h)	SA (μM)	Priming time‡	Carotenoid (μg g^{-1} FW)	Chlorophyll *a* (mg g^{-1} FW)	Chlorophyll *b* (mg g^{-1} FW)
48	0	B	140.93	3.45	6.45
48	0	A	142.40	3.41	6.37
48	0	B + A	141.00	3.36	6.24
48	300	B	153.40	4.39	7.04
48	300	A	165.03	5.49	8.59
48	300	B + A	169.33	6.13	9.06
48	600	B	158.27	4.79	7.58
48	600	A	163.43	5.29	8.39
48	600	B + A	174.67	6.20	9.14
72	0	B	132.23	2.40	5.25
72	0	A	131.30	2.41	5.29
72	0	B + A	131.70	2.46	5.38
72	300	B	155.03	4.59	7.29
72	300	A	160.17	5.09	8.19
72	300	B + A	184.47	6.26	9.39
72	600	B	150.70	4.19	7.00
72	600	A	167.43	5.70	8.79
72	600	B + A	179.67	6.22	9.19
LSD (5% level)			*1.120*	*0.088*	*6.453*

†The plants (with roots) were removed at the 8-leaf stage (28 days after emergence).
‡B: before performing the AA test, A: after performing the AA test, B + A: double priming (both before and after performing the AA test).

the best combination for increasing carotenoid, chlorophyll *a*, and chlorophyll *b* contents.

The experimental findings may be attributed to SA metabolism. In fact, it seems that special adaptability to aging conditions in SA-primed seeds is induced by stimulating the signal reactions to improve cellular resistance against reactive oxygen species. In this context, increased germination rate and mitigated electrolyte leakage using SA priming have been observed (Lee, Kim, and Park 2010; Zhang et al. 2011).

We found a strong negative correlation between MDA content and carotenoid content ($r = -0.99$, $P \leq 0.01$) under AA (Figure 1). The same trend was also observed for chlorophyll *a* ($r = -0.99$, $P \leq 0.01$) and chlorophyll *b* ($r = -0.99$, $P \leq 0.01$) (Figure 2).

Under AA, correlation between MDA content and MET was positive and significant ($r = 0.95$, $P \leq 0.01$) (Figure 3). By contrast, the correlation between CAT activity and MET was found to be negative ($r = -0.97$, $P \leq 0.01$) (Figure 4). Similar relationships were also observed for POX and MET ($r = -0.96$, $P \leq 0.01$), APX and MET ($r = -0.98$, $P \leq 0.01$), GPX and MET ($r = -0.97$, $P \leq 0.01$), GR and MET ($r = -0.96$, $P \leq 0.01$), and SOD and MET ($r = -0.83$, $P \leq 0.01$) activity (Figure 4).

Generally, a positive correlation between MDA content with MET in aged soybean seed indicates the importance of storage lipids in improving seedling establishment. Along with lipid peroxidation, reduced enzyme

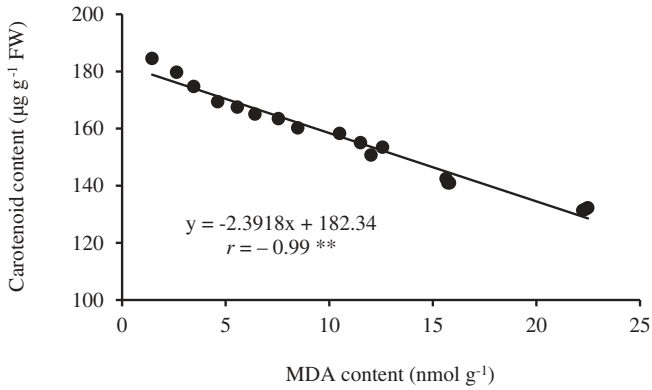

Figure 1. Correlation between malondialdehyde (MDA) content with carotenoid content after performing the accelerated aging test. The asterisks ** indicate statistical differences at $P \leq 0.01$.

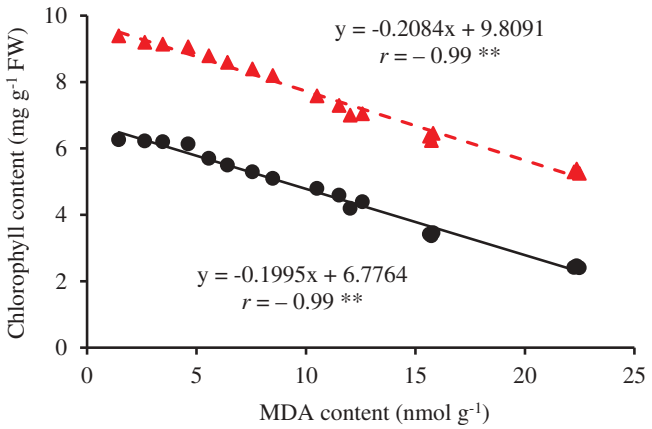

Figure 2. Correlation between malondialdehyde (MDA) content with chlorophyll *a* (triangles) and chlorophyll *b* (circles) contents after performing the accelerated aging test. The asterisks ** indicate statistical differences at $P \leq 0.01$.

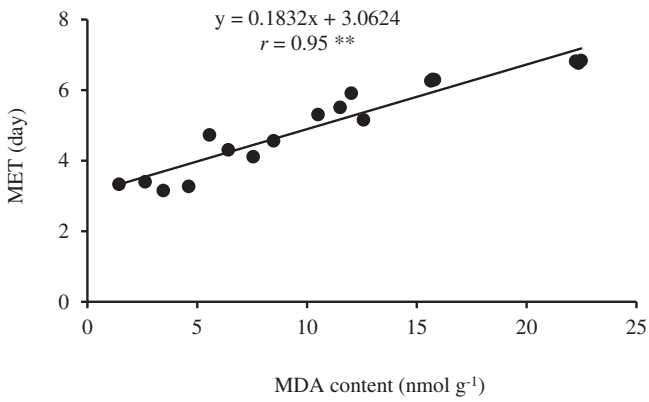

Figure 3. Correlation between malondialdehyde (MDA) content with mean emergence time (MET) after performing the accelerated aging test. The asterisks ** indicate statistical differences at $P \leq 0.01$.

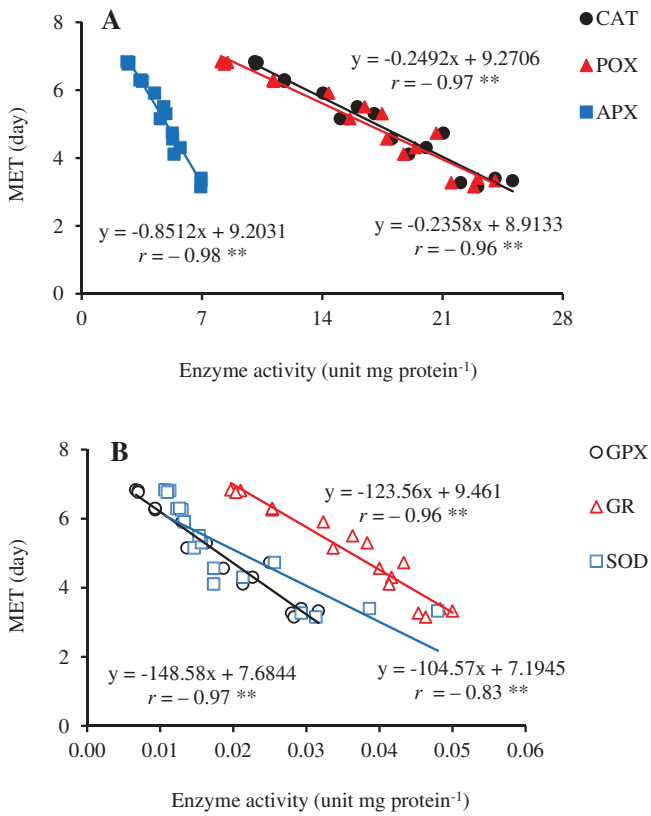

Figure 4. Correlation between CAT, POX and APX activity with MET (A) and between GPX, GR and SOD activity with MET (B) after performing the accelerated aging test. The asterisks ** indicate statistical differences at $P \leq 0.01$. (CAT: catalase, POX: peroxidase, APX: ascorbate peroxidase, GPX: glutathione peroxidase, GR: glutathione reductase, SOD: superoxide dismutase, and MET: mean emergence time).

activities may be attributable to the accumulation of reactive oxygen species, which ultimately affect the seedling vigor and establishment. In this regard, a significant reduction in seed reserves, especially unsaturated fatty acids, during the AA process, was observed in canola (Seyyedi, Tavakkol Afshari, and Daneshmandi 2018).

Conclusions

Based on the results of this investigation, seed germination and seedling growth were negatively affected by the AA process, which was mainly attributable to increased MDA content and degraded chlorophyll structure. Nevertheless, the adverse effects of seed aging were alleviated with the application of SA solution, especially with double priming. It seems that information on the biological metabolism of enzyme activity and lipid peroxidation in deteriorated seedlings can properly predict the

seeding establishment potential under field conditions. Overall, considering the three-way interaction, double priming of SA at 300 μM on 48- or 72-h aged seeds is suggested the best combination for increasing seedling vigor in soybean. However, more comprehensive studies are needed to understand the functional mechanism of SA priming at genetic, cellular and molecular levels.

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