Industrial Crops & Products 153 (2020) 112605

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

# Exogenous γ-aminobutyric acid can alleviate the adverse effects of seed aging on fatty acids composition and heterotrophic seedling growth in medicinal pumpkin

Reza Tavakkol Afshari*, Seyyed Mohammad Seyyedi

*Department of Agrotechnology, Faculty of Agriculture, Ferdowsi University of Mashhad, P.O. Box 91779-48974, Mashhad, Iran*

ARTICLE INFO

*Keywords:*
Electrolyte leakage
GABA priming
Mean germination time
Saturated fatty acids
Seed reserve utilization efficiency
Unsaturated fatty acids

ABSTRACT

Accelerated oil oxidation and degraded fatty acids (FA) composition are considered as well-known consequences in oilseed crops resulting from the imposed aging conditions. Hence, evaluating the effective techniques and understanding the biological mechanisms associated to mitigating seed deterioration process in medicinal pumpkin (*Cucurbita pepo* subsp. Pepo. *Convar*. Pepo var. *styriaca* Greb) may be necessary. In the current study, different durations of artificial accelerated aging (0, 48, 72, and 96 h) and priming solutions (water priming as control and γ-aminobutyric acid (GABA) priming at 1, 2, and 3 mmol $L^{-1}$) were tested. From the results, a significant decrease in oil percentage and seedling dry weight was observed with increasing seed aging duration, whereas malondialdehyde content and electrolyte leakage significantly increased. Moreover, with increasing seed deterioration severity, percentage of saturated FA (SFA) significantly increased, while mono- and poly-unsaturated FA (MUFA and PUFA) decreased. In contrast, GABA priming alleviated the deleterious effects of seed deterioration on seedling growth and FA composition. For instance, after 96-h aging period, GABA priming at 3 mmol $L^{-1}$ decreased SFA/MUFA and SFA/PUFA by 14.2 and 13.3%, respectively, compared with control. Overall, the results obtained here may confirm the crucial role of GABA priming to improve the successful establishment of seedlings raised from medicinal pumpkin seeds.

## 1. Introduction

Medicinal pumpkin (*Cucurbita pepo* subsp. Pepo. *Convar*. Pepo var. *styriaca* Greb), an annual oilseed crop from the Cucurbitaceae family (Younis et al., 2000), is mainly cultivated in semi-arid regions characterized by hot and long summer season (Koocheki et al., 2019; Yavuz et al., 2015). A wide range of bioactive components, including tocopherols, phytosterols, and squalene, have been identified from medicinal pumpkin seeds (Rabrenović et al., 2014; Sedghi et al., 2008). As well as, linoleic and oleic found as dominant fatty acids (FA) in those seeds (Sabudak, 2007; Younis et al., 2000). The crucial importance of medicinal pumpkin in pharmaceutical and pharmacological industries has been reported due to its specific anti-diabetic (Yoshinari et al., 2009), anti-tumor and (Sim et al., 2008) anti-inflammatory (Karpagam et al., 2011) activities.

Medicinal pumpkin is known as a relatively compatible plant to stressful environments (Koocheki et al., 2019). However, the poor establishment of pumpkin seedlings, especially under adverse soil conditions, is one of the most important limiting factors in achieving optimum plant density (Dutra and Vieira, 2006; Ghaderi-Far et al., 2011). This can emphasize the need for producing certified seed or improving seed quality to help successful medicinal pumpkin production.

Strategies for improving the performance of oilseeds depend on nutrient reserves (Trawatha et al., 1995), especially FA composition (Balešević-Tubić et al., 2007; Seyyedi et al., 2018). On the other hand, the maximum seed vigor index occurs at the maturity stage and then gradually declines under natural conditions (Ellis et al., 1991; Flakelar et al., 2018). Accordingly, determining the potential of target seeds, especially in terms of vigor status, is usually recognized as the first priority during the crop production cycle.

In general, biochemical processes involved in natural aging occur over the long-term (Eisvand et al., 2010; Hang et al., 2015). Hence, the accelerated aging test, described as imposing artificial deterioration at high temperature and relative humidity (Mavi and Demir, 2007), is known as an alternative approach to accurately estimate seed performance in a wide range of environmental conditions (Demir and Mavi, 2008; Simić et al., 2004). In this regard, destroyed FA structure

* Corresponding author.
*E-mail address:* tavakolafshari@um.ac.ir (R. Tavakkol Afshari).

https://doi.org/10.1016/j.indcrop.2020.112605
Received 2 February 2020; Received in revised form 17 May 2020; Accepted 19 May 2020
0926-6690/ 

(Seyyedi et al., 2018), accelerated lipid peroxidation (Bailly et al., 1996; Nazari et al., 2020; Tatić et al., 2012), and increased electrolyte leakage (Gidrol et al., 1989; Mohammadi et al., 2012) are well-known consequences associated with natural or artificial aging processes in oilseed crops.

When cellular metabolic functions are exposed to artificial aging, seed priming by exogenous bioactive compounds, such as γ-aminobutyric acid (GABA), can be considered as a practical technique to enhance seedling establishment potential (Hu et al., 2015; Vijayakumari and Puthur, 2016). GABA, a specific plant growth regulator, acts as an endogenous signal molecule in responses to environmental stresses (Song et al., 2010). GABA plays a crucial role in alleviating oxidative damage (Song et al., 2010) and stabilizing cell performance (Hu et al., 2015; Rezaei-Chiyaneh et al., 2018; Shelp et al., 1999). The positive effect of GABA-primed seeds on improving germination performance and emergence rate in white clover (Cheng et al., 2018) and tomato (Islam et al., 2006) has been previously reported. However, the functional mechanism of GABA priming on aged oilseeds, especially in terms of FA profile, has not been well understood.

The current research was aimed to investigate how GABA priming affects germination and emergence performance in deteriorated medicinal pumpkin seeds. The induced changes in FA composition were also investigated to better understand biochemical metabolisms resulting from GABA-primed seeds.

## 2. Materials and methods

### 2.1. Experimental design

The study was conducted as a series of laboratory experiments based on completely randomized design arranged in factorial with four replicates at Faculty of Agriculture, Ferdowsi University of Mashhad, Iran, in 2018. Seed exposure for different periods of accelerated aging (0, 48, 72, and 96 h) and priming solutions (water priming as control and GABA priming at 1, 2, and 3 mmol $L^{-1}$) were considered as experimental factors, respectively.

### 2.2. Plant materials

Medicinal pumpkin seeds were obtained from Agricultural Research Station, Ferdowsi University of Mashhad, Iran. The seeds were produced in 2018 with standard germination 93%.

### 2.3. Seed priming technique

Seeds were immersed in Erlenmeyer flask containing different GABA (Sigma-Aldrich, A2129; $NH_2(CH_2)_3COOH$; CAS: 56-12-2) solutions (prepared at concentrations of 1, 2, and 3 mmol $L^{-1}$). The ratio of seed weight to solution volume was fixed at 1:3 (Jisha and Puthur, 2016). Similarly, distilled water-primed seeds were considered as control (Shailasree et al., 2001). The Erlenmeyer flasks were sealed with aluminum foil to prevent evaporation. The solutions, kept at 20 °C under dark conditions for 12 h, were swirled gently and intermittently to ensure appropriate aeration (Jisha and Puthur, 2016). Then, the primed seeds were washed with distilled water, surface dried with absorbent paper and then placed in an oven at 25 °C for 48 h (Zheng et al., 2016).

### 2.4. Accelerated seed aging process

The accelerated aging test was performed after seed priming (Hacisalihoglu, 2008). Seed samples were placed in plastic mesh boxes for 0, 48, 72, and 96 h, respectively (Dutra and Vieira, 2006). The aging process was carried out at 41 °C and 100% relative humidity (Dutra and Vieira, 2006).

**Table 1**
Effects of aging durations and GABA priming on electrical conductivity (EC), oil, N, and P contents in medicinal pumpkin seeds.

| Experimental treatments | EC (μs $cm^{-1}$ $g^{-1}$) | Oil (%) | N (g $kg^{-1}$) | P (g $kg^{-1}$) |
|---|---|---|---|---|
| Aging durations (h) | | | | |
| 0 | 16.03 | 38.54 | 32.54 | 8.14 |
| 48 | 22.66 | 36.89 | 31.56 | 7.56 |
| 72 | 25.49 | 34.30 | 27.70 | 7.24 |
| 96 | 27.99 | 30.71 | 26.04 | 6.42 |
| *LSD (0.05)* | *0.5453* | *0.7985* | *0.5277* | *0.0777* |
| GABA priming (mmol $L^{-1}$) | | | | |
| Control | 24.70 | 33.51 | 28.10 | 7.14 |
| 1 | 23.42 | 34.84 | 29.73 | 7.33 |
| 2 | 22.28 | 35.64 | 30.26 | 7.42 |
| 3 | 21.76 | 36.44 | 29.75 | 7.45 |
| *LSD (0.05)* | *0.5453* | *0.7985* | *0.5277* | *0.0777* |
| Aging durations × GABA priming | | | | |
| 0 × control | 15.59 | 38.25 | 32.67 | 8.08 |
| 0 × 1 | 16.36 | 39.00 | 32.88 | 8.19 |
| 0 × 2 | 16.16 | 38.71 | 32.40 | 8.06 |
| 0 × 3 | 16.01 | 38.22 | 32.22 | 8.22 |
| 48 × control | 23.89 | 35.08 | 28.91 | 7.31 |
| 48 × 1 | 23.17 | 36.59 | 33.42 | 7.53 |
| 48 × 2 | 21.88 | 37.08 | 32.60 | 7.67 |
| 48 × 3 | 21.72 | 38.81 | 31.32 | 7.71 |
| 72 × control | 27.20 | 32.86 | 26.75 | 6.93 |
| 72 × 1 | 25.79 | 34.26 | 27.36 | 7.25 |
| 72 × 2 | 24.94 | 34.94 | 28.49 | 7.40 |
| 72 × 3 | 24.01 | 35.13 | 28.19 | 7.37 |
| 96 × control | 32.13 | 27.86 | 24.06 | 6.26 |
| 96 × 1 | 28.37 | 29.52 | 25.27 | 6.38 |
| 96 × 2 | 26.15 | 31.84 | 27.57 | 6.53 |
| 96 × 3 | 25.30 | 33.61 | 27.27 | 6.51 |
| *LSD (0.05)* | *1.0905* | *1.5969* | *1.0554* | *0.1555* |
| Aging durations | ** | ** | ** | ** |
| GABA priming | ** | ** | ** | ** |
| Aging durations × GABA priming | ** | ** | ** | ** |

**: significant at $P \leq 0.01$.

### 2.5. Crude oil extraction and determination of FA

Following the aging treatments, seed oil percentage was measured according to soxhlet method (AOAC, 2000). To determine FA, samples of 5 g were ground and mixed with n-hexane (1:4 w/v). The samples were then placed on a shaker (120 rpm) in a dark place (23 − 25 °C) for 48 h. Then, the solvent was subjected to evaporation at 40 °C (Farhoosh et al., 2011). The FA were determined by gas chromatography (YOUNG LIN – Acme 6000 GC, The Republic of Korea) equipped with the fame-ionization detector and capillary column (60 m × 0.32 mm i.d.; film thickness was 0.25 μm). Injector, detector, and oven temperatures were 300, 320, and 250 °C, respectively.

### 2.6. Determination of N and P concentrations

N content was determined through titration method (AOAC, 2000) using a Kjeltec-PECO-Psu 55 Analyzer. P content was also measured by colorimetric method (Murphy and Riley, 1962) using a spectrophotometer (Jenway 6305).

### 2.7. Determination of electrolyte leakage (EL)

To determine EL, 50 seeds were weighed and then soaked in Erlenmeyer flask containing 250 mL double-distilled water (Seyyedi et al., 2018). The samples were kept at 20 °C under dark conditions for 24 h (Seyyedi et al., 2018). As suggested by Hajiabbasi et al. (2015), the electrical conductivity (EC) of soaked seeds was determined (using a Jenway 4510 conductivity meter) and expressed as μS $cm^{-1}$ $g^{-1}$ (Eq.

**Table 2**
Effects of aging durations and GABA priming on saturated fatty acids composition in medicinal pumpkin seed.

| Experimental treatments | SFA (g 100 g$^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Lauric | Myristic | Palmitic | Margaric | Stearic | Arachidic | Behenic | Lignoceric |
| Aging durations | | | | | | | | |
| 0 | 0.021 | 0.091 | 10.02 | 0.063 | 4.46 | 0.024 | 0.244 | 0.113 |
| 48 | 0.023 | 0.093 | 13.74 | 0.071 | 6.34 | 0.033 | 0.342 | 0.124 |
| 72 | 0.026 | 0.096 | 16.19 | 0.073 | 6.82 | 0.036 | 0.346 | 0.134 |
| 96 | 0.028 | 0.098 | 18.29 | 0.077 | 7.34 | 0.037 | 0.358 | 0.137 |
| *LSD (0.05)* | *0.0011* | *0.0012* | *0.2732* | *0.0012* | *0.2497* | *0.0011* | *0.0131* | *0.0039* |
| GABA priming (mmol L$^{-1}$) | | | | | | | | |
| Control | 0.024 | 0.094 | 15.33 | 0.073 | 6.55 | 0.034 | 0.330 | 0.129 |
| 1 | 0.024 | 0.095 | 14.84 | 0.071 | 6.30 | 0.033 | 0.328 | 0.129 |
| 2 | 0.025 | 0.094 | 14.15 | 0.070 | 6.07 | 0.032 | 0.316 | 0.126 |
| 3 | 0.025 | 0.095 | 13.91 | 0.070 | 6.04 | 0.031 | 0.316 | 0.124 |
| *LSD (0.05)* | *0.0011* | *0.0012* | *0.2732* | *0.0012* | *0.2497* | *0.0011* | *0.0131* | *0.0039* |
| Aging durations × GABA priming | | | | | | | | |
| 0 × control | | | 10.26 | 0.062 | 4.53 | 0.023 | 0.234 | |
| 0 × 1 | | | 10.02 | 0.064 | 4.40 | 0.024 | 0.253 | |
| 0 × 2 | | | 9.78 | 0.064 | 4.41 | 0.025 | 0.257 | |
| 0 × 3 | | | 10.01 | 0.062 | 4.49 | 0.024 | 0.233 | |
| 48 × control | | | 14.92 | 0.073 | 6.71 | 0.035 | 0.337 | |
| 48 × 1 | | | 14.56 | 0.072 | 6.33 | 0.032 | 0.357 | |
| 48 × 2 | | | 13.11 | 0.070 | 6.09 | 0.032 | 0.323 | |
| 48 × 3 | | | 12.37 | 0.068 | 6.21 | 0.031 | 0.350 | |
| 72 × control | | | 16.40 | 0.075 | 7.43 | 0.038 | 0.370 | |
| 72 × 1 | | | 16.21 | 0.073 | 7.06 | 0.037 | 0.350 | |
| 72 × 2 | | | 16.07 | 0.073 | 6.60 | 0.035 | 0.333 | |
| 72 × 3 | | | 16.09 | 0.072 | 6.19 | 0.034 | 0.330 | |
| 96 × control | | | 19.75 | 0.081 | 7.51 | 0.039 | 0.380 | |
| 96 × 1 | | | 18.58 | 0.076 | 7.42 | 0.037 | 0.353 | |
| 96 × 2 | | | 17.65 | 0.075 | 7.19 | 0.037 | 0.350 | |
| 96 × 3 | | | 17.16 | 0.076 | 7.25 | 0.036 | 0.350 | |
| *LSD (0.05)* | | | *0.5464* | *0.0023* | *0.4993* | *0.0023* | *0.0263* | |
| Aging durations | ** | ** | ** | ** | ** | ** | ** | ** |
| GABA priming | *NS* | *NS* | ** | ** | ** | ** | ** | ** |
| Aging durations × GABA priming | *NS* | *NS* | * | ** | ** | ** | ** | *NS* |

**: significant at $P \le 0.01$; *: significant at $0.01 < P \le 0.05$; *NS*: non-significant ($0.05 < P$).
SFA: saturated fatty acids.

(6)).

$$\text{EC } (\mu\text{S cm}^{-1}\text{ g}^{-1}) = \text{EC for each sample } (\mu\text{S cm}^{-1})/\text{weight of seed sample (g)} \quad (6)$$

## 2.8. Seed vigor-related traits

To determine vigor-related traits, the treated seeds were subjected to standard germination. The 25 seeds (with 8 replicates) were placed on Whatman filter paper in 15-cm Petri dishes. Then, the standard germination test was carried out at 25 °C in a germinator. The germinated seeds, defined by 2 mm radicle growth, were counted at 24-h intervals for 8 days (ISTA, 2011). At the end of the germination period, mean germination time (MGT) was calculated according to the following Eq. (Khajeh-Hosseini et al., 2009):

$$MGT = \Sigma n.t \, / \, \Sigma n \quad (1)$$

Where n: number of seeds newly germinated at each day, t: number of days from sowing.

## 2.9. Heterotrophic seedling growth (HSG)

Four replicates of each treatment were randomly selected to determine the components of HSG: the weight of mobilized seed reserve (WMSR) and seed reserve utilization efficiency (SRUE). The calculation methods were according to Eqs. (2) and (3) (Soltani et al., 2006). The rest of replicates were considered for fresh tissue-related traits.

$$\text{WMSR (mg seed}^{-1}) = \text{Initial seed dry weight} - \text{un-utilized seed dry weight} \quad (2)$$

$$\text{SRUE (\%)} = (\text{Seedling dry weight/WMSR}) \times 100 \quad (3)$$

As described by Seyyedi et al. (2015), initial seed dry weight was measured. Briefly, the samples were weight and then dried at 70 °C for 48 h. Other dry weight measurements were performed under the same conditions.

## 2.10. Determination of malondialdehyde content

Lipid peroxidation was estimated by measuring malondialdehyde (MDA) concentration. As suggested by Heath and Packer (1968), the seedlings were randomly selected and MDA content (nmol g$^{-1}$ FW) was determined using the thiobarbituric acid method.

## 2.11. Statistical analysis

All the data were analyzed from the analysis of variance using SAS 9.3 (SAS, 2011). To determine the statistical differences between treatments, the least significant difference (LSD) was performed ($P \le 0.05$).

# 3. Results

## 3.1. Electrolyte leakage, oil percentage and N and P contents

The individual effects of aging durations and GABA priming were significant on electrolyte leakage, oil percentage and N and P contents (Table 1). Also, the interaction of experimental factors was found to be

**Table 3**
Effects of aging durations and GABA priming on mono and polyunsaturated fatty acids composition in medicinal pumpkin seed.

| Experimental treatments | MUFA (g 100 g$^{-1}$) | | | | | PUFA (g 100 g$^{-1}$) | | SFA / MUFA | SFA / PUFA | SFA / UFA |
|---|---|---|---|---|---|---|---|---|---|---|
| | Myristoleic | Palmitoleic | Oleic | Paullinic | Erucic | Linoleic | Linolenic | | | |
| Aging durations (h) | | | | | | | | | | |
| 0 | 0.169 | 0.143 | 36.72 | 0.110 | 0.074 | 47.45 | 0.308 | 0.404 | 0.315 | 0.177 |
| 48 | 0.151 | 0.137 | 33.39 | 0.107 | 0.059 | 45.13 | 0.272 | 0.614 | 0.458 | 0.263 |
| 72 | 0.143 | 0.129 | 32.91 | 0.096 | 0.051 | 42.71 | 0.234 | 0.712 | 0.553 | 0.311 |
| 96 | 0.135 | 0.122 | 31.39 | 0.092 | 0.050 | 41.66 | 0.191 | 0.830 | 0.631 | 0.358 |
| *LSD (0.05)* | *0.0027* | *0.0022* | *0.3405* | *0.0010* | *0.0010* | *0.3013* | *0.0074* | *0.0183* | *0.0117* | *0.0070* |
| GABA priming (mmol L$^{-1}$) | | | | | | | | | | |
| Control | 0.147 | 0.130 | 33.37 | 0.099 | 0.056 | 43.40 | 0.235 | 0.679 | 0.525 | 0.296 |
| 1 | 0.149 | 0.132 | 33.43 | 0.101 | 0.058 | 44.06 | 0.250 | 0.654 | 0.499 | 0.283 |
| 2 | 0.150 | 0.135 | 33.52 | 0.103 | 0.060 | 44.88 | 0.260 | 0.624 | 0.468 | 0.267 |
| 3 | 0.152 | 0.134 | 34.08 | 0.102 | 0.060 | 44.60 | 0.260 | 0.604 | 0.464 | 0.263 |
| *LSD (0.05)* | *0.0027* | *0.0022* | *0.3405* | *0.0010* | *0.0010* | *0.3013* | *0.0074* | *0.0183* | *0.0117* | *0.0070* |
| Aging durations × GABA priming | | | | | | | | | | |
| 0 × control | 0.165 | 0.144 | 36.68 | 0.110 | 0.073 | 47.20 | 0.309 | 0.412 | 0.323 | 0.181 |
| 0 × 1 | 0.171 | 0.144 | 36.47 | 0.110 | 0.075 | 47.73 | 0.307 | 0.406 | 0.312 | 0.177 |
| 0 × 2 | 0.168 | 0.147 | 36.68 | 0.111 | 0.074 | 47.75 | 0.309 | 0.397 | 0.307 | 0.173 |
| 0 × 3 | 0.171 | 0.138 | 37.05 | 0.109 | 0.073 | 47.11 | 0.309 | 0.401 | 0.317 | 0.177 |
| 48 × control | 0.151 | 0.133 | 33.13 | 0.103 | 0.053 | 43.87 | 0.238 | 0.666 | 0.506 | 0.288 |
| 48 × 1 | 0.145 | 0.135 | 33.25 | 0.107 | 0.057 | 44.44 | 0.277 | 0.641 | 0.483 | 0.276 |
| 48 × 2 | 0.153 | 0.140 | 33.23 | 0.109 | 0.062 | 46.16 | 0.290 | 0.590 | 0.428 | 0.248 |
| 48 × 3 | 0.157 | 0.141 | 33.93 | 0.109 | 0.063 | 46.04 | 0.285 | 0.561 | 0.416 | 0.239 |
| 72 × control | 0.139 | 0.124 | 32.91 | 0.093 | 0.049 | 41.90 | 0.215 | 0.738 | 0.583 | 0.326 |
| 72 × 1 | 0.144 | 0.127 | 32.98 | 0.095 | 0.051 | 42.38 | 0.234 | 0.718 | 0.563 | 0.316 |
| 72 × 2 | 0.144 | 0.131 | 32.56 | 0.097 | 0.053 | 43.39 | 0.241 | 0.709 | 0.536 | 0.305 |
| 72 × 3 | 0.143 | 0.132 | 33.17 | 0.097 | 0.052 | 43.18 | 0.247 | 0.684 | 0.529 | 0.298 |
| 96 × control | 0.132 | 0.117 | 30.76 | 0.090 | 0.047 | 40.65 | 0.180 | 0.900 | 0.686 | 0.390 |
| 96 × 1 | 0.135 | 0.122 | 31.01 | 0.091 | 0.050 | 41.69 | 0.183 | 0.851 | 0.638 | 0.365 |
| 96 × 2 | 0.136 | 0.124 | 31.60 | 0.094 | 0.051 | 42.23 | 0.201 | 0.799 | 0.603 | 0.344 |
| 96 × 3 | 0.138 | 0.124 | 32.18 | 0.094 | 0.051 | 42.08 | 0.201 | 0.772 | 0.595 | 0.336 |
| *LSD (0.05)* | *0.0055* | *0.0044* | *0.6810* | *0.0020* | *0.0020* | *0.6026* | *0.0147* | *0.0367* | *0.0234* | *0.0139* |
| Aging durations | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| GABA priming | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| Aging durations × GABA priming | ** | ** | * | ** | ** | ** | ** | ** | ** | ** |

**: significant at $P \leq 0.01$; *: significant at $0.01 < P \leq 0.05$.
SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UFA: unsaturated fatty acids.

significant for these traits (Table 1).

Regardless of GABA priming, a significant decrease in oil percentage and N and P contents was observed with increasing seed aging durations, whereas electrolyte leakage significantly increased (Table 1). For instance, in 96-h-aged seeds, electrolyte leakage was up to 23.5% higher than 48-h-aged seeds (Table 1).

After imposing the artificial aging durations, GABA priming was considered as an effective technique to protect the nutrient reserves in aged seeds. For example, when 96-h-aged seeds were primed with 3 mmol L$^{-1}$ GABA, oil percentage was 20.6% higher than the same seeds primed with water (Table 1). Similarly, a significant increase in N contents by 13.3% as well as a significant decrease in electrolyte leakage by 21.3% were observed (Table 1).

### 3.2. FA-related traits

Saturated, monounsaturated, and polyunsaturated FA (SFA, MUFA, and PUFA) accounted for 21.5, 34.0, and 44.5% of total FA, respectively. Moreover, linoleic and oleic acids, which constituted 44.2 and 33.6% of total FA, respectively, were identified as the most important FA in medicinal pumpkin seeds (Tables 2 and 3).

Aging periods showed a significant effect on all SFA, MUFA, and PUFA (Tables 2 and 3). GABA priming showed significant influence on these traits, except for lauric and myristic acids. Moreover, the interaction between experimental factors was not statistically significant on lauric, myristic, and lignoceric acids (Table 3).

With increasing the intensity of seed deterioration severity, SFA percentage significantly increased, while MUFA and PUFA percentage decreased. Moreover, SFA/MUFA, SFA/PUFA, and SFA/UFA significantly increased due to accelerated aging process (Tables 2 and 3). For instance, when the aging duration increased from 0 to 96 h, oleic and linoleic acids decreased by 14.5 and 12.2%, respectively (Table 3).

Considering the accelerated aging, GABA priming alleviated the negative effects of seed deterioration on FA composition. For instance, under 96-h aging duration, GABA priming at 2 mmol L$^{-1}$ decreased SFA/MUF, SFA/PUFA, and SFA/UFA by 11.2, 12.1, and 11.8%, respectively, compared with control (Table 3). Similarly, the values were 14.2, 13.3, and 13.9%, respectively, when 3 mmol L$^{-1}$ GABA was applied.

### 3.3. Seedling-related traits

The individual and interaction effects of aging duration and GABA priming were significant on all seedling traits except for germination percentage (Table 4).

Irrespective of priming treatment, an increase in aging durations significantly increased MGT and MDA content, while seedling dry weight, WMSR, and SRUE decreased (Table 4). For instance, MGT increased by 11.3% after imposing 96-h artificial aging, compared with 48-h artificial aging. Under similar conditions, WMSR and SRUE reduced by 13.4 and 20.2%, respectively (Table 4).

Despite the harmful effects of seed deterioration on seedling-related indices, GABA priming mitigated these effects. For example, after the seeds were aged for 96 h, GABA priming at 3 mmol L$^{-1}$ decreased MDA content by 22.3% and increased seedling dry weight by 41.1%, compared with water priming (Table 4). Similarly, a considerable increase in SRUE (up to 39.1%) was recorded (Table 4).

**Table 4**
Effects of aging durations and GABA priming on seedling-related traits in medicinal pumpkin.

| Experimental treatments | Germination (%) | MGT (day) | Seedling dry weight (mg) | WMSR (mg seed$^{-1}$) | SRUE (%) | MDA content (nmol g$^{-1}$ FW) |
|---|---|---|---|---|---|---|
| Aging durations (h) | | | | | | |
| 0 | 91.38 | 4.18 | 111.56 | 122.55 | 91.02 | 11.31 |
| 48 | 92.13 | 4.32 | 101.59 | 120.98 | 83.95 | 12.84 |
| 72 | 91.50 | 4.60 | 87.92 | 119.66 | 73.44 | 14.33 |
| 96 | 91.13 | 4.81 | 73.10 | 118.88 | 61.46 | 15.29 |
| *LSD (0.05)* | *1.4034* | *0.1582* | *1.1880* | *0.2091* | *0.9676* | *0.7021* |
| GABA priming (mmol L$^{-1}$) | | | | | | |
| Control | 91.63 | 4.59 | 86.42 | 119.66 | 72.05 | 15.05 |
| 1 | 91.63 | 4.52 | 91.23 | 120.36 | 75.66 | 13.99 |
| 2 | 91.38 | 4.41 | 95.38 | 120.64 | 78.95 | 12.47 |
| 3 | 91.50 | 4.39 | 101.14 | 121.41 | 83.22 | 12.26 |
| *LSD (0.05)* | *1.4034* | *0.1582* | *1.1880* | *0.2091* | *0.9676* | *0.7021* |
| Aging durations × GABA priming | | | | | | |
| 0 × control | | 4.22 | 109.53 | 121.99 | 89.79 | 12.30 |
| 0 × 1 | | 4.18 | 110.75 | 122.40 | 90.48 | 11.24 |
| 0 × 2 | | 4.17 | 112.40 | 122.62 | 91.67 | 11.12 |
| 0 × 3 | | 4.15 | 113.54 | 123.20 | 92.15 | 10.59 |
| 48 × control | | 4.46 | 96.72 | 119.94 | 80.64 | 14.16 |
| 48 × 1 | | 4.38 | 99.36 | 120.81 | 82.24 | 14.01 |
| 48 × 2 | | 4.26 | 103.78 | 121.08 | 85.72 | 11.38 |
| 48 × 3 | | 4.19 | 106.49 | 122.10 | 87.21 | 11.79 |
| 72 × control | | 4.70 | 77.89 | 118.74 | 65.59 | 16.13 |
| 72 × 1 | | 4.61 | 85.23 | 119.43 | 71.37 | 15.09 |
| 72 × 2 | | 4.55 | 90.85 | 119.79 | 75.84 | 13.09 |
| 72 × 3 | | 4.55 | 97.72 | 120.70 | 80.96 | 13.01 |
| 96 × control | | 4.98 | 61.54 | 117.98 | 52.17 | 17.60 |
| 96 × 1 | | 4.89 | 69.58 | 118.81 | 58.56 | 15.63 |
| 96 × 2 | | 4.68 | 74.48 | 119.06 | 62.55 | 14.30 |
| 96 × 3 | | 4.68 | 86.81 | 119.64 | 72.55 | 13.65 |
| *LSD (0.05)* | | *0.3163* | *2.3759* | *0.4182* | *1.9352* | *1.4041* |
| Aging durations | *NS* | ** | ** | ** | ** | ** |
| GABA priming | *NS* | * | ** | ** | ** | ** |
| Aging durations × GABA priming | *NS* | * | * | ** | ** | * |

**: significant at $P \leq 0.01$; *: significant at $0.01 < P \leq 0.05$; *NS*: non-significant ($0.05 < P$).
MGT: mean germination time; WMSR: weight of mobilized seed reserve; SRUE: seed reserve utilization efficiency; MDA: malondialdehyde.

### 3.4. Correlation between biochemical parameters

According to Fig. 1, there was a significant correlation between SFA/MUFA with MGT ($r^2 = 0.90$), WMSR ($r^2 = 0.92$), and SRUE ($r^2 = 0.87$). The same response was also observed between SFA/PUFA with MGT ($r^2 = 0.93$), WMSR ($r^2 = 0.93$), and SRUE ($r^2 = 0.89$). Moreover, the correlation between oleic or linoleic acid with MGT ($r^2 = 0.77$ and 0.93) was found to be negative (Fig. 2).

Based on the results, EC of soaked seeds showed a negative correlation with oleic ($r^2 = 0.93$) or linoleic ($r^2 = 0.92$) acid. The same correlation was observed between MDA content with oleic ($r^2 = 0.63$) or linoleic ($r^2 = 0.82$) acid (Fig. 3).

## 4. Discussion

Seed aging, as a relatively progressive and irreversible process (Hajiabbasi et al., 2020; Mohaddes Ardebili et al., 2019), involves a series of biochemical mechanisms that considerably reduces seedling establishment potential (Demir and Mavi, 2008; Oenel et al., 2017). On the other hand, due to the poor accuracy of standard germination test to evaluate seedling performance, especially under adverse soil conditions (Noli et al., 2008; Seyyedi et al., 2017), the importance of seed vigor tests such as accelerated aging are particularly emphasized (Eisvand et al., 2010; Woltz and TeKrony, 2001).

The accelerated aging test is one of the specialized techniques used to estimate seedling vigor potential in response to different environmental conditions and provide important information related to adaptation mechanisms (Mavi and Demir, 2007; Oenel et al., 2017). In this regard, a significant reduction in seedling dry weight, WMSR, and SRUE has been observed due to artificially accelerated aging (Seyyedi et al., 2018). Similarly, degraded cell membrane (Hwangbo et al., 2003) and impaired FA structure (Balešević-Tubić et al., 2007) have been observed.

When plants are exposed to unfavorable environmental conditions, self-adaptive mechanisms are induced by endogenous signals to stabilize cellular processes (Chen et al., 2009; He et al., 2019; Koocheki and Seyyedi, 2019). However, the higher intensity of abiotic stresses may lead to a gradual decrease in the efficiency of molecular signals (Ebrahimian et al., 2019; Tamás et al., 2006). On the other hand, the deleterious effects of environmental stresses, especially heat and drought, can be simultaneously exacerbated by oxidative stresses (Sharma and Dubey, 2005; Talaat et al., 2015). This can be recognized as one of the main mechanisms associated with seed deterioration caused by prolonged aging. In this context, MDA, a molecular marker of seed aging, is known as a lipid peroxidation product that seriously affects seed vigor and viability (Parkhey et al., 2012). It has been found that prolonging the aging process significantly increased MGT and MDA content in sunflower (Bailly et al., 1996).

GABA, a four-carbon non-protein amino acid (Hu et al., 2015) is endogenously synthesized in plants to activate the defense responses and regulate the biochemical functions, especially under stressful conditions (Bor et al., 2009; Malekzadeh et al., 2014; Serraj et al., 1998). After exposure to salinity stress, increasing GABA accumulation in medicinal pumpkin leaves significantly reduced lipid peroxidation and improved antioxidant enzymes activities (Nejad-Alimoradi et al., 2019). The positive role of GABA priming in enhancing tolerance to osmotic stress in black pepper varieties has been observed (Vijayakumari and Puthur, 2016). As reported by Cheng et al. (2018), the exogenous application of GABA alleviated salt-induced damage during the germination process in white clover trough activating antioxidant defense and reducing oxidative stress. Similarly, GABA priming has been considered as an efficient technique to mitigate the negative effects of heat

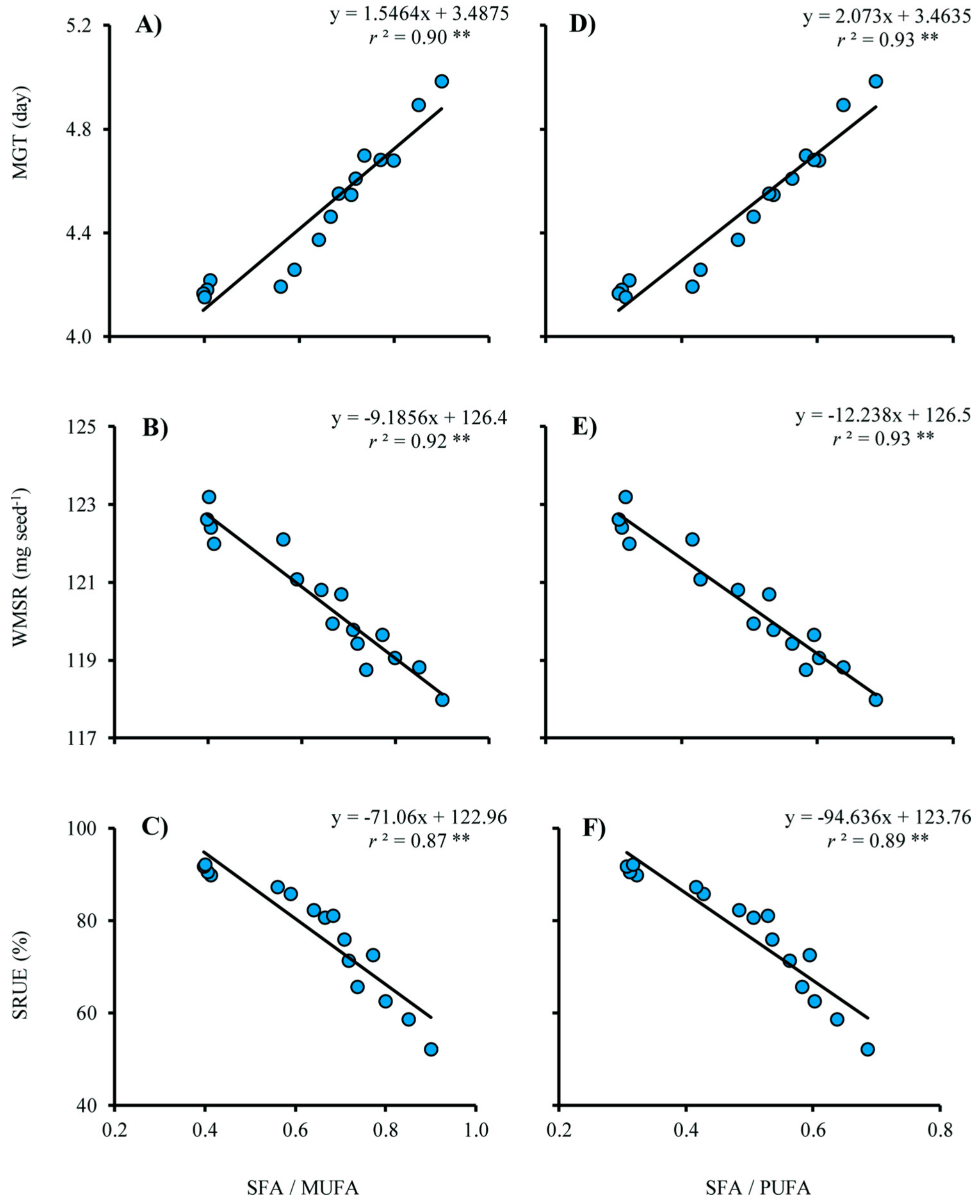


**Fig. 1.** Correlation between SFA/MUFA with MGT (A), WMSR (B), and SRUE (C), and between SFA/PUFA with MGT (D), WMSR (E), and SRUE (F) after performing the accelerated aging test. **: statistical differences at $P \leq 0.01$.
SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; MGT: mean germination time; WMSR: weight of mobilized seed reserve; SRUE: seed reserve utilization efficiency.

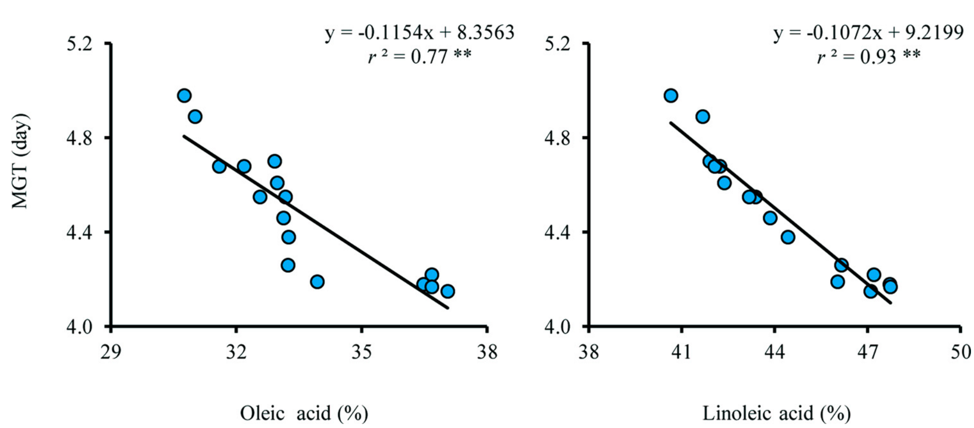


**Fig. 2.** Correlation between oleic and linoleic acids with MGT after performing the accelerated aging test. **: statistical differences at $P \leq 0.01$.
MGT: mean germination time.

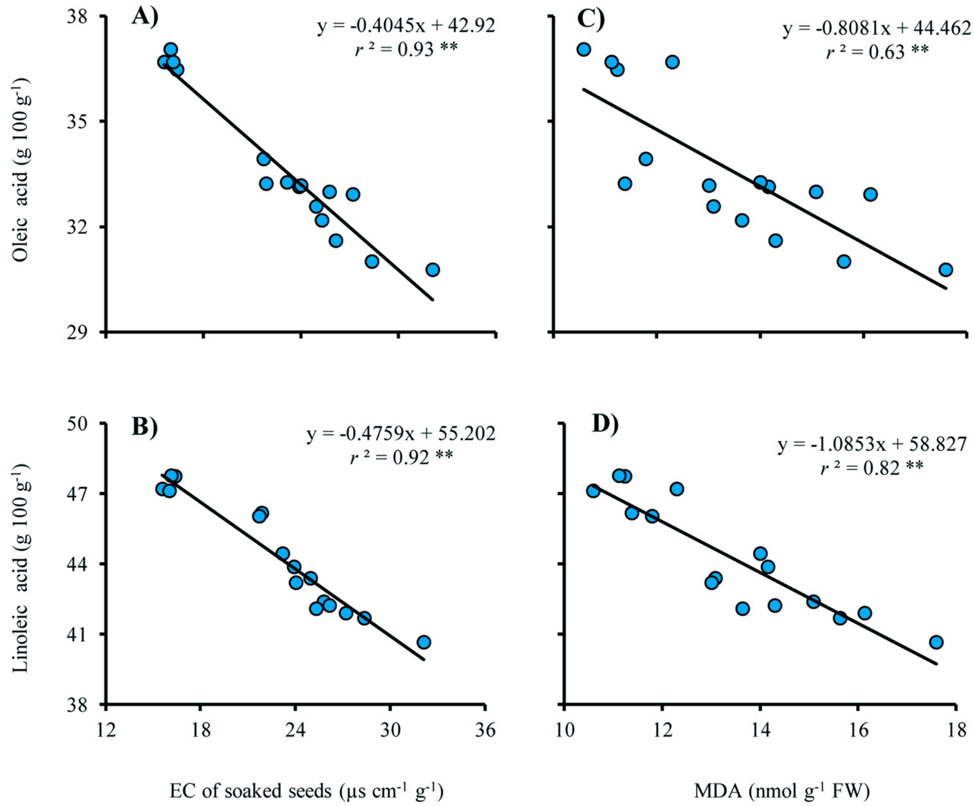


**Fig. 3.** Correlation between electrolyte leakage with oleic acid (A) and linoleic acid (B) as well as between MDA content with oleic acid (C) and linoleic acid (D) after performing the accelerated aging test. **: statistical differences at $P \leq 0.01$.
EC: electrical conductivity; MDA: malondialdehyde.

and drought stresses (Krishnan et al., 2013; Li et al., 2016).

Oil content and FA composition are found to be the most important factors for assessing seedling vigor in oilseeds (González Belo et al., 2014; Seyyedi et al., 2015). Considering the high oil content in medicinal pumpkin seeds up to 45.40 (Lazos, 1986) or 48.15% (Habibi et al., 2014), it seems that seed germination and seedling growth are highly dependent on oil quality and FA profile.

As mentioned already, SFA/MUFA, SFA/PUFA, and SFA/UFA significantly increased as a result of prolonged aging. Moreover, with increasing MDA content, oleic and linoleic acids considerably decreased. The oxidized seed reserves are recognized as one of the most important consequences associated with accelerated seed aging process (Oenel et al., 2017; Seyyedi et al., 2018). Accordingly, reducing the oil percentage after imposing a 96-h aging duration, compared to a 48-h aging duration, can justify the mechanism of reserves oxidation. On the other hand, the oil content considerably reduced along with the destruction of SFA, MUFA, and PUFA structure. But it is noteworthy that SFA is probably less oxidized than MUFA and PUFA. This may be considered as one reason why SFA/MUFA or SFA/PUFA gradually increases as a result of the accelerated aging process.

From the results obtained here, SFA/MUFA showed a negative correlation with WMSR and SRUE. The same response was found between SFA/PUFA with WMSR and SRUE. HSG, which depends on the quality of seed reserves (Brown and Huber, 1988; Erbaş et al., 2016; Seyyedi et al., 2018), is defined by two functionally different components: WMSR and SRUE (Soltani et al., 2006). Understanding the biochemical mechanisms during the heterotrophic growth process can be useful in predicting successful seedling establishment, especially under adverse environmental conditions (Elamrani et al., 1992; Seyyedi et al., 2015). Therefore, it seems that after decreasing oil content and increasing SFA/MUFA or SFA/PUFA, seed reserve depletion percentage declines during the germination process, which eventually leads to reduced SRUE and weakened seedling vigor.

## 5. Conclusion

According to the results, the increase in accelerated aging durations significantly increased MGT, MDA, electrolyte leakage, and SFA/UFA, while WMSR and SRUE decreased. Nonetheless, GABA priming mitigated the harmful effects of seed aging on HSG through decreasing MDA content and increasing oil percentage. These results may highlight the crucial role of exogenous GABA as a cellular regulator through activating the defense-related responses and regulating the biochemical metabolisms. Furthermore, a positive correlation between SFA/MUFA or SFA/PUFA with MGT, as well as a negative correlation between MDA content with oleic or linoleic acid can confirm the crucial importance of FA composition in controlling seed deterioration. The findings suggest SFA/MUFA and SFA/PUFA as important biochemical traits for assessing the seed vigor of medicinal pumpkin and other oilseed crops.

## CRediT authorship contribution statement

**Reza Tavakkol Afshari:** Methodology, Supervision, Writing - review & editing. **Seyyed Mohammad Seyyedi:** Investigation, Software, Formal analysis, Writing - original draft.

## Declaration of competing interest

The authors declare no conflict of interest.

## References

AOAC, 2000. Official Methods of Analysis, 17th ed. Association of Official Analytical Chemists, Gaithersburg, Maryland, USA.
Bailly, C., Benamar, A., Corbineau, F., Côme, D., 1996. Changes in malondialdehyde

content and in superoxide dismutase, catalase and glutathione reductase activities in sunflower seeds as related to deterioration during accelerated aging. Physiol. Plant. 97, 104–110.
Balešević-Tubić, S., Tatić, M., Miladinović, J., Pucarević, M., 2007. Changes of fatty acids content and vigor of sunflower seed during natural aging. Helia 30, 61–68.
Bor, M., Seckin, B., Ozgur, R., Yılmaz, O., Ozdemir, F., Turkan, I., 2009. Comparative effects of drought, salt, heavy metal and heat stresses on gamma-aminobutryric acid levels of sesame (*Sesamum indicum* L.). Acta Physiol. Plant. 31, 655–659.
Brown, C.S., Huber, S.C., 1988. Reserve mobilization and starch formation in soybean (*Glycine max*) cotyledons in relation to seedling growth. Physiol. Plant. 72, 518–524.
Chen, X.Y., Ding, X., Xu, S., Wang, R., Xuan, W., Cao, Z.Y., Chen, J., Wu, H.H., Ye, M.B., Shen, W.B., 2009. Endogenous hydrogen peroxide plays a positive role in the upregulation of heme oxygenase and acclimation to oxidative stress in wheat seedling leaves. Journal of Integr. Plant Biol. 51, 951–960.
Cheng, B., Li, Z., Liang, L., Cao, Y., Zeng, W., Zhang, X., Ma, X., Huang, L., Nie, G., Liu, W., Peng, Y., 2018. The γ-aminobutyric acid (GABA) alleviates salt stress damage during seeds germination of white clover associated with Na+/K+ transportation, dehydrins accumulation, and stress-related genes expression in white clover. Int. J. Mol. Sci. 19, 2520.
Demir, I., Mavi, K., 2008. Controlled deterioration and accelerated aging tests to estimate the relative storage potential of cucurbit seed lots. HortScience 43, 1544–1548.
Dutra, A.S., Vieira, R.D., 2006. Accelerated ageing test to evaluate seed vigor in pumpkin and zucchini seeds. Seed Sci. Technol. 34, 209–214.
Ebrahimian, E., Seyyedi, S.M., Bybordi, A., Damalas, C.A., 2019. Seed yield and oil quality of sunflower, safflower, and sesame under different levels of irrigation water availability. Agric. Water Manag. 218, 149–157.
Eisvand, H.R., Tavakkol Afshari, R., Sharifzadeh, F., Maddah Arefi, H., Hesamzad-e-Hejazi, S., M, 2010. Effects of hormonal priming and drought stress on activity and isozyme profiles of antioxidant enzymes in deteriorated seed of tall wheatgrass (*Agropyron elongatum* Host). Seed Sci. Technol. 38, 280–297.
Elamrani, A., Raymond, P., Saglio, P., 1992. Nature and utilization of seed reserves during germination and heterotrophic growth of young sugar beet seedlings. Seed Sci. Res. 2, 1–8.
Ellis, R.H., Hong, T.D., Roberts, E.H., Soetisna, U., 1991. Seed storage behaviour in *Elaeis guineensis*. Seed Sci. Res. 1, 99–104.
Erbaş, S., Tonguç, M., Karakurt, Y., Şanli, A., 2016. Mobilization of seed reserves during germination and early seedling growth of two sunflower cultivars. J. Appl. Bot. Food Qual. 89, 217–222.
Farhoosh, R., Tavassoli-Kafrani, M.H., Sharif, A., 2011. Antioxidant activity of the fractions separated from the unsaponifiable matter of bene hull oil. Food Chem. 126, 583–589.
Flakelar, C.L., Doran, G.S., Howitt, J.A., Luckett, D.J., Prenzler, P.D., 2018. Effects of storage temperature and duration on bioactive concentrations in the seed and oil of *Brassica napus* (Canola). Eur. J. Lipid Sci. Technol. 120, 1700335.
Ghaderi-Far, F., Soltani, A., Sadeghipour, H.R., 2011. Changes in seed quality during seed development and maturation in medicinal pumpkin (*Cucurbita pepo*subsp. pepo.*Convar*. Pepo var. *styriaca* Greb). J. Herb. Spice. Med. Plant. 17, 249–257.
Gidrol, X., Serghini, H., Noubhani, A., Mocouot, B., Mazliak, P., 1989. Biochemical changes induced by accelerated aging in sunflower seeds. I. Lipid peroxidation and membrane damage. Physiol. Plant. 76, 591–597.
González Belo, R., Tognetti, J., Benech-Arnold, R., Izquierdo, N.G., 2014. Germination responses to temperature and water potential as affected by seed oil composition in sunflower. Ind. Crop. Prod. 62, 537–544.
Habibi, A., Heidari, G., Sohrabi, Y., Badakhshan, H., Mohammadi, K., 2014. Grain quality of medicinal pumpkin (*Cucurbita pepo* subsp. Pepo var. *styriaca*) affected by type and amount of fertilizers. J. Essent. Oil Bear. Plant. 17, 533–537.
Hacisalihoglu, G., 2008. Responses of three switchgrass (*Panicum virgatum* L.) cultivars to seed priming and differential aging conditions. Acta Agr. Scand. S.B- Soil Plant Sci. 58, 280–284.
Hajiabbasi, M., Tavakkol Afshari, R., Abbasi, A., 2015. Effects of salicylic acid and ethylene on germination improvement of deteriorated seed of *Glycine max* (L.). Crop Res. 50, 86–94.
Hajiabbasi, M., Tavakkol Afshari, R., Abbasi, A., Seyyedi, S.M., 2020. Germination and gene expression as affected by aminocyclopropane-1-carboxylic acid in deteriorated soybean seed. J. Crop Improv. https://doi.org/10.1080/15427528.2020.1737612.
Hang, N.T., Lin, Q., Liu, L., Liu, X., Liu, S., Wang, W., Li, L., He, N., Liu, Z., Jiang, L., Wan, J., 2015. Mapping QTLs related to rice seed storability under natural and artificial aging storage conditions. Euphytica 203, 673–681.
He, Y., Ye, Z., Ying, Q., Ma, Y., Zang, Y., Wang, H., Yu, Y., Zhu, Z., 2019. Glyoxylate cycle and reactive oxygen species metabolism are involved in the improvement of seed vigor in watermelon by exogenous $GA_3$. Sci. Hortic. 247, 184–194.
Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts. I-kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125, 189–198.
Hu, X., Xu, Z., Xu, W., Li, J., Zhao, N., Zhou, Y., 2015. Application of γ-aminobutyric acid demonstrates a protective role of polyamine and GABA metabolism in muskmelon seedlings under Ca $(NO_3)_2$ stress. Plant Physiol. Biochem. 92, 1–10.
Hwangbo, J.-K., Kim, J.-S., Lim, J.-H., Baek, M.-H., Chung, B.-Y., 2003. Alterations in seed vigour and viability of soybean related with accelerated seed aging and low dose gamma irradiation. Korean J. Crop Sci. 48, 334–338.
Islam, M.O., Islam, M.S., Azad-Ud-Doula Prodhan, A.K.M., 2006. Seed germination and seedling growth of brinjal, tomato and chilli treated with GA3 and GABA. J. Bangladesh Agric. Univ. 4, 43–49.
ISTA, 2011. International Rules for Seed Testing. The International Seed Testing Association, Bassersdorf, Switzerland.
Jisha, K.C., Puthur, J.T., 2016. Seed priming with beta-amino butyric acid improves abiotic stress tolerance in rice seedlings. Rice Sci. 23, 242–254.
Karpagam, T., Varalakshmi, B., Bai, J.S., Gomathi, S., 2011. Effect of different doses of *Cucurbita pepo* linn extract as an anti-Inflammatory and analgesic nutraceautical agent on inflamed rats. Int. J. Pharm. Res. Dev. 3, 184–192.
Khajeh-Hosseini, M., Lomholt, A., Matthews, S., 2009. Mean germination time in the laboratory estimates the relative vigour and field performance of commercial seed lots of maize (*Zea mays* L.). Seed Sci. Technol. 37, 446–456.
Koocheki, A., Seyyedi, S.M., 2019. Mother corm origin and planting depth affect physiological responses in saffron (*Crocus sativus* L.) under controlled freezing conditions. Ind. Crop. Prod. 138, 111468.
Koocheki, A., Rezvani Moghaddam, P., Seyyedi, S.M., 2019. Saffron-pumpkin/watermelon: a clean and sustainable strategy for increasing economic land equivalent ratio under limited irrigation. J. Clean. Prod. 208, 1327–1338.
Krishnan, S., Laskowski, K., Shukla, V., Merewitz, E.B., 2013. Mitigation of drought stress damage by exogenous application of a non-protein amino acid γ–aminobutyric acid on perennial ryegrass. J. Am. Soc. Hortic. Sci. 138, 358–366.
Lazos, E.S., 1986. Nutritional, fatty acid, and oil characteristics of pumpkin and melon seeds. J. Food Sci. 51, 1382–1383.
Li, Z., Peng, Y., Huang, B., 2016. Physiological effects of γ-aminobutyric acid application on improving heat and drought tolerance in creeping bentgrass. J. Am. Soc. Hortic. Sci. 141, 76–84.
Malekzadeh, P., Khara, J., Heydari, R., 2014. Alleviating effects of exogenous Gamma-aminobutiric acid on tomato seedling under chilling stress. Physiol. Mol. Biol. Plants 20, 133–137.
Mavi, K., Demir, I., 2007. Controlled deterioration and accelerated aging tests predict relative seedling emergence potential of melon seed lots. HortScience 42, 1431–1435.
Mohaddes Ardebili, Z., Abbaspour, H., Tavakkol Afshari, R., Nabavi Kalat, S.M., 2019. Evaluation of germination and antioxidant activity in $GA_3$-primed deteriorated wheat seed. Russ. J. Plant Physiol. 66, 958–965.
Mohammadi, H., Soltani, A., Sadeghipour, H.R., Zeinali, E., 2012. Effects of seed aging on subsequent seed reserve utilization and seedling growth in soybean. Int. J. Plant Prod. 5, 65–70.
Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36.
Nazari, R., Parsa, S., Tavakkol Afshari, R., Mahmoodi, S., Seyyedi, S.M., 2020. Salicylic acid priming before and after accelerated aging process increases seedling vigor in aged soybean seed. J. Crop Improv. 34, 218–237.
Nejad-Alimoradi, F., Nasibi, F., Kalantari, K.M., 2019. 24-epibrassinolide pre-treatment alleviates the salt-induced deleterious effects in medicinal pumpkin (*Cucurbita pepo*) by enhancement of GABA content and enzymatic antioxidants. S. Afr. J. Bot. 124, 111–117.
Noli, E., Casarini, E., Urso, G., Conti, S., 2008. Suitability of three vigour test procedures to predict field performance of early sown maize seed. Seed Sci. Technol. 36, 168–176.
Oenel, A., Fekete, A., Krischke, M., Faul, S.C., Gresser, G., Havaux, M., Mueller, M.J., Berger, S., 2017. Enzymatic and non-enzymatic mechanisms contribute to lipid oxidation during seed aging. Plant Cell Physiol. 58, 925–933.
Parkhey, S., Naithani, S.C., Keshavkant, S., 2012. ROS production and lipid catabolism in desiccating *Shorea robusta* seeds during aging. Plant Physiol. Biochem. 57, 261–267.
Rabrenović, B.B., Dimić, E.B., Novaković, M.M., Tešević, V.V., Basić, Z.N., 2014. The most important bioactive components of cold pressed oil from different pumpkin (*Cucurbita* pepo L.) seeds. LWT Food Sci. Technol. 55, 521–527.
Rezaei-Chiyaneh, E., Seyyedi, S.M., Ebrahimian, E., Moghaddam, S.S., Damalas, C.A., 2018. Exogenous application of gamma-aminobutyric acid (GABA) alleviates the effect of water deficit stress in black cumin (*Nigella sativa* L.). Ind. Crop. Prod. 112, 741–748.
Sabudak, T., 2007. Fatty acid composition of seed and leaf oils of pumpkin, walnut, almond, maize, sunflower and melon. Chem. Nat. Compd. 43, 465–467.
Sedghi, M., Gholipouri, A., Sharifi, R.S., 2008. Gama-tocopherol accumulation and floral differentiation of medicinal pumpkin (*Cucurbita pepo* L.) in response to plant growth regulators. Not. Bot. Horti Agrobot. Cluj. 36, 80–84.
Serraj, R., Shelp, B.J., Sinclair, T.R., 1998. Accumulation of γ-aminobutyric acid in nodulated soybean in response to drought stress. Physiol. Plant. 102, 79–86.
Seyyedi, S.M., Khajeh-Hosseini, M., Rezvani Moghaddam, P., Shahandeh, H., 2015. Effects of phosphorus and seed priming on seed vigor, fatty acids composition and heterotrophic seedling growth of black seed (*Nigella sativa* L.) grown in a calcareous soil. Ind. Crop. Prod. 74, 939–949.
Seyyedi, S.M., Rezvani Moghaddam, P., Khajeh-Hossieni, M., Shahandeh, H., 2017. Improving of seed quality of black seed (*Nigella sativa* L.) by increasing seed phosphorus content in a calcareous soil. J. Plant Nutr. 40, 197–206.
Seyyedi, S.M., Tavakkol Afshari, R., Daneshmandi, M.S., 2018. The relationships between fatty acids and heterotrophic seedling growth in winter canola cultivars during accelerated seed aging process. S. Afr. J. Bot. 119, 353–361.
Shailasree, S., Sarosh, B.R., Vasanthi, N.S., Shetty, H.S., 2001. Seed treatment with β-aminobutyric acid protects *Pennisetum glaucum* systemically from *Sclerospora graminicola*. Pest Manag. Sci. 57, 721–728.
Sharma, P., Dubey, R.S., 2005. Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. Plant Growth Regul. 46, 209–221.
Shelp, B.J., Bown, A.W., McLean, M.D., 1999. Metabolism and functions of gamma-aminobutyric acid. Trends Plant Sci. 4, 446–452.
Sim, H.S., Jang, B.C., Park, H.M., Jeng, B.Y., Oh, M.J., 2008. Isolation of cucurbitacin E from sprouted pumpkin seed and analysis of its anti-cancer and anti-inflammatory activities. J. Korean Soc. Food Sci. Nutr. 37, 834–840.
Simić, A., Sredojević, S., Todorović, M., Đukanović, L., Radenović, C., 2004. Studies on the relationship between the content of total phenolics in exudates and germination ability of maize seed during accelerated aging. Seed Sci. Technol. 32, 213–218.

Soltani, A., Gholipoor, M., Zeinali, E., 2006. Seed reserve utilization and seedling growth of wheat as affected by drought and salinity. Environ. Exp. Bot. 55, 195–200.
Song, H., Xu, X., Wang, H., Wang, H., Tao, Y., 2010. Exogenous γ-aminobutyric acid alleviates oxidative damage caused by aluminium and proton stresses on barley seedlings. J. Sci. Food Agric. 90, 1410–1416.
Talaat, N.B., Shawky, B.T., Ibrahim, A.S., 2015. Alleviation of drought-induced oxidative stress in maize (*Zea mays* L.) plants by dual application of 24-epibrassinolide and spermine. Environ. Exp. Bot. 113, 47–58.
Tamás, L., Huttová, J., Mistrík, I., Šimonovic, M., Široká, B., 2006. Aluminium-induced drought and oxidative stress in barley roots. J. Plant Physiol. 163, 781–784.
Tatić, M., Balešević-Tubić, S., Đorđević, V., Nikolić, Z., Đukić, V., Vujaković, M., Cvijanovic, G., 2012. Soybean seed viability and changes of fatty acids content as affected by seed aging. Afr. J. Biotechnol. 11, 10310–10316.
Trawatha, S.E., TeKrony, D.M., Hildebrand, D.F., 1995. Relationship of soybean seed quality to fatty acid and C6-aldehyde levels during storage. Crop Sci. 35, 1415–1422.
Vijayakumari, K., Puthur, J.T., 2016. γ-aminobutyric acid (GABA) priming enhances the osmotic stress tolerance in *Piper nigrum* Linn. Plants subjected to PEG-induced stress. Plant Growth Regul. 78, 57–67.
Woltz, J.M., TeKrony, D.M., 2001. Accelerated aging test for corn seed. Proc. Int. Symp. Seed-up Serv. Technol. Railw. Maglev Syst. Stech 23, 21–34.
Yavuz, D., Yavuz, N., Seymen, M., Türkmen, Ö., 2015. Evapotranspiration, crop coefficient and seed yield of drip irrigated pumpkin under semi-arid conditions. Sci. Hortic. 197, 33–40.
Yoshinari, O., Sato, H., Igarashi, K., 2009. Anti-diabetic effects of pumpkin and its components, trigonelline and nicotinic acid, on Goto-Kakizaki rats. Biosci. Biotechnol. Biochem. 73, 103–1041.
Younis, Y.M.H., Ghirmay, S., Al-Shihry, S.S., 2000. African *Cucurbita pepo* L.: properties of seed and variability in fatty acid composition of seed oil. Phytochem 54, 71–75.
Zheng, M., Tao, Y., Hussain, S., Jiang, Q., Peng, S., Huang, J., Cui, K., Nie, L., 2016. Seed priming in dry direct-seeded rice: consequences for emergence, seedling growth and associated metabolic events under drought stress. Plant Growth Regul. 78, 167–178.