



# The relationships between fatty acids and heterotrophic seedling growth in winter canola cultivars during accelerated seed aging process

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## ABSTRACT

Oil content and fatty acid composition are important traits for assessing and modeling germination in oilseeds. On the other hand, seed germination and seedling growth are affected by the weight of mobilized seed reserves and the conversion efficiency of mobilized seed reserve to seedlings tissues. In order to examine seeds physiological aspects and heterotrophic seedlings growth of 12 winter canola cultivars (Fornax, Karaj 1, Karaj 2, Karaj 3, L73, L201, Licord, Modena, Okapi, Opera, SLM046, and Zarfam) as related to seed reserves, series of laboratory experiments were conducted in Ferdowsi University of Mashhad, Mashhad, Iran. At the first and the second stages (before and after the performing accelerated aging test), significant differences were found among cultivars in terms of oil percentage. From the results, N, P and crude protein contents in seeds were decreased due to accelerated aging. Moreover, a positive correlation was found between electrical conductivity (EC) of seed soaking solution and mean germination time (MGT). By contrast, the correlation between weight of mobilized seed reserve (WMSR) and seed reserve utilization efficiency (SRUE) with MGT were significantly negative. Accelerated aging in canola seeds increased saturated fatty acids percentage and reduced unsaturated fatty acids percentage. There was a positive and significant relationship between oleic acid and SRUE. By contrast, the relationship between linolenic or linoleic acid and SRUE was found to be negative. According to the results, it seems that seed vigor and heterotrophic seedlings growth in canola highly depends on fatty acids composition.

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## 1. Introduction

Canola (*Brassica napus* L.), one of the most important oilseed crops in the world, is cultivated in many regions of Iran (Mousavi-Avval et al., 2011). The harvested area and seed production have been estimated about 40,200 ha and 58,700 tons, respectively (Agricultural statistics, 2016). There are many canola genotypes with different oil content and fatty acids composition adapted to diverse ecological conditions (Burton et al., 2004). Depending on the climate conditions and genotype, canola oil content varies from 21 to 46% (Sana et al., 2003; Sharafi et al., 2015).

Despite the relatively widespread cultivation of canola in most regions of Iran, the canola average yield is relatively low; so that according to available statistics, the average yield in irrigated and rainfed fields have been recorded as 1.53 and 1.09 t. ha<sup>-1</sup>, respectively (Agricultural statistics, 2016). In this regard, one of the most important problems in canola production, especially in arid and semiarid regions, where low soil moisture is the most serious issue, is poor seedling emergence and establishment (Zhang et al., 2012; Torabi and Rabii, 2013). Therefore,

depending on climatic conditions and facilities, desired genotypes should be selected with higher vigor germination rate. On the other hand, due to prevailing the arid and semi-arid climate in the most regions of Iran (Modarres et al., 2007), seed vigor must be considered as the main criteria for the identification and selection of canola genotypes under such stressful conditions.

Among factors affecting seed quality, seed vigor and germination are considered as physiological aspects of seed (Ellis, 1992; Marcos-Filho, 2015). On the other hand, heterotrophic seedling growth is an important indicator in determining seed vigor (Seyyedi et al., 2015). Based on physiological mechanisms during seed germination, heterotrophic seedling growth can be described by two components: (1) 'the weight of mobilized seed reserves, and (2) the conversion efficiency of mobilized seed reserve to seedling tissue, i.e. production of seedling dry matter per unit of usage of seed reserve' (Soltani et al., 2006). Accordingly, seed reserves are mainly affected by two factors: initial seed weight and the fraction of seed reserve which is mobilized (Seyyedi et al., 2015). Hence, determining seed quality and investigating factors affecting seed vigor are so important.

Heterotrophic seedling growth which directly affects seeds vigor may predict germination and seedling establishment, especially under stressful environments (Seyyedi et al., 2015). In oilseed crops, seed

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germination and heterotrophic seedling growth are affected by seed reserves, especially oil content and fatty acid composition (Priestley and Leopold, 1983; Wang et al., 2001; Parkhey et al., 2012). On the other hand, the vegetable oils with a higher percentage of polyunsaturated fatty acids, especially linolenic acid, are more sensitive to oxidation (Mohdaly et al., 2011; Rezvani Moghaddam and Seyyedi, 2016). During germination of oilseeds, storage lipids are metabolized to supply the required energy for cell reactions and formation of new structural constituents (Mostafa et al., 1987; Shi et al., 2010). In this context, it has been reported that total lipid content of flax (*Linum usitatissimum* L.) seeds was reduced four times at the end of the eighth day after initiation of germination as compared to ungerminated seeds (Wanasundara et al., 1999).

According to our hypothesis, fatty acid structure would affect canola seeds vigor through affecting the seed reserve utilization efficiency. Accordingly, the current experiment was aimed to compare different canola cultivars in terms of seed oil content, fatty acids composition and some mineral nutrients such as nitrogen (N) and phosphorus (P). In addition, the relationship between seed reserves and fatty acids content with seed vigor in canola cultivars were studied. In order to test this hypothesis, the above-mentioned relationships were evaluated before and after performing the accelerated aging test.

## 2. Material and methods

### 2.1. Experiments layout

The current study was conducted as series of laboratory experiments based on completely randomized design with 12 treatments and 4 replicates at Faculty of Agriculture, Ferdowsi University of Mashhad, Iran in 2017. The experimental treatments consisted of 12 winter canola (*Brassica napus* L.) cultivars (Fornax, Karaj 1, Karaj 2, Karaj 3, L73, L201, Licord, Modena, Okapi, Opera, SLM046, and Zarfam).

### 2.2. Seed materials

The canola seeds were obtained from East Azerbaijan Research Center for Agriculture and Natural Resources, Tabriz, Iran. The research center provides optimal seed production conditions for each cultivar. Seeds of all cultivars were produced in the summer of 2017.

### 2.3. Determination of N, P, oil and crude protein contents

First, 1000-seed weight, N and P concentrations in the seeds were determined. Nitrogen content was determined through titration method by a Kjeltac-PECO-Psu 55 Analyzer (AOAC, 2000). Phosphorus content was determined by calorimetric method using a spectrophotometer, UNICO S-2100-Vis (Murphy and Riley, 1962). Moreover, oil percentage was measured using soxhlet method (Allen et al., 1986).

### 2.4. Crude oil extraction and determination of fatty acid composition

In order to determine initial fatty acid composition, the seeds were ground and then oil extracted with n-hexane (1:4 wt/vol) by agitation in a dark place at room temperature (25 °C) for 48 h. The solvent was evaporated in vacuo at 40 °C to dryness (Farhoosh et al., 2009). The fatty acids composition of the canola oil were determined by gas chromatography (YOUNG LIN – Acme 6000 GC, The Republic of Korea) with the fame-ionization detector and capillary column (60 m × 0.32 mm i.d.; film thickness was 0.25 µm). Injector and detector temperatures were 300 and 320 °C, respectively. Oven temperature was at 250 °C. Fatty acids composition was calculated from the compound peak areas (Nzikou et al., 2009).

### 2.5. Determination of seed physiological quality

In order to evaluate seed physiological quality, germination and vigor tests were performed (on Whatman filter paper in 9-cm Petri dishes) using standard germination (ISTA, 2011). The 25 seeds from each treatment (with four replicates) were germinated at 20 °C in dark condition. The germinated seeds (with 2 mm radicle growth) were counted daily for 7 days. Mean germination time (MGT) was calculated using the following equation (ISTA, 2011):

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

Where n = number of seeds germinated at each day, t = number of days from the beginning of germination.

After 7 days, seedlings were dried at 70 °C for 48 h to determine seedling dry weight. Then, the weight of mobilized seed reserve (WMSR) and seed reserve utilization efficiency (SRUE) were calculated according to following equations (Soltani et al., 2006):

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

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

The unutilized seed dry weight is seed residues dry weight at the end of germination period. To determine initial seed dry weight, 25 seeds from each treatment (with four replicates) were weighed. Then, samples (seed fresh weight) were dried at 70 °C for 48 h and weighed again to calculate seed moisture content (Eq. 4). In the following, initial seed dry weight was calculated according to Eq. 5 (Seyyedi et al., 2015).

$$\text{Seed moisture content} = \text{seed fresh weight} - \text{seed dry weight} \quad (4)$$

$$\text{Initial seed dry weight} = \text{seed fresh weight} - \text{seed moisture content} \quad (5)$$

### 2.6. Electrical conductivity (EC) test

For EC measurement, 4 replicates of 50 pre-weighed seeds were soaked in Erlenmeyer flask containing 250 mL distilled water at 20 °C in the dark for 24 h. The EC soaked seeds was measured using an EC meter (JENWAY, 4510 Conductivity Meter) and expressed as  $\mu\text{S cm}^{-1} \text{ g}^{-1}$  according to Eq. 6. (Hajiabbasi et al., 2015).

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

### 2.7. Accelerated aging test

Appropriate temperature and time for an accelerated aging test were set by utilizing the procedure suggested by Abdolahi et al. (2012): an aging temperature of 40 °C and 100% relative humidity for 96 h. After performing the accelerated aging test, all traits have been determined again.

### 2.8. Statistical analyses

The data were subjected to analysis of variance using SAS 9.3 software (SAS, 2011). To determine the difference among means, the least significant difference (LSD) was used at 0.05 probability level. The first and second stage data (before and after aging, respectively) were separately analyzed. After determining the significant level, a linear equation was fit to the data using Microsoft Excel 2013 Software.

**Table 1**

Mean comparison of some physical and chemical characteristics of seed in winter canola cultivars.

Winter canola cultivars	100 seed weight (g)	Oil percentage		N content (g 100 g <sup>-1</sup> )		Crude protein content (%)		P content (g 100 g <sup>-1</sup> )	
		Before aging	After aging	Before aging	After aging	Before aging	After aging	Before aging	After aging
Fornax	4.83	51.82	46.71	3.32	2.85	20.72	17.82	0.38	0.34
Karaj 1	4.15	56.14	50.77	3.33	2.77	20.81	17.30	0.37	0.32
Karaj 2	3.64	34.72	31.06	3.30	2.74	20.61	17.11	0.39	0.34
Karaj 3	3.85	59.88	54.51	3.33	2.64	20.83	16.49	0.38	0.32
L73	3.35	39.91	36.07	3.32	2.73	20.73	17.03	0.40	0.34
L201	3.35	38.80	35.08	3.28	2.62	20.50	16.40	0.38	0.32
Licord	4.66	43.68	39.63	3.30	2.82	20.64	17.64	0.40	0.37
Modena	3.79	42.37	38.04	3.29	2.72	20.58	16.99	0.38	0.32
Okapi	4.25	41.25	37.15	3.34	2.78	20.85	17.39	0.40	0.34
Opera	4.36	48.51	43.64	3.29	2.79	20.56	17.44	0.39	0.34
SLM046	3.35	62.69	56.96	3.30	2.67	20.63	16.70	0.39	0.33
Zarfam	4.46	38.22	34.18	3.29	2.81	20.56	17.53	0.37	0.33
LSD (0.05)	0.140	3.979	4.235	–	–	–	–	–	–
Treatment	**	**	**	ns	ns	ns	ns	ns	ns

\*\*and ns are significant at the 0.01 and 0.05 levels of probability and no significant, respectively.

### 3. Results

There were significant differences among canola cultivars in terms of 1000-seed weight (Table 1). For instance, Fornax, Licord and Zarfam showed the highest 1000-seed weight. By contrast, the lowest 1000-seed weight (3.35 g) was related to L73, L201 and SLM046 cultivars (Table 1).

At the first and the second stages (before and after performing the accelerated aging test), there were significant differences among cultivars in terms of oil percentage. At both stages, the highest and the lowest oil percentage were achieved from SLM046 and K2 cultivars, respectively (Table 1).

At the first and the second stages, there was no significant difference among cultivars in terms of N, P and crude protein contents. However, all these traits decreased when accelerated aging test was performed (Table 1). For instance, after performing accelerated seed aging, seed N and P loss in SLM046 cultivar were recorded as 19.09 and 15.39%, respectively (Table 1).

According to Table 2, there was a significant difference among cultivars for all physiological seed traits, except for germination percentage at the first stage.

At the first stage, germination percentage in all studied cultivars was found to be 100%. However, there was a significant difference between cultivars in terms of germination percentage when the accelerated

aging test was performed (Table 2). At this time, the highest and lowest germination percentages were obtained in Fornax and K3 cultivars, respectively. Furthermore, before and after performing the accelerated aging test, the lowest MGT and highest seedlings dry weight were related to Fornax cultivar (Table 2).

At the second stage, the highest WMSR was related to Fornax and Licord cultivars. Interestingly, WMSR in Fornax cultivar was higher (63.7%) than K3 cultivar when the accelerated aging test was performed (Table 2). Furthermore, at the second stage, the highest and lowest SRUE were achieved from Fornax and K3 cultivars, respectively (Table 2).

As mentioned already, at the second stage, there was no significant difference between cultivars in terms of seed N, P and crude protein contents. However, there was a positive and significant correlation between seed crude protein, N and P loss with MGT (Fig. 1A,C,D). Moreover, a strong positive correlation was found between EC of the seed soaking solution with MGT (Fig. 2). By contrast, the correlation between WMSR and SRUE with MGT (Fig. 3) and between EC of the seed soaking solution with WMSR and SRUE (Fig. 4) were significantly negative.

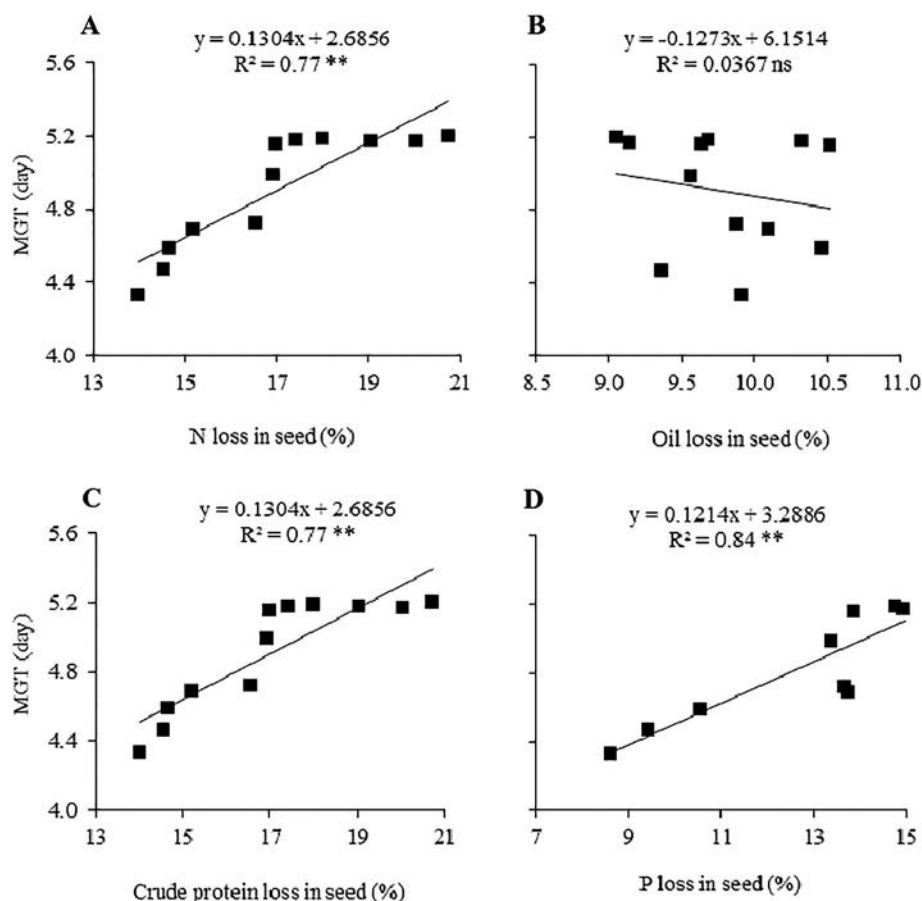
At both stages, there was no significant difference among cultivars in terms of saturated fatty acids (Table 3). However, there was a significant difference among cultivars for unsaturated fatty acids, except for palmitoleic and heptadecenoic acids (Table 4). The highest oleic

**Table 2**

Mean comparison of some physiological aspects of seed in winter canola cultivars.

Winter canola cultivars	Germination (%)		MGT (day)		Seedling dry weight (mg)		Weight of mobilized seed reserve (mg seed <sup>-1</sup> )		Seed reserve utilization efficiency (%)		EC test (μS cm <sup>-1</sup> g <sup>-1</sup> )	
	Before aging	After aging	Before aging	After aging	Before aging	After aging	Before aging	After aging	Before aging	After aging	Before aging	After aging
Fornax	100.00	96.00	3.74	4.33	3.17	2.94	3.54	3.41	89.62	86.28	11.47	22.57
Karaj 1	100.00	76.00	4.03	4.99	2.58	2.00	3.44	2.93	75.17	69.20	14.33	34.37
Karaj 2	100.00	74.00	4.58	5.16	1.81	1.77	2.74	2.71	65.70	65.28	18.35	29.20
Karaj 3	100.00	70.00	4.10	5.20	1.62	1.34	2.98	2.56	54.63	52.70	16.80	39.70
L73	100.00	75.00	4.06	5.19	2.09	1.72	2.84	2.60	73.96	67.09	15.83	34.63
L201	100.00	72.00	4.07	5.17	2.17	1.76	2.93	2.64	74.39	67.12	15.63	33.70
Licord	100.00	92.00	3.86	4.47	2.76	2.52	3.37	3.33	82.50	75.66	13.50	29.87
Modena	100.00	74.00	4.06	5.18	1.89	1.79	2.87	2.76	65.21	64.67	16.13	37.23
Okapi	100.00	78.00	3.98	4.72	2.45	2.07	3.22	3.00	76.01	69.61	15.60	32.17
Opera	100.00	81.00	3.94	4.69	2.26	2.09	2.89	2.81	78.08	74.28	14.33	34.67
SLM046	100.00	75.00	4.07	5.17	1.88	1.73	2.61	2.59	71.89	66.72	16.00	36.60
Zarfam	100.00	86.00	3.91	4.59	2.97	2.37	3.62	3.20	82.13	74.05	11.60	25.60
LSD (0.05)	–	9.412	0.351	0.138	0.315	0.271	0.278	0.304	11.421	12.800	0.320	4.142
Treatment	ns	*	*	*	**	**	**	**	**	**	**	**

\*, \*\* and ns are significant at the 0.01 and 0.05 levels of probability and no significant, respectively.



**Fig. 1.** Relationship between N loss in seed with MGT (A), oil loss in seed with MGT (B), crude protein loss in seed with MGT (C), and P loss in seed with MGT (D). The asterisks \*\* indicate statistical differences at  $P \leq .01$ . MGT was determined after aging.

percentage and lowest linoleic and linolenic percentages were obtained in Fornax cultivars. Furthermore, before and after performing the accelerated aging test, the highest linoleic and linolenic percentages were related to Karaj 3 cultivar (Table 4).

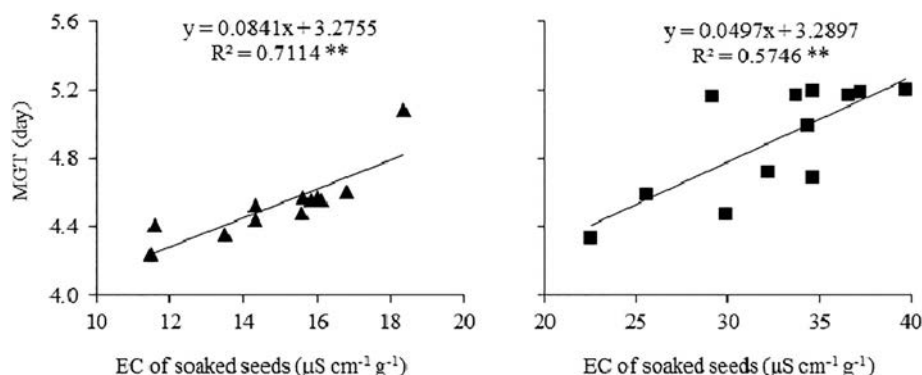
In general, accelerated aging in canola seeds increased saturated fatty acids percentage and reduced unsaturated fatty acids percentage (Tables 3 and 4). For example, at the first stage (before accelerated aging), the average saturated and unsaturated fatty acids constitute 8.69 and 91.31%, respectively, whereas at the second stage (after accelerated aging), the average values were found to be 26.76 and 73.23%, respectively. Furthermore, at the first stage, oleic acid, linoleic acid, and linolenic acid constitute 63.14, 20.65 and 7.20% of the oil, respectively. At the second stage, the average values of these fatty acids were found to be 56.93, 12.86 and

3.28%, respectively (Tables 3 and 4). Moreover, in all canola cultivars, the unsaturated to saturated fatty acid ratio decreased due to accelerated aging test (Fig. 5).

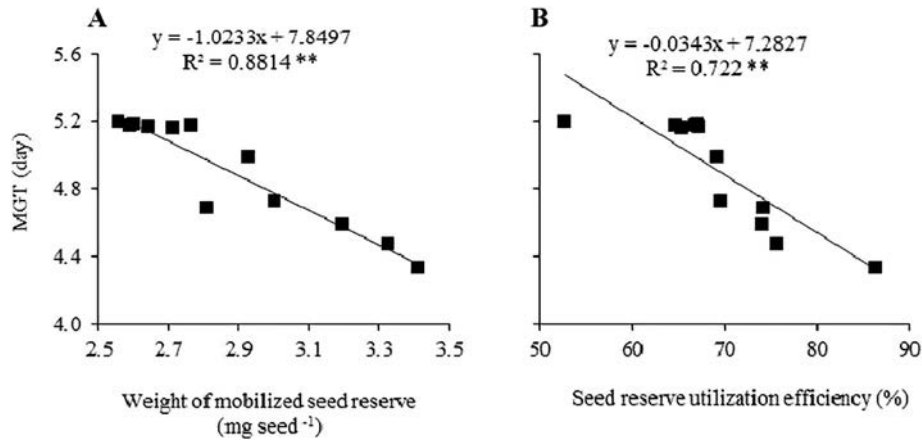
At both stages, there was a positive and significant relationship between oleic acid and SRUE (Fig. 6A). By contrast, the relationship between linolenic or linoleic acid and SRUE was found to be negative (Fig. 6B,C).

#### 4. Discussion

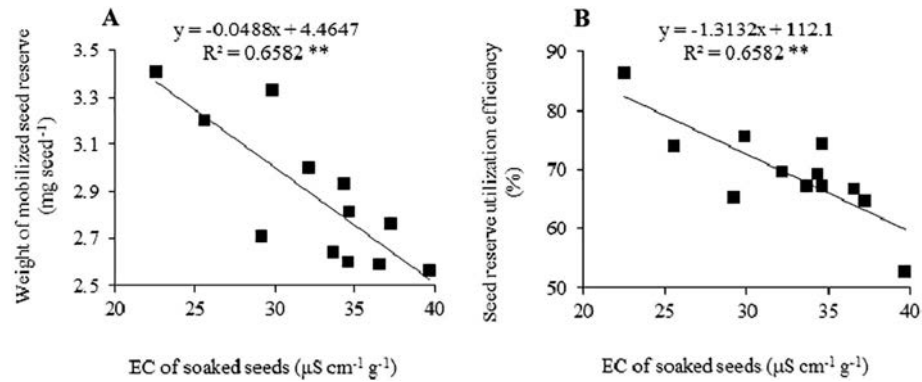
In addition to soil conditions, germination and seedling growth depend on seed reserves (Elamrani et al., 1992; Peltonen-Sainio et al., 2006; Faize et al., 2015). On the other hand, 1000-seed weight, which represents seed size, directly affects seed reserves content, germination



**Fig. 2.** Relationship between EC test with MGT. The asterisks \*\* indicate statistical differences at  $P \leq .01$ . First stage (triangle): before aging; second stage (square): after aging.



**Fig. 3.** Relationship between weight of mobilized seed reserve with MGT (A), and seed reserve utilization efficiency with MGT (B). The asterisks \*\* indicate statistical differences at  $P \leq .01$ . MGT, weight of mobilized seed reserve and seed reserve utilization efficiency were determined after aging.



**Fig. 4.** Relationship between EC test with weight of mobilized seed reserve (A), and seed reserve utilization efficiency (B). The asterisks \*\* indicate statistical differences at  $P \leq .01$ . EC test, weight of mobilized seed reserve and seed reserve utilization efficiency were determined after aging.

rate, and seedling growth rate (Liao and Yan, 1999). In this regard, a positive relationship between seedling vigor and seed size in cotton (*Gossypium hirsutum*) cultivars has been reported by Snider et al. (2014). Therefore, it can be concluded that higher 1000-seed weight in Fornax cultivar has a reason for its more seed vigor, as compared to other cultivars.

As mentioned already, seed vigor and seedling establishment in oil-seeds are strongly affected by seed reserves including carbohydrate, proteins, and fat (Soltani et al., 2006; Nonogaki, 2008). On the other hand, chemical composition and quality of these compounds are determined by mineral nutrient contents such as N and P (Naegle et al., 2005; Lamont and Groom, 2013). Nitrogen is considered as an essential

**Table 3**  
Mean comparison of saturated fatty acids of seed in winter canola cultivars.

Winter canola cultivars	Lauric (%)		Myristic (%)		Palmitic (%)		Margaric (%)		Stearic (%)		Arachidic (%)		Behenic (%)		Lignoceric (%)	
	Before aging	After aging	Before aging	After aging	Before aging	After aging	Before aging	After aging	Before aging	After aging	Before aging	After aging	Before aging	After aging	Before aging	After aging
Fornax	0.022	0.130	0.163	0.213	6.24	19.68	0.042	0.085	0.437	1.27	1.22	3.63	0.223	0.517	0.313	0.587
Karaj 1	0.022	0.123	0.130	0.230	6.14	20.36	0.044	0.082	0.450	1.27	1.23	3.64	0.243	0.563	0.353	0.550
Karaj 2	0.023	0.130	0.140	0.230	6.22	20.45	0.044	0.078	0.443	1.27	1.24	3.66	0.233	0.537	0.320	0.550
Karaj 3	0.024	0.127	0.157	0.220	6.28	20.47	0.042	0.083	0.430	1.27	1.22	3.62	0.233	0.550	0.397	0.580
L73	0.022	0.127	0.163	0.240	6.20	20.58	0.043	0.081	0.443	1.27	1.22	3.62	0.233	0.527	0.340	0.507
L201	0.025	0.137	0.150	0.223	6.22	20.68	0.044	0.076	0.427	1.27	1.24	3.65	0.253	0.570	0.320	0.550
Licord	0.025	0.137	0.157	0.217	6.26	20.38	0.042	0.084	0.433	1.29	1.24	3.64	0.230	0.533	0.340	0.550
Modena	0.024	0.123	0.167	0.220	6.16	20.68	0.043	0.081	0.430	1.28	1.24	3.63	0.237	0.550	0.333	0.540
Okapi	0.024	0.123	0.167	0.223	6.27	20.26	0.044	0.083	0.413	1.27	1.23	3.62	0.237	0.540	0.273	0.547
Opera	0.022	0.127	0.147	0.213	6.23	19.95	0.045	0.086	0.450	1.27	1.23	3.64	0.240	0.543	0.373	0.523
SLM046	0.024	0.127	0.150	0.223	6.31	20.07	0.044	0.082	0.440	1.28	1.23	3.61	0.247	0.557	0.320	0.533
Zarfam	0.023	0.120	0.153	0.223	6.22	20.50	0.043	0.083	0.450	1.29	1.22	3.57	0.243	0.553	0.327	0.530
Average	0.02	0.13	0.15	0.22	6.23	20.34	0.04	0.08	0.44	1.28	1.23	3.63	0.24	0.55	0.33	0.55
LSD (0.05)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Treatment	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

\*, \*\* and ns are significant at the 0.01 and 0.05 levels of probability and no significant, respectively.

**Table 4**

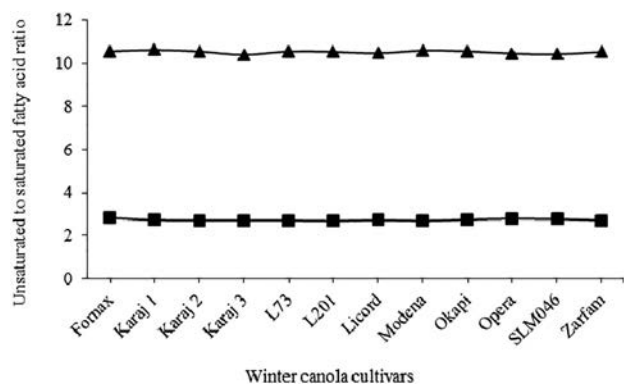
Mean comparison of unsaturated fatty acid of seed in winter canola cultivars.

Winter canola cultivars	Palmitoleic (%)		Heptadecenoic (%)		Oleic (%)		Linoleic (%)		Linolenic (%)		Erucic (%)	
	Before aging	After aging	Before aging	After aging	Before aging	After aging	Before aging	After aging	Before aging	After aging	Before aging	After aging
Fornax	0.194	0.111	0.051	0.038	66.79	60.97	18.30	10.85	5.93	1.90	0.073	0.011
Karaj 1	0.193	0.109	0.057	0.044	62.94	57.51	20.90	12.70	7.21	2.81	0.093	0.011
Karaj 2	0.195	0.112	0.056	0.043	62.30	55.82	21.35	13.42	7.38	3.70	0.061	0.011
Karaj 3	0.190	0.115	0.053	0.037	60.63	53.26	21.87	14.12	8.38	5.54	0.096	0.010
L73	0.196	0.113	0.050	0.040	60.99	54.19	21.84	14.07	8.18	4.61	0.083	0.011
L201	0.192	0.116	0.056	0.043	62.00	55.04	21.36	13.77	7.67	3.86	0.054	0.009
Licord	0.193	0.118	0.055	0.042	66.11	59.55	18.59	11.35	6.26	2.10	0.065	0.009
Modena	0.192	0.115	0.052	0.041	61.33	54.88	21.78	13.78	7.95	4.06	0.059	0.011
Okapi	0.190	0.121	0.053	0.041	62.89	57.87	20.99	12.66	7.15	2.62	0.074	0.009
Opera	0.190	0.114	0.053	0.039	64.53	58.73	19.92	12.40	6.50	2.35	0.065	0.008
SLM046	0.197	0.118	0.051	0.047	62.07	56.02	21.31	13.72	7.50	3.60	0.096	0.011
Zarfam	0.194	0.117	0.054	0.033	65.15	59.31	19.59	11.49	6.28	2.16	0.053	0.010
Average	0.19	0.11	0.05	0.04	63.14	56.93	20.65	12.86	7.20	3.28	0.07	0.01
LSD (0.05)	–	–	–	–	0.561	1.040	0.578	0.505	0.220	0.550	0.0042	0.0017
Treatment	ns	ns	ns	ns	**	**	**	**	**	**	**	**

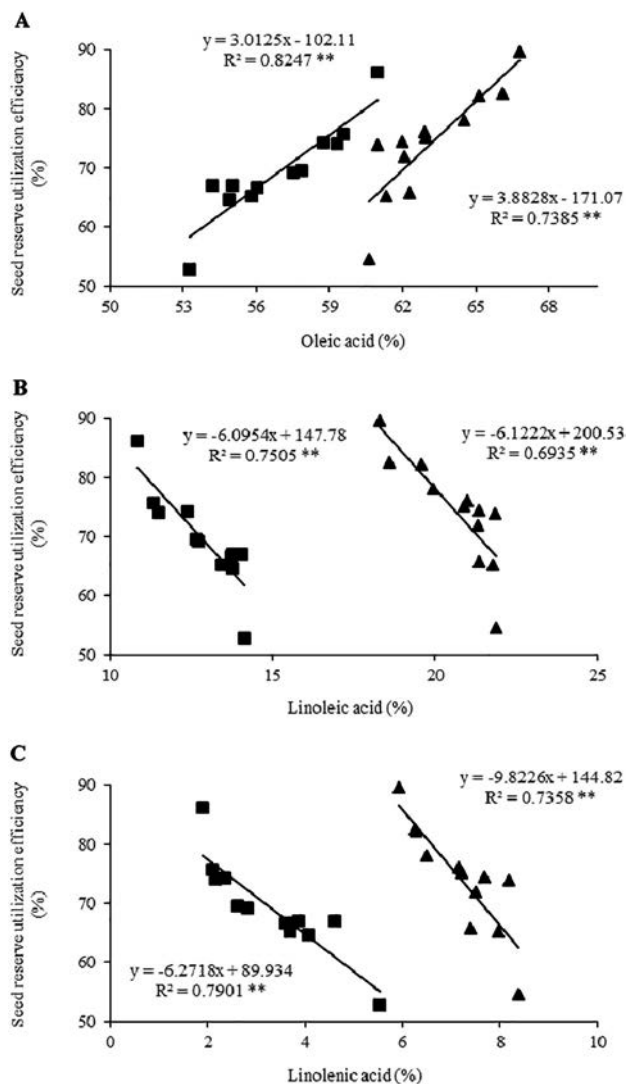
\*, \*\* and ns are significant at the 0.01 and 0.05 levels of probability and no significant, respectively.

element for formation of amino acids, the building blocks of protein, plant cell division, energy reactions and seedling growth (Hara and Toriyama, 1998; Monaco et al., 2003). Phosphorus is also one of the major macronutrients and plays a crucial role in energy transfer and is an essential component of phospholipids and proteins (Nicanuzia Dos Prazeres et al., 2004; White and Veneklaas, 2012). Hence, seed N and P concentration are among the most important factors affecting seed germination, seedling growth and establishment (Modi, 2002; Peltonen-Sainio, et al., 2006; Seyyedi et al., 2015). In this context, the importance of these elements in improving seeds vigor in soybean (*Glycine max* L.), common bean (*Phaseolus vulgaris* L.) and cotton has been previously reported (Bishnoi et al., 2007; Sawan et al., 2009, 2011; Pacheco et al., 2012). Therefore, a positive correlation between seed N and P loss with MGT represents the importance of N and P in increasing canola seed vigor. In other words, the loss of these elements during accelerated seed aging process means reducing the possibility of normal seedling establishment in the soil.

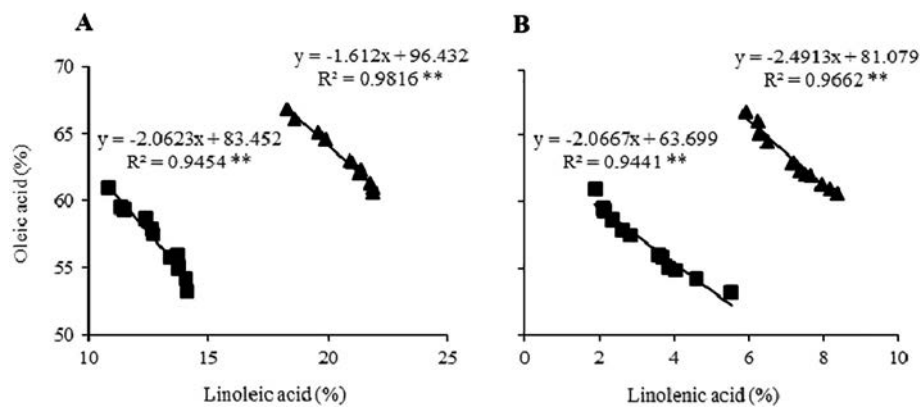
In general, standard germination test is not able to predict seeds vigor accurately, especially in unfavorable conditions (Noli et al., 2008), therefore, EC and accelerated aging tests are among the most important indexes in predicting seeds vigor and seedlings emergence (Wang et al., 1996; Vieira et al., 2004; Abdolahi et al., 2012). On the other hand, seed aging often reduces water absorption and germination capacity and increases lipid peroxidation, deformation of the membrane structure and cellular secretions (Gidrol et al., 1989; Basra et al., 2003;



**Fig. 5.** Unsaturated to saturated fatty acid ratio in winter canola cultivars. First stage (triangle): before aging; second stage (square): after aging.



**Fig. 6.** Relationship between oleic acid with seed reserve utilization efficiency (A), linoleic acid with seed reserve utilization efficiency (B), and linolenic acid with seed reserve utilization efficiency (C). The asterisks \*\* indicate statistical differences at  $P \leq .01$ . First stage (triangle): before aging; second stage (square): after aging.



**Fig. 7.** Relationship between oleic acid with linoleic acid (A) and oleic acid with linolenic acid (B). The asterisks \*\* indicate statistical differences at  $P \leq .01$ . First stage (triangle): before aging; second stage (square): after aging.

Hsu et al., 2003; Balešević-Tubić et al., 2005; Veselovsky and Veselova, 2012), so that it can greatly increase the vulnerability of seedlings against environmental stresses (Hailstones and Smith, 1988; Khaliliaqdam et al., 2013). Accordingly, the imposition of artificial stress on the seeds as a result of the implementation of accelerated aging test can predict successful establishment of seedlings under unfavorable conditions.

During the accelerated aging process, part of the seed reserves may be oxidized and destroyed (Gidrol et al., 1989; Pinzino, 1999; Tatić et al., 2012). For instance, a significant reduction in phospholipids in soybean seeds during accelerated aging process was observed by Priestley and Leopold (1979). In this regard, a strong correlation between seed N and P loss with MGT and between EC test with MGT represents accelerated aging and EC test importance in assessing seed vigor and successful establishment of canola seedlings. Such results, not only indicate crucial effects of nutrient reserves on seed vigor but also demonstrate that reduction in WMSR during seed aging process plays an important role in improving seeds vigor and heterotrophic seedling growth, especially under unfavorable conditions.

Upon water uptake and activation of enzymes, seeds reserves are hydrolyzed during biochemical processes (Muccifora and Bellani, 2013). In the next phase, quick transfer of energy and nutrients to radicles leads to successful completion of seedling emergence (Welbaum et al., 1998; Nonogaki et al., 2010). Accordingly, reduction in WMSR during germination affect normal emergence through an effect on heterotrophic seedling growth.

From the results, the negative and significant correlation between WMSR and MGT and also between SRUE and MGT can justify the above-mentioned description. In other words, canola cultivars with a smaller percentage of nutrients lost during the accelerated aging process have likely higher WMSR and SRUE. Therefore, it seems that canola seeds vigor highly depends on heterotrophic seedling growth.

Oleic, linoleic and linolenic acids were found to be the most important unsaturated fatty acids found in canola seeds because, at the first stage, these fatty acids constituted 91% of the total fatty acids. Hence, it is likely that these fatty acids play a key role in seed vigor in canola cultivars. In addition, it can be concluded that seed vigor in canola is more affected by unsaturated fatty acids than saturated ones.

The response of seed vigor to fatty acids composition is not clearly known. Generally, oilseeds with high lipids content have lower seed longevity (Balešević-Tubić et al., 2010). Besides, with increasing the degree of unsaturation, the fatty acid resistance usually decreases. Since oils with higher linoleic and linolenic acids and lower oleic acid content are oxidized more quickly (Yun and Surh, 2012; Seyyedi et al., 2015), higher linoleic and linolenic acids content might affect oil quality and lead to reduce seed vigor in canola cultivars.

As mentioned before, accelerated aging in canola seeds increases saturated fatty acid percentage, decreases unsaturated fatty acid percentage, oil percentage and unsaturated to saturated fatty acid ratio. It seems that accelerated aging causes oil oxidation by destroying unsaturated and saturated fatty acids structure. But the important point is that probably unsaturated fatty acids are more likely to be degraded than saturated fatty acids that is why unsaturated-to-saturated fatty acid ratio would decrease due to accelerated aging processes. Similar results were found when sunflower (*Helianthus annuus* L.) cultivars were studied in order to increase oleic acid and decrease linoleic acid content (González Belo et al., 2014). As reported by Balešević-Tubić et al. (2007), the severity of seed deterioration in sunflower is influenced by the degree of fatty acid unsaturation; so that oleic acid was more resistant to oxidation than linoleic acid. According to Seyyedi et al. (2015), among black seed fatty acids, linolenic acid showed the highest correlation with seed vigor, so that seed vigor significantly decreased with increasing seeds linolenic acid percentage. Moreover, due to a negative correlation between oleic and linoleic acids (Fig. 7A) and also between oleic and linolenic acids (Fig. 7B), it seems that any environmental factors that could lead to an increase in oleic acid percentage, possibly could increase seed vigor. In this regard, a negative correlation between linolenic and oleic acids percentage was reported by Were et al. (2006). Similarly, Rezvani Moghaddam and Seyyedi (2016) have reported that MGT decreased with increasing oleic acid percentage in sesame cultivars, however, increase in linoleic or linolenic acid percentage was parallel with the increase in MGT.

A negative correlation between linolenic acid and SRUE, as well as between linoleic acid and SRUE and also a positive correlation between oleic acid and SRUE represent this fact that unsaturated fatty acids content strongly affect heterotrophic seedling growth in canola. In other words, cultivars with high oleic acid content and low linoleic and linolenic acids content showed lower MGT and higher seeds vigor. As reported by González Belo et al. (2014), breeding for higher oleic acid and lower linoleic acid content in sunflower can improve seed vigor.

## 5. Conclusions

It can be concluded that seed reserves play a crucial role in increasing canola seed vigor. In fact, MGT decreases when nutrient reserves are less impacted and destroyed by an accelerated aging process which finally increases successful establishment of normal seedlings. On the other hand, negative correlations between EC of the seed soaking solution with SRUE and between SRUE with MGT suggest this fact that decrease in losses of WMSR strongly affects heterotrophic seedling growth in canola cultivars. Moreover, a strong correlation was found between oleic, linoleic and linolenic acids content and SRUE; therefore, it seems that canola seed vigor highly depends on fatty acids composition.

## Conflict of interest

The authors declare that they have no conflict of interest.

## References

- Abdollahi, M., Andelibi, B., Zangani, E., Shekari, F., Jamaati-E-Somarin, S., 2012. Effect of accelerated aging and priming on seed germination of rapeseed (*Brassica napus* L.) cultivars. *International Research Journal of Applied and Basic Sciences* 3, 499–508.
- Agricultural Statistics, 2016. Iran's Minister of Agriculture, Department of Planning and Economy. <http://www.majir.ir/>.
- Allen, S.E., Grimshaw, H.M., Rowland, A.P., 1986. In: Moore, P.D., Chapman, S.B. (Eds.), *Chemical Analysis, in Methods in Plant Ecology*. Blackwell Scientific, Oxford.
- AOAC, 2000. Official methods of analysis. 17th ed. Association of Official Analytical Chemists, Gaithersburg, Maryland, USA.
- Balešević-Tubić, S., Tatić, M., Miladinović, J., 2005. Influence of natural aging on the dynamics of water absorption by sunflower seed. *Seed Science and Technology* 33, 255–258.
- 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.
- Balešević-Tubić, S., Tatić, M., Đorđević, V., Nikolić, Z., Đukić, V., 2010. Seed viability of oil crops depending on storage conditions. *Helia* 33, 153–160.
- Basra, S.M.A., Ahmad, N., Khan, M.M., Iqbal, N., Cheema, M.A., 2003. Assessment of cottonseed deterioration during accelerated ageing. *Seed Science and Technology* 31, 531–540.
- Bishnoi, U.R., Kaur, G., Khan, M.H., 2007. Calcium, phosphorus, and harvest stages effects soybean seed production and quality. *Journal of Plant Nutrition* 30, 2119–2127.
- Burton, W.A., Ripley, V.L., Potts, D.A., Salisbury, P.A., 2004. Assessment of genetic diversity in selected breeding lines and cultivars of canola quality *Brassica juncea* and their implications for canola breeding. *Euphytica* 136, 181–192.
- Elamrani, A., Raymond, P., Saglio, P., 1992. Nature and utilization of seed reserves during germination and heterotrophic growth of young sugar beet seedlings. *Seed Science Research* 2, 1–8.
- Ellis, R.H., 1992. Seed and seedling vigour in relation to crop growth and yield. *Plant Growth Regulation* 11, 249–255.
- Faize, M., Nicolás, E., Faize, L., Díaz-Vivancos, P., Burgos, L., Hernández, J.A., 2015. Cytosolic ascorbate peroxidase and Cu, Zn-superoxide dismutase improve seed germination, plant growth, nutrient uptake and drought tolerance in tobacco. *Theoretical and Experimental Plant Physiology* 27, 215–226.
- Farhoosh, R., Haddad Khodaparast, M.H., Sharif, A., 2009. Bene hull oil as a highly stable and antioxidative vegetable oil. *European Journal of Lipid Science and Technology* 111, 1259–1265.
- Gidrol, X., Serghini, H., Noubhani, A., Mocnot, B., Mazliak, P., 1989. Biochemical changes induced by accelerated aging in sunflower seeds. I. Lipid peroxidation and membrane damage. *Physiologia Plantarum* 76, 591–597.
- González Belo, R., Tognetti, J., Benec-Arnold, R., Izquierdo, N.G., 2014. Germination responses to temperature and water potential as affected by seed oil composition in sunflower. *Industrial Crops and Products* 62, 537–544.
- Hailstones, M.D., Smith, M.T., 1988. Lipid peroxidation in relation to declining vigour in seeds of soya (*Glycine max* L.) and cabbage (*Brassica oleracea* L.). *Journal of Plant Physiology* 133, 452–456.
- Hajjabbasi, M., Tavakkol Afshari, R., Abbasi, A., 2015. Effects of salicylic acid and ethylene on germination improvement of deteriorated seed of *Glycine max* (L.). *Crop Research* 50, 86–94.
- Hara, Y., Toriyama, K., 1998. Seed nitrogen accelerates the rates of germination, emergence, and establishment of rice plants. *Soil Science and Plant Nutrition* 44, 359–366.
- Hsu, C.C., Chen, C.L., Chen, J.J., Sung, J.M., 2003. Accelerated aging-enhanced lipid peroxidation in bitter melon seeds and effects of priming and hot water soaking treatments. *Scientia Horticulturae* 98, 201–212.
- ISTA, 2011. International Rules for Seed Testing. The International Seed Testing Association, Bassersdorf, Switzerland.
- Khaliliaqdam, N., Soltani, A., Latifi, N., Ghaderi Far, F., 2013. Soybean seed aging and environmental factors on seedling growth. *Communications in Soil Science and Plant Analysis* 44, 1786–1799.
- Lamont, B.B., Groom, P.K., 2013. Seeds as a source of carbon, nitrogen, and phosphorus for seedling establishment in temperate regions: a synthesis. *American Journal of Plant Sciences* 4, 30–40.
- Liao, H., Yan, X., 1999. Seed size is closely related to phosphorus use efficiency and photosynthetic phosphorus use efficiency in common bean. *Journal of Plant Nutrition* 22, 877–888.
- Marcos-Filho, J., 2015. Seed vigor testing: an overview of the past, present and future perspective. *Scientia Agricola* 72, 363–374.
- Modarres, R., De, Paulo, Rodrigues Da Silva, V., 2007. Rainfall trends in arid and semi-arid regions of Iran. *Journal of Arid Environments* 70, 344–355.
- Modi, A.T., 2002. Wheat seed quality in response to molybdenum and phosphorus. *Journal of Plant Nutrition* 25, 2409–2419.
- Mohdaly, A.A.A., Smetanska, I., Ramadan, M.F., Sarhan, M.A., Mahmoud, A., 2011. Antioxidant potential of sesame (*Sesamum indicum*) cake extract in stabilization of sunflower and soybean oils. *Industrial Crops and Products* 34, 952–959.
- Monaco, T.A., Mackown, C.T., Johnson, D.A., Jones, T.A., Norton, J.M., Norton, J.B., Redinbaugh, M.G., 2003. Nitrogen effects on seed germination and seedling growth. *Journal of Range Management* 56, 646–653.
- Mostafa, M.M., Rahma, E.H., Rady, A.H., 1987. Chemical and nutritional changes in soybean during germination. *Food Chemistry* 23, 257–275.
- Mousavi-Avval, S.H., Rafiee, S., Jafari, A., Mohammadi, A., 2011. Energy flow modeling and sensitivity analysis of inputs for canola production in Iran. *Journal of Cleaner Production* 19, 1464–1470.
- Muccifora, S., Bellani, L.M., 2013. Effects of copper on germination and reserve mobilization in *Vicia sativa* L. seeds. *Environmental Pollution* 179, 68–74.
- Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* 27, 31–36.
- Naegle, E.R., Burton, J.W., Carter, T.E., Rufty, T.W., 2005. Influence of seed nitrogen content on seedling growth and recovery from nitrogen stress. *Plant and Soil* 271, 329–340.
- Nicanuzia Dos Prazeres, J., Ferreira, C.V., Aoyama, H., 2004. Acid phosphatase activities during the germination of *Glycine max* seeds. *Plant Physiology and Biochemistry* 42, 15–20.
- Noli, E., Casarini, E., Urso, G., Conti, S., 2008. Suitability of three vigor test procedures to predict field performance of early sown maize seed. *Seed Science and Technology* 36, 168–176.
- Nonogaki, H., 2008. Seed germination and reserve mobilization. *Encyclopedia of Life Sciences*. John Wiley & Sons, Ltd, Chichester.
- Nonogaki, H., Bassel, G.W., Bewley, J.D., 2010. Germination—Still a mystery. *Plant Science* 179, 574–581.
- Nzikou, J.M., Matos, L., Bouanga-Kalou, G., Ndangui, C.B., Pambou-Tobi, N.P.G., Kimbonguila, A., Silou, T., Linder, M., Desobry, S., 2009. Chemical composition on the seeds and oil of sesame (*Sesamum indicum* L.) grown in Congo-Brazzaville. *Advances Journal of Food Science and Technology* 1, 6–11.
- Pacheco, R.S., Brito, L.F., Stralio, R., Pérez, D.V., Araújo, A.P., 2012. Seeds enriched with phosphorus and molybdenum as a strategy for improving grain yield of common bean crop. *Field Crops Research* 136, 97–106.
- Parkhey, S., Naithani, S.C., Keshavkant, S., 2012. ROS production and lipid catabolism in desiccating *Shorea robusta* seeds during aging. *Plant Physiology and Biochemistry* 57, 261–267.
- Peltonen-Sainio, P., Kontturi, M., Peltonen, J., 2006. Phosphorus seed coating enhancement on early growth and yield components in oat. *Agronomy Journal* 98, 206–211.
- Pinzino, C., 1999. Aging, free radicals, and antioxidants in wheat seeds. *Journal of Agricultural and Food Chemistry* 47, 1333–1339.
- Priestley, D.A., Leopold, A.C., 1979. Absence of lipid oxidation during accelerated aging of soybean seeds. *Plant Physiology* 63, 726–772.
- Priestley, D.A., Leopold, A.C., 1983. Lipid changes during natural aging of soybean seeds. *Physiologia Plantarum* 59, 467–470.
- Rezvani Moghaddam, P., Seyyedi, S.M., 2016. Evaluation of germination characteristics of sesame cultivars (*Sesamum indicum* L.) seeds as related to fatty acids composition. *Iranian Journal of Seed Science and Technology* 5, 119–131.
- Sana, M., Ali, A., Malik, M.A., Saleem, M.F., Rafiq, M., 2003. Comparative yield potential and oil contents of different canola cultivars (*Brassica napus* L.). *Pakistan Journal of Agronomy* 2, 1–7.
- SAS, 2011. SAS for Windows Version 9.3. SAS Institute Inc, Cary, NC, USA.
- Sawan, Z.M., Fahmy, A.H., Yousef, S.E., 2009. Direct and residual effects of nitrogen fertilization, foliar application of potassium and plant growth retardant on Egyptian cotton growth, seed yield, seed viability and seedling vigor. *Acta Ecologica Sinica* 29, 116–123.
- Sawan, Z.M., Fahmy, A.H., Yousef, S.E., 2011. Effect of potassium, zinc and phosphorus on seed yield, seed viability and seedling vigor of cotton (*Gossypium barbadense* L.). *Archives of Agronomy and Soil Science* 57, 75–90.
- 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. *Industrial Crops and Products* 74, 939–949.
- Sharafi, Y., Majidi, M.M., Goli, S.A.H., Rashidi, F., 2015. Oil content and fatty acids composition in *Brassica* species. *International Journal of Food Properties* 18, 2145–2154.
- Shi, H., Nam, P.K., Ma, Y., 2010. Comprehensive profiling of isoflavones, phytosterols, tocopherols, minerals, crude protein, lipid, and sugar during soybean (*Glycine max*) germination. *Journal of Agricultural and Food Chemistry* 58, 4970–4976.
- Snider, J.L., Collins, G.D., Whitaker, J., Chapman, K.D., Horn, P., Grey, T.L., 2014. Seed size and oil content are key determinants of seedling vigor in *Gossypium hirsutum*. *Journal of Cotton Science* 18, 1–9.
- Soltani, A., Gholipoor, M., Zeinali, E., 2006. Seed reserve utilization and seedling growth of wheat as affected by drought and salinity. *Environmental and Experimental Botany* 55, 195–200.
- Tatić, M., Balešević-Tubić, S., Đorđević, V., Nikolić, Z., Đukić, V., Vujaković, M., Cvijanović, G., 2012. Soybean seed viability and changes of fatty acids content as affected by seed aging. *African Journal of Biotechnology* 11, 10310–10316.
- Torabi, B., Rabii, A., 2013. Germination response of canola (*Brassica napus* L.) to pre-soaking duration. *International Journal of Agriculture and Crop Sciences* 5, 421–425.
- Veselovsky, V.A., Veselova, T.V., 2012. Lipid peroxidation, carbohydrate hydrolysis, and Amadori-Maillard reaction at early stages of dry seed aging. *Russian Journal of Plant Physiology* 59, 763–770.
- Vieira, R.D., Neto, A.S., de Bittencourt, S.R.M., Panobianco, M., 2004. Electrical conductivity of the seed soaking solution and soybean seedling emergence. *Scientia Agricola* 61, 164–168.
- Wanasundara, P.K.J.P.D., Wanasundara, U.N., Shahidi, F., 1999. Changes in flax (*Linum usitatissimum* L.) seed lipids during germination. *Journal of the American Oil Chemists' Society* 76, 41–48.
- Wang, Y.R., Yu, L., Nan, Z.B., 1996. Use of seed vigor tests to predict field emergence of lucerne (*Medicago sativa*). *New Zealand Journal of Agricultural Research* 39, 255–262.
- Wang, T., Harp, T., Hammond, E.G., Burris, J.S., Fehr, W.R., 2001. Seed physiological performance of soybeans with altered saturated fatty acid contents. *Seed Science Research* 11, 93–97.

- Welbaum, G.E., Bradford, K.J., Yim, K.-O., Booth, D.T., Oluoch, M.O., 1998. Biophysical, physiological and biochemical processes regulating seed germination. *Seed Science Research* 8, 161–172.
- Were, B.A., Onkware, A.O., Gudu, S., Welander, M., Carlsson, A.S., 2006. Seed oil content and fatty acid composition in East African sesame (*Sesamum indicum* L.) accessions evaluated over 3 years. *Field Crops Research* 97, 254–260.
- White, P.J., Veneklaas, E.J., 2012. Nature and nurture: the importance of seed phosphorus content. *Plant and Soil* 357, 1–8.
- Yun, J.M., Surh, J.H., 2012. Fatty acid composition as a predictor for the oxidation stability of Korean vegetable oils with or without induced oxidative stress. *Preventive Nutrition and Food Science* 17, 158–165.
- Zhang, W., Chiwocha, S.D.S., Trischuk, R., Gusta, L.V., 2012. Profile of plant hormones and their metabolites in germinated and ungerminated canola (*Brassica napus*) seeds imbibed at 8°C in either GA<sub>4+7</sub>, ABA, or a saline solution. *Journal of Plant Growth Regulation* 29, 91–105.