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SEED FATTY ACID PROFILES: POTENTIAL RELATIONS BETWEEN SEED GERMINATION UNDER TEMPERATURE STRESS IN SELECTED VEGETABLE SPECIES

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Abstract. The study was undertaken to determine characterization of the fatty acid profile of pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), radish (*Raphanus sativus* L.) and cabbage (*Brassica oleracea* var. *capitata*) seeds as well as relations between fatty acid profile and seed germination under temperature stress in controlled conditions. Germination tests were conducted using four replicates of 50 seeds from each species at low, optimum and high-temperatures (5, 10, 15, 20, 25 30, 35 and 40°C). Germination percentage of pepper, eggplant, radish and cabbage in last count ranged from 1.28 to 72.10, from 1.28 to 74.88, from 22.51 to 88.72 and from 1.28 to 74.94, respectively. Palmitic (C16:0), oleic (C18:1n-9) and linoleic (C18:2n-6), acids were sequentially the highest in concentration followed by stearic acid (C18:0) at less than 5% and miristic, palmitoleic, margaric, arachidic, erucic, behenic and nervonic acids at an even lower content (<1%) in pepper and eggplant. Erucic acid (C22:1n-9) was the principal fatty acid followed by oleic, linoleic, gadoleic and behenic acids and miristic, palmitic, palmitoleic, arachidonic and stearic acids at a lower content between <1% and 5% in radish and cabbage seeds. The simple correlation coefficients and stepwise multiple regression analysis showed that the low or high amount of fatty acids in tested species such as palmitic (C16:0), palmitoleic (C16:1n-7), margaric (C17:0), stearic (C18:0), oleic (C18:1n-9), linoleic (C18:2n-6), arachidic (C20:0), gadoleic (C20:1n-9), arachidonic (C20:4n-6), behenic (C22:0), MUFA, n-6 PUFA and total oil might play a major role in seed germination under low and high temperatures.

Key words: fatty acids, pepper, eggplant, radish, cabbage, seed germination

INTRODUCTION

Various definitions of seed germination have been proposed, and it is important to understand their distinctions. To the seed physiologist, germination is defined as the

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emergence of the radicle through the seed coat or is considered to be completed at the time of visible radicle emergence [Copeland and Mcdonald 1985, Bewley and Black 1994]. And, germination of higher plants seeds as vegetables involves a complex series of metabolic processes such as water imbibition, respiration, mobilization of food reserves, nucleic acid and protein synthesis, and cell differentiation and growth [Wanasundara et al. 1999]. This complex process, seed germination is involving many individual reactions and phases, each of which is affected by temperature. This effect on germination can be expressed in terms of cardinal temperatures [Copeland and Mcdonald 1985]. For example, peppers have a prolonged germination period and an optimum germination temperature of about 30°C [O'Sullivan and Bouw 1984]. The applicable germination temperatures for cabbage and radish, and eggplant are 17ºC and 25°C, respectively [Taylor 1997]. In other words, the response to temperature depends on the species, variety, growing region, and duration of time from harvest. Also, as a general rule, the optimum germination temperature for most seeds is between 15 and 30°C [Copeland and Mcdonald 1985].

There are two groups of seeds based on their storage reserves: starch and lipid storing [Taylor 1997, Da Silva et al. 1998]. Especially, seeds belonging to Cucurbitaceae family are also known to be as rich in oil as cottonseed, soya beans or corn [De Mello et al. 2001]. In addition, most of the small-seeded vegetable crops except for large-seeded crops such as common bean and pea contain high levels of starch with little lipid, accumulate mostly lipid and proteins, and total lipid content of small-seeded vegetables range between 7% (*Spinacia oleracea* L.) and 50% (Cucurbits) [Al-Khalifa 1996, Taylor 1997]. And, total lipid content of pepper, radish and cabbage are 22%, 36% and 38%, respectively [Taylor 1997]. Thus, Wanasundara et al. [1999] reported that during germination, storage lipids are metabolized to supply the required energy for the high-energy demanding processes and structural lipids also change quantitatively due to new membrane formation.

Although a number of papers exist about characterization of the seeds oils and fatty acid composition of different vegetable crops' seeds [Al-Khalifa 1996, De Mello 2000, Mandal et al. 2002, Barthet 2008], most do not cover the relations between fatty acid composition and seed germination under various temperatures. Therefore, the present study was undertaken to determine not only characterization of the fatty acid profile of pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), radish (*Raphanus sativus* L.) and cabbage (*Brassica oleracea* var. *capitata*) seeds but also relations between fatty acid profile and low and high-temperatures seed germination under controlled conditions.

MATERIALS AND METHODS

This study was conducted out in growth chambers and Gas Chromatography-Mass Spectrometry laboratory in Atatürk University in 2009 and 2010, to determine the relations between fatty acid composition and seed germination of different vegetable species under temperature stress. In this study, pepper (*Capsicum annuum* L. cv. 'Doru-16'), eggplant (*Solanum melongena* L. cv. 'Pala-49'), radish (*Raphanus sativus* L.

cv. 'Siyah') and cabbage (*Brassica oleracea* var. *capitata* cv. 'Ekici-79') seeds were used as plant material and seeds of vegetable species were supplied by vegetables seed companies in Turkey. Species were selected to their non-cold-germinating and cold-germinating abilities as their cool and warm climate species members.

Germination tests were conducted using four replicates of 50 seeds from each species in 9 cm Petri dishes in the dark. They were placed in growth chamber for a period of 10 days for radish and cabbage, and 14 days for pepper and eggplant [ISTA 1996] at low, optimum and high-temperatures (5, 10, 15, 20, 25 30, 35 and 40°C). The seeds were incubated between two filter papers saturated with water containing Benlate 1 g l^{-1} to prevent fungal growth. Visible-radicle protrusion, 2 mm length of radicle, was the criterion of germination [Demir et al. 2008, Kaymak 2012].

Germinated seeds were recorded and discarded at 24 hour intervals during 10 (radish and cabbage) and 14 (pepper and eggplant) days [ISTA 1996] and the results were expressed as final germination percentage. Germination speed was calculated according to the equation (Germination speed = Germination percentage on 1^{st} day/1 + ….. + Germination percentage n^{th} day/n) of Kaymak et al. [2009] and Kaymak [2012].

Lipid extraction of seeds of pepper (*Capsicum annuum* L. cv. 'Doru-16'), eggplant (*Solanum melongena* L. cv. 'Pala-49'), radish (*Raphanus sativus* L. cv. 'Siyah') and cabbage (*Brassica oleracea* var. *capitata* cv. 'Ekici-79') (*c.* 1 g) was made according to Folch et al. [1957]. Chloroform/methanol (2:1 v/v) including 0.01% (w/v) of butylated hydroxytoluene ('Sigma' ≥ 99.0% (GC), product No. B1378) as antioxidant 20 vol. (w/v) for one minute was used to become homogeneous of the tested seed samples. Homogenization was performed in ice and other media (filtration, incubation etc.) at 20–22°C. The organic solvent was released moisture under flow freely of nitrogen and total lipid was established gravimetrically. Metcalfe and Schmitz [1961] method was used to prepare for fatty acid methyl esters (FAMEs) from lipids. By using NaOH in methanol saponification was carried out and FAMEs were supplied through boron trifluoride (BF_3) in methanol. FAMEs were gained by means of a HP (Hewlet Packard, USA) "Agilent 6890 N" model gas chromatography (GC), which has a flame ionization detector and suited with a DB 23 capillary column (60 m, 0.25 mm i.d. and 0.25 μm) ejector and detector whose temperature program was 190°C for 35 min than increases at 30°C per min up to 220°C, where it was preserved for 5 min. Hydrogen gas (2 ml min^{-1} and split ratio was 30:1) was used as carrier. By comparing their retention times and peak with a standard mix of fatty acids (FAs) ("Supelco 37" component FAME mix, Cat No. 47885-U) the characteristic FAs were recognized and they were obtained in quantity [David et al. 2003].

Germination tests were conducted in a randomized complete block design including four replications. The data were subjected to ANOVA and means were compared by using Duncan's multiple range test. Arcsin transformation was made for percentage data before statistical analysis. Additionally, stepwise multiple regression and the correlation coefficients (r) between fatty acid profile and germination percentage and germination speed were determined for all temperatures except for 40ºC owing to the fact that no germination was observed in all species.

Data were presented as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA). The significant means were compared using Duncan's multiple range tests at $\alpha = 0.05$ level (n = 4).

RESULTS

The major fatty acids of the oil extracted from the seeds from pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), radish (*Raphanus sativus* L.) and cabbage (*Brassica oleracea* var. *capitata*) are shown in Table 1. The fatty acids content was significantly different among species' seed samples. Palmitic (C16:0), oleic (C18:1n-9) and linoleic (C18:2n-6), acids were sequentially the highest in concentration followed by stearic acid (C18:0) at less than 5% and miristic, palmitoleic, margaric, arachidic, erucic, behenic and nervonic acids at an even lower content (<1%) in pepper and eggplant. Erucic acid (C22:1n-9) was the principal fatty acid followed by oleic, linoleic, gadoleic and behenic acids and miristic, palmitic, palmitoleic, arachidonic and stearic acids at a lower content between <1% and 5% in radish and cabbage seeds (tab. 1).

Table 1. Fatty acid profile of pepper, eggplant, radish and cabbage seeds (%)

Fatty Acids	Pepper cv 'Doru-16'	Eggplant cv 'Pala-49'	Radish cv. 'Siyah'	Cabbage cv. 'Ekici-79'
C14:0	0.380 ± 0.105 a	0.267 ± 0.132 ab	0.201 ± 0.021 b	0.254 ± 0.027 ab
C14:1	–	–	0.021 ± 0.002 a	0.020 ± 0.001 b
C15:0	0.018 ± 0.001 a	–	0.014 ± 0.004 b	0.009 ± 0.004 c
C15:1	0.038 ± 0.011 b	0.060 ± 0.012 a	0.018 ± 0.002 c	0.011 ± 0.008
C16:0	10.844 ± 0.077 a	7.967 ± 0.098 b	5.244 ± 0.055 c	3.936 ± 0.188 d
C16:1n-7	0.219 ± 0.001 ab	0.235 ± 0.024 a	0.208 ± 0.007 b	0.182 ± 0.005 c
C17:0	0.064 ± 0.039 a	0.076 ± 0.002 a	0.029 ± 0.007 b	0.019 ± 0.005 b
C17:1	0.131 ± 0.002 a	–	0.056 ± 0.004 b	0.059 ± 0.001 b
C18:0	2.422 ±0 .036 b	3.474 ± 0.102 a	1.790 ± 0.006 c	0.924 ± 0.020 d
C18:1n-9	8.683 ± 0.008 d	12.622 ± 0.136 c	29.658 ± 0.242 a	14.388 ± 0.321 b
C18:2n-6	76.050 ± 0.554 a	74.245 ± 0.217 b	12.587 ± 0.145 d	13.312 ± 0.109 c
C20:0	0.311 ± 0.006 b	1.004 ± 0.026 a	–	-
C20:1n-9	–	–	11.458 ± 0.161 a	9.903 ± 0.012 b
C20:4n-6	–	–	1.002 ± 0.048 a	0.570 ± 0.017 b
C22:0	0.172 ± 0.115 c	–	10.321 ± 0.163 a	5.630 ± 0.068 b
C22:1n-9	0.206 ± 0.001 c	0.043 ± 0.026 c	27.395 ± 0.358 b	50.787 ± 0.046 a
C24:1n-9	0.116 ± 0.053	–	–	-

C14:0 – miristic acid, C16:0 – palmitic acid, C16:1n-7 – palmitoleic acid, C17:0 – margaric acid, C18:0 – stearic acid, C18:1n-9 – oleic acid, C18:2n-6 – linoleic acid, C20:0 – arachidic acid, C20:1n-9 – gadoleic acid, C20:4n-6 – arachidonic acid, C22:0 – behenic acid, C22:1n-9 – erucic acid C24:1n-9 – nervonic acid; means followed by different letters in line are significantly different at $P = 0.05$

Table 2. SFA, MUFA, n-6 PUFA and total oil of pepper, eggplant, radish and cabbage seeds

Species	SFA (%)	MUFA (%)	n-6 PUFA (%)	Total oil (%)
Pepper (cv 'Doru-16')	14.210 ± 0.158 b	9.392 ± 0.036 d	76.050 ± 0.554 a	15.530 ± 0.124 b
Eggplant (cv 'Pala-49')	12.788 ± 0.096 c	12.960 ± 0.126 c	74.245 ± 0.217 b	13.986 ± 0.528 b
Radish (cv. 'Siyah')	17.597 ± 0.134 a	68.813 ± 0.056 b	13.589 ± 0.193 c	28.809 ± 2.737 a
Cabbage (cv. 'Ekici-79')	10.771 ± 0.251 d	75.348 ± 0.341 a	13.882 ± 0.092 c	26.672 ± 0.725 a

SFA – saturated fatty acids, n-6 PUFA – poly-unsaturated fatty acids, MUFA – mono-unsaturated fatty acids; means in column followed by different letters are significantly different at $P = 0.05$

The total SFA, MUFA, n-6 PUFA and total oil contents were also different (tab. 2). Poly-unsaturated fatty acids composed nearly 75% of total fatty acids in pepper (76.05%) and eggplant (74.24%). Mono-unsaturated and saturated fatty acids contributed to 9.39% and 14.21% in pepper, and 12.96% and 12.78% in eggplant of the total fatty acids. On the other hand, mono-unsaturated composed between 68.81% and 75.34% of total fatty acids in radish and cabbage, respectively. Saturated fatty acids contributed to 17.59% and 10.77% of total fatty acids in radish and cabbage, respectively. Poly-unsaturated fatty acids composed with a concentration of nearly 13% of total fatty acids in radish and cabbage. However, seeds of all tested species contained high concentrations of oil (13.53–28.80%). The highest and lowest oil content were found in the seeds of *Raphanus sativus* L. cv. 'Siyah' and *Solanum melongena* L. cv 'Pala-49', respectively.

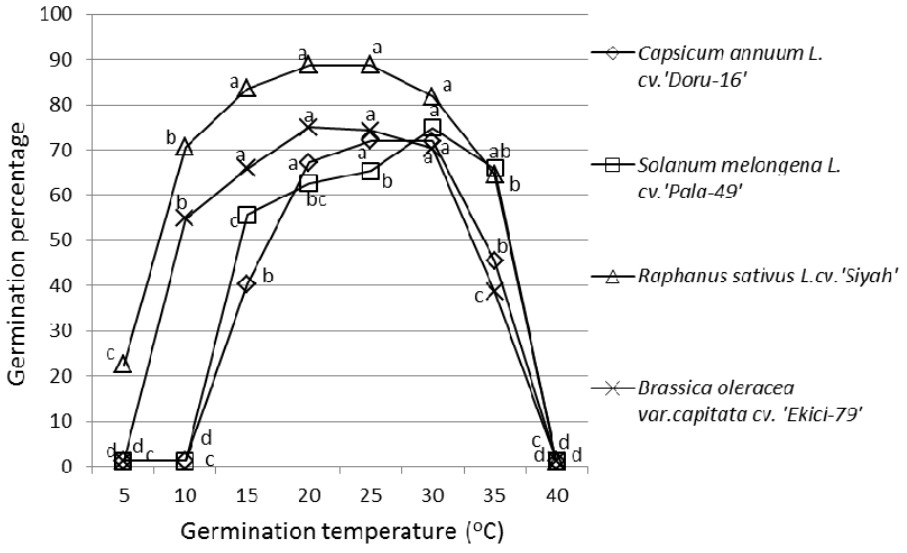

Fig. 1. Germination percentage of pepper, eggplant, radish and cabbage seeds for each species, means with different letters are significantly different at $P = 0.01$

The data on germination percentage, viability, of tested species at temperatures between 5°C and 40°C are presented in Fig. 1. There were statistically significant differences among germination temperatures in germination percentage ($P = 0.01$). Germination percentage of pepper, eggplant, radish and cabbage ranged from 1.28 to 72.10%, from 1.28 to 74.88%, from 22.51 to 88.72% and from 1.28 to 74.94%, respectively. Increasing temperature from 10°C to 30°C, and 20°C to 30°C increased germination for radish and cabbage, and pepper and eggplant, respectively. Increasing the temperature above 30°C resulted in decreased germination across species. The highest germination percentages of pepper and eggplant seeds were found at 25°C and 30°C and, for radish and cabbage at 20°C and 25°C.

Seed germination speed of four species at temperatures between 5°C and 40°C is shown in Fig. 2. There were statistically significant differences among germination temperatures in germination speed ($P = 0.01$). The highest germination speeds of species for pepper, eggplant, radish and cabbage were 21.35% (25°C), 20.48% (25°C), 30.33% (20°C and 25°C) and 25.84% (20°C), respectively. While increasing temperature from 5°C to 25°C resulted in increased germination speed, increasing temperature from 25°C to 35°C resulted in decreased germination speed across species. Additionally, temperatures between 20°C and 30°C were more effective than low or high temperatures on germination speed.

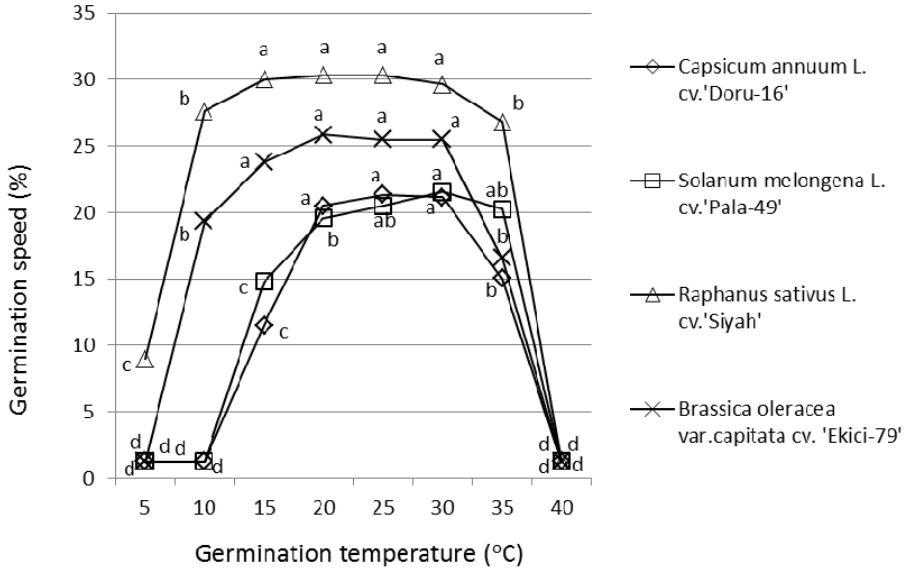

Fig. 2. Germination speed of pepper, eggplant, radish and cabbage seeds for each species, means with different letters are significantly different at $P = 0.01$

The correlation coefficients between fatty acid profile, total oil and seed germination percentage and speed at various temperatures for all species are shown in Table 3 and 4.

Table 3. Simple correlation coefficients (r) between fatty acid profile and final germination percentage at various temperatures

Fatty acids	Germination percentage at						
	5°C	10°C	15°C	20°C	25°C	30°C	35°C
C14:0	-0.423 NS	-0.513*	-0.613**	-0.662**	-0.437*	-0.444*	-0.332 NS
C14:1	0.599**	0.985**	0.878**	0.800**	0.762**	0.375 NS	-0.090 NS
C15:0	0.311 NS	0.182 NS	-0.009 NS	0.346 NS	0.433*	-0.142 NS	-0.473*
C15:1	-0.374 NS	-0.799**	-0.572*	-0.770**	-0.622**	-0.003 NS	0.457*
C16:0	-0.379 NS	-0.854**	-0.832**	-0.586**	-0.462*	-0.314 NS	0.002 NS
C16:1n-7	-0.057 NS	-0.634**	-0.406 NS	-0.540*	-0.323 NS	0.261 NS	0.551*
C17:0	-0.334 NS	-0.751**	-0.592**	-0.466*	-0.487*	-0.052 NS	0.286 NS
C17:1	-0.065 NS	-0.093 NS	-0.348 NS	0.034 NS	0.151 NS	-0.450*	-0.657**
C18:0	-0.223 NS	-0.772**	-0.520*	-0.550*	-0.510*	0.136 NS	0.609**
C18:1n-9	0.959**	0.829**	0.920**	0.849**	0.901**	0.841**	0.480*
C18:2n-6	-0.579**	-0.979**	-0.884**	-0.788**	-0.732**	-0.344 NS	0.123 NS
C20:0	-0.459*	-0.784**	-0.541*	-0.688**	-0.724**	-0.027 NS	0.502*
C20:1 n-9	0.654**	0.992**	0.910**	0.826**	0.791**	0.408 NS	-0.072 NS
C20:4n-6	0.820**	0.983**	0.940**	0.894**	0.903**	0.596**	0.126 NS
C22:0	0.843**	0.975**	0.946**	0.900**	0.919**	0.605**	0.127 NS
C22:1n-9	0.210 NS	0.825**	0.662**	0.546*	0.431*	-0.008 NS	-0.403 NS
C24:1n-9	-0.301 NS	-0.513*	-0.664**	-0.250 NS	-0.189 NS	-0.435*	-0.407 NS
SFA	0.865**	0.318 NS	0.424 NS	0.572*	0.755**	0.733**	0.518*
MUFA	0.509*	0.960**	0.856**	0.743**	0.672**	0.284 NS	-0.167 NS
n-6 PUFA	-0.576**	-0.978**	-0.883**	-0.785**	-0.729**	-0.340 NS	0.127 NS
Total oil	0.667**	0.968**	0.880**	0.826**	0.806**	0.357 NS	-0.139 NS

* – significant at $P = 0.05$, ** – significant at $P = 0.01$ and NS – not significant
For abbreviations see Table 1 and 2

Significant correlations were observed for the seed germination and fatty acid contents at various temperatures. Oleic (C18:1n-9) acid content was positively correlated with germination percentage in all temperatures from 5°C to 35°C. Plus, gadoleic (C20:1n-9) and, arachidonic (C20:4n-6) and behenic (C22:0) acids were positively correlated with germination percentage increasing temperature from 5°C to 25°C and 5°C to 30°C, respectively. Similarly, increasing temperature from 5°C to 25°C, linoleic (C18:2n-6) and arachidic (C20:0) acids were negatively correlated with germination percentage. In addition, C15:1, palmitic (C16:0), margaric (C17:0) and stearic (C18:0) were negatively correlated when temperature increased from 10°C to 25°C and other a few significant correlations were seen clearly in Table 3.

As seen in Table 4, C14:1, oleic (C18:1n-9), gadoleic (C20:1n-9), arachidonic (C20:4n-6), behenic (C22:0) acids, MUFA and total oil were positively; linoleic (C18:2n-6) and n-6 PUFA were negatively correlated with germination speed in all temperatures. In addition, miristic (C14:0), C15:1, palmitic (C16:0), palmitoleic (C16:1n-7) and margaric (C17:0) acids were negatively correlated with germination speed increasing temperature from 10°C to 30°C.

In addition to simple correlation coefficients between fatty acids, total oil and germination percentage and speed under various temperatures for all species; similar results were obtained in stepwise multiple regression analysis (tabs 5, 6). In other words, near to direct effect of fatty acids and total oil, indirect effects of them were determined.

Table 4. Simple correlation coefficients (r) between fatty acid profile and germination speed at various temperatures

Fatty acids	Germination speed at						
	5°C	10°C	15°C	20°C	25°C	30°C	35°C
C14:0	-0.408 NS	-0.519*	-0.601**	-0.573*	-0.524*	-0.527*	-0.527*
C14:1	0.582**	0.970**	0.948**	0.920**	0.911**	0.913**	0.505*
C15:0	0.327 NS	0.202 NS	0.035 NS	0.288 NS	0.275 NS	0.217 NS	-0.130 NS
C15:1	-0.364 NS	-0.772**	-0.674**	-0.782**	-0.716**	-0.695**	-0.151 NS
C16:0	-0.374 NS	-0.824**	-0.862**	-0.735**	-0.708**	-0.772**	-0.486*
C16:1n-7	-0.045 NS	-0.584**	-0.502*	-0.574*	-0.472*	-0.460*	0.075 NS
C17:0	-0.320 NS	-0.724**	-0.655**	-0.654**	-0.626**	-0.632**	-0.216 NS
C17:1	-0.061 NS	-0.093 NS	-0.264 NS	-0.031 NS	-0.031 NS	-0.128 NS	-0.518*
C18:0	-0.220 NS	-0.731**	-0.632**	-0.669**	-0.640**	-0.630**	0.014 NS
C18:1n-9	0.946**	0.869**	0.899**	0.895**	0.924**	0.925**	0.911**
C18:2n6	-0.571*	-0.962**	-0.943**	-0.910**	-0.895**	-0.913**	-0.490*
C20:0	-0.452*	-0.769**	-0.647**	-0.759**	-0.750**	-0.703**	-0.105 NS
C20:1n-9	0.645**	0.982**	0.962**	0.942**	0.933**	0.945**	0.545*
C20:4n-6	0.803**	0.993**	0.979**	0.982**	0.989**	0.984**	0.706**
C22:0	0.832**	0.989**	0.976**	0.983**	0.994**	0.991**	0.709**
C22:1n-9	0.206 NS	0.778**	0.746**	0.689**	0.650**	0.681**	0.153 NS
C24:1n-9	-0.296	-0.504*	-0.611**	-0.418 NS	-0.408 NS	-0.496*	-0.586**
SFA	0.854**	0.392 NS	0.383 NS	0.501*	0.563*	0.500*	0.664**
MUFA	0.502*	0.936**	0.918**	0.874**	0.853**	0.877**	0.438*
n-6 PUFA	-0.567*	-0.961**	-0.941**	-0.909**	-0.893**	-0.911**	-0.486*
Total oil	0.667**	0.961**	0.929**	0.931**	0.925**	0.932**	0.492*

* – significant at $P = 0.05$, ** – significant at $P = 0.01$ and NS – not significant
For abbreviations see Table 1 and 2.

Table 5. Stepwise multiple regression analysis between fatty acid profile and final germination percentage at various temperatures

Temperatures		Unstandardized coefficients		Standardized coefficients		
		B	std. error	Beta	t	*P* values
5°C	constant	-20.774	1.466		-14.174	0.000
	C18:1n-9	0.849	0.097	0.742	8.791	0.000
	SFA	1.793	0.302	0.490	5.933	0.000
	total oil	0.395	0.036	0.289	10.929	0.000
	C14:1	-678.3	45.718	-0.758	-14.837	0.000
	C18:0	-4.928	0.871	-0.503	-5.659	0.000
	C17:1	-38.315	12.364	-0.195	-3.099	0.013
	R square = 0.998					
	Y = -20.774 + (0.849 × C18:1n-9) + (1.793 × SFA) + (0.395 × total oil) – (678.3 × C14:1) – (4.928 × C18:0) – (38.315 × C17:1)					
10°C	constant	1.667	0.735		2.267	0.043
	C20:1n-9	3.514	0.354	0.596	9.920	0.000
	C20:4n-6	76.109	17.036	1.015	4.467	0.001
	C22:0	-4.491	1.568	-0.608	-2.863	0.014
	R square = 0.996					
	Y = 1.667 + (3.514 × C20:1n-9) + (76.109 × C20:4n-6) – (4.491 × C22:0)					
15°C	constant	53.647	1.157		46.369	0.000
	C22:0	9.276	1.823	2.391	5.088	0.000
	C17:1	-108.661	13.041	-0.303	-8.333	0.000
	C20:4n-6	-57.938	18.522	-1.472	-3.128	0.009
	R square = 0.981					
	Y = 53.647 + (9.276 × C22:0) – (108.661 × C17:1) – (57.938 × C20:4n-6)					
20°C	constant	76.522	3.025		25.295	0.000
	C22:0	1.419	0.227	0.576	6.255	0.000
	C14:0	-49.346	9.993	-0.462	-4.938	0.000
	C15:0	508.168	121.74	0.343	4.174	0.001
	R square = 0.929					
	Y = 76.522 + (1.419 × C22:0) – (49.346 × C14:0) + (508.168 × C15:0)					
25°C	constant	51.448	1.716		29.983	0.000
	C22:0	2.687	0.116	1.354	23.251	0.000
	C16:0	1.824	0.186	0.571	9.795	0.000
	R square = 0.979					
	Y = 51.448 + (2.687 × C22:0) + (1.824 × C16:0)					
30°C	constant	45.735	4.507		10.149	0.000
	C18:1n-9	0.556	0.056	0.924	9.878	0.000
	C16:1n-7	93.34	20.007	0.436	4.665	0.000
	R square = 0.874					
	Y = 45.735 + (0.556 × C18:1n-9) + (93.34 × C16:1n-7)					
35°C	constant	29.777	5.174		5.755	0.000
	C17:1	-212.916	19.093	-0.782	-11.151	0.000
	SFA	3.741	0.368	0.738	10.158	0.000
	Total oil	-0.638	0.136	-0.337	-4.704	0.001
	R square = 0.929					
	Y = 29.777 – (212.916 × C17:1) + (3.741 × SFA) – (0.638 × total oil)					

For abbreviations see Table 1 and 2

Table 6. Stepwise multiple regression analysis between fatty acid profile and germination speed at various temperatures

Temperatures		Unstandardized coefficients		Standardized coefficients		
		B	std. error	Beta	t	*P* values
5°C	constant	-8.429	1.231		-6.845	0.000
	C18:1n-9	0.294	0.035	0.686	8.312	0.000
	SFA	0.494	0.113	0.361	4.373	0.001
	R square = 0.951					
	Y = -8.429 + (0.294 × C18:1n-9) + (0.494 × SFA)					
10°C	constant	8.796	1.287		6.835	0.000
	C20:4n-6	20.4	1.305	0.743	15.63	0.000
	C18:2n-6	-0.1	0.018	-0.268	-5.648	0.000
	R square = 0.996					
	Y = 8.796 + (20.4 × C20:4n-6) – (0.1 × C18:2n-6)					
15°C	constant	17.431	0.563		30.941	0.000
	C15:0	-161.293	17.926	-0.156	-8.998	0.000
	C22:0	1.584	0.045	0.921	35.47	0.000
	C16:0	-0.336	0.072	-0.121	-4.658	0.001
	R square = 0.998					
	Y = 17.431 – (161.293 × C15:0) + (1.584 × C22:0) – (0.336 × C16:0)					
20°C	constant	21.034	0.525		40.086	0.000
	C22:0	0.36	0.188	0.351	1.916	0.079
	C15:1	-71.912	17.288	-0.338	-4.16	0.001
	C18:1n-9	0.232	0.078	0.421	2.99	0.011
	R square = 0.983					
	Y = 21.034 + (0.36 × C22:0) – (71.912 × C15:1) + (0.232 × C18:1n-9)					
25°C	constant	19.186	0.432		44.457	0.000
	C22:0	0.992	0.029	1.085	34.114	0.000
	C16:0	0.175	0.047	0.119	3.733	0.003
	R square = 0.994					
	Y = 19.186 + (0.992 × C22:0) + (0.175 × C16:0)					
30°C	constant	21.041	0.177		118.759	0.000
	C22:0	0.832	0.030	0.991	27.615	0.000
	R square = 0.981					
	Y = 21.041 + (0.832 × C22:0)					
35°C	constant	8.406	0.726		11.572	0.000
	C18:1n-9	0.614	0.034	1.103	18.022	0.000
	C20:0	4.439	0.662	0.411	6.710	0.000
	R square = 0.956					
	Y = 8.406 + (0.614 × C18:1n-9) + (4.439 × C20:0)					

For abbreviations see Table 1 and 2

Regression equations, R square and other details were shown in Table 5 and 6 separately for germination percentage and germination speed, respectively. As it was in correlation analysis, most of the fatty acids and total oil's indirect effect were determined and clearly seen in related tables.

DISCUSSION

It was determined that there were wide variations among the contents of major fatty acids of tested seeds. The total saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) fatty acids and total oil contents were also different. Such differences, may be due to various factors including harvest time, level of maturity, seasonal variation, drying conditions, variety, cultivar, source, soil and storage conditions [Al-Khalifa 1996, De Mello 2000]. In addition, some reports are available on the composition of fatty acids of pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), radish (*Raphanus sativus* L.) and cabbage (*Brassica oleracea* var. *capitata*) from different regions of the world. For example, Mandal et al. [2002] reported that palmitic, stearic, linoleic, gadoleic and erucic acids are the seven major fatty acids present in the oil extracted from members of the Brassicaceae, and the oleic acid values were changed according to the species such as 29.99%, in *Raphanus caudatus*, 25.25% in *Raphanus sativus*, 23.64%, % in *Sinapis alba* and the saturated fatty acids in tested Brassicaceae members i.e. palmitic and stearic acid were found to be present within the range of 2.23 to 4.71% and 0.99 to 1.68%, respectively. For all that radish seeds contained higher amounts of palmitic, oleic, and linolenic + eicosenoic acids and lower amounts of linoleic and erucic acids compared to cauliflower and turnip seeds [Ahuja et al. 1987]. In addition, the fatty acid composition of the Brassicaceae oil is genetically more variable than probably the composition of any other major vegetable oil [Mandal et al. 2002]. Similarly, Barthet [2008] reported that tested *Brassica* species had very different relative fatty acid compositions. In addition to these reports about fatty acid contents of Brassica species, although there limited data about eggplant, Pérez-Gálvez et al. [1999] reported that the major fatty acids accumulated in the pepper are palmitic, oleic, linoleic, and linolenic. Demir et al. [2008] similarly declared that linoleic acid was the most abundant fatty acid and constituted the maximum amount in total lipid content in pepper seeds. Also reported that oleic and palmitic acid contents of pepper seeds showed similar values, oleic acid content changed between 6.4% and 13.6% in fresh and between 9.3% and 10.7% in dried seeds. Plus, in mature pepper seeds, the most abundant fatty acid was linoleic acid (62–67%), followed by oleic acid and palmitic acid [Xu and Kafkafi 2003]. Results of this work were similar and confirmative previous mentioned studies.

Germination of seeds is the first developmental step in the life cycle of a plant to produce a new generation [Bewley 1997]. Vegetable seeds that had high germination under optimal laboratory conditions and germination percentages of seeds under optimum conditions could give up to 90% germination. And, a complex metabolic process, germination of higher plants seeds as vegetables, is involving many individual reactions and phases, each of which is affected by cardinal temperatures [Copeland and Mcdonald

1985, Wanasundara et al. 1999]. Several investigations have reported about the germinability of vegetable species under low and high temperatures for different aims. For example, the germination of pepper is slow at room temperature, and further delayed by cooler conditions (15–18°C). At 25°C, pepper required 3.5 days for radical emergence, while at 15°C, 9 days were required and, seed germination range of pepper types is between 15°C and 35°C, an optimum germination temperature of about 30°C [O'Sullivan and Bouw 1984, Swiader et al. 1992]. Similarly, eggplant seeds germinate slowly and susceptible to adverse temperatures [Swiader et al. 1992]. Plus, Nascimento and Lima [2008] also reported that low temperatures affected the germination percentage and germination rate of eggplant. Although the temperature markedly influences seed germination, the radish seeds were not affected by adverse temperatures and showed satisfactory germination (above 90%) at temperatures ranging from 10 to 35°C. However, high temperatures (35°C) negatively influenced the germination [Steiner et al. 2009]. Similarly, Cavusoglu and Kabar [2007] declared that high temperature both delayed and inhibited the germination of radish. The optimum germination temperature for cabbage seeds was 20°C, and that germination decreased remarkably when seeds were germinated at more than 35°C or less than 15°C [Wang et al. 2008]. Beyond, White [2000] reported that germination of cabbage was slower in the 15°C (only 1%) and the 23.5 and 27°C had from 7 to 81% germination on day 4, final germination taken at day 18 was similar for all temperatures and treatments with a range from 91 to 97% germination.

The statistical results according to the simple correlation coefficients and stepwise multiple regression analysis clearly revealed that fatty acid composition had a significant effect on the germination percentage and speed of the tested species and that germination percentage and germination were closely related to extra or deficient of fatty acids. Results of this study confirm previous studies owing to the fact that triglycerides, which are the major form of stored lipids in seeds, are hydrolyzed by lipases to diglycerides, monoglycerides, and then to glycerol and fatty acids. Glycerol is metabolized through glycosis after its oxidation to triose phosphates. The fatty acids are further oxidized through the □-oxidation pathway by sequentially removing two carbon atoms in the form of acetyl acetyl coenzyme A. Triglycerides comprise >90% of common plant seed oils, in which they play a major role as energy storage molecules [Krist et al. 2005]. In other words, the free fatty acids are further degraded by one of two processes: 1- α-oxidation plays a minor role in germinating seeds. 2- β-oxidation plays a major role during germination period with the aid β-oxidase, yielding acetyl coenzyme A and energy in the form of adenosine triphosphate (ATP) [Copeland and McDonald 1985]. Beyond, Huang and Grunwald [1990] and Wanasundara et al. [1999] reported that during germination of oilseeds, storage lipids provide fatty acids that serve as an energy source to produce ATP and soluble carbohydrates for the growth of new cells. In addition to these reports, Kaymak [2012] showed that palmitoleic (C16:1n-7), oleic (C18:1n-9), linoleic (C18:2n-6), arachidic (C20:0) acids, total saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) fatty acids and total oil were significantly correlated with germination percentage and speed in cucurbit seeds.

CONCLUSIONS

1. Total oil and fatty acid profile, most likely connected with species, influence germination percentage and speed of tested species and this effect in seed germination may have results of low or high contents of fatty acids under various temperatures.

2. Simple correlation coefficients and stepwise multiple regression analysis showed that the low or high amount of fatty acids in tested species such as palmitic (C16:0), palmitoleic (C16:1n-7), margaric (C17:0), stearic (C18:0), oleic (C18:1n-9), linoleic (C18:2n-6), arachidic (C20:0), gadoleic (C20:1n-9), arachidonic (C20:4n-6), behenic (C22:0), MUFA, n-6 PUFA and total oil may play a major role in seed germination under low and high temperatures.

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REFERENCES

Ahuja K.L., Singh H. Raheja R.K., Labana K.S., 1987. The oil content and fatty acid composition of various genotypes of cauliflower, turnip and radish. Plant Food Hum. Nutr. 37, 33–40.

Al-Khalifa A.S., 1996. Physicochemical characteristics, fatty acid composition, and lipoxygenase activity of crude pumpkin and melon seed oils. J. Agr. Food Chem. 44, 964–966.

Barthet V.J., 2008. (n-7) and (n-9) cis-monounsaturated fatty acid contents of 12 *Brassica* species. Phytochemistry 69, 411–417.

Bewley J.D., 1997. Seed germination and dormancy. Plant Cell. 9, 1055–1066.

Bewley J.D., Black M., 1994. Seeds: physiology of development and germination. Plenum Press, New York.

Cavusoglu K., Kabar K., 2007. Comparative effects of some plant growth regulators on the germination of barley and radish seeds under high temperature stress. EurAsia J. BioSci. 1, 1–10.

Copeland L.O., McDonald M.B., 1985. Principle of seed science and technology, 2nd edition. New York.

Da Silva T.R.G., Cortelazzo A.L., De Campos D.S.M., 1998. Variations in storage compounds during germination and early plantlet growth of *Dalbergia miscolobium*. Rev. Bras. Fisiol. Veg. 10, 119–124.

David F., Sandra P., Wylie P.L., 2003. Improving the analysis of fatty acid methyl esters using retention time locked methods and retention time databases. Application Note. http://www.chem.agilent.com/Library/applications/5988-5871EN.pdf.

De Mello M.L.S., Narain N., Bora P.S., 2000. Characterisation of some nutritional constituents of melon (*Cucumis melo* hybrid AF-522) seeds. Food Chem. 68, 411–414.

De Mello M.L.S., Narain N., Bora P.S., 2001. Fatty and amino acids composition of melon (*Cucumis melo* var. *saccharinus*) seeds. J. Food Compos. Anal. 14, 69–74.

Demir I., Tekin A., Okmen Z.A., Okçu G., Kenanoglu B.B., 2008. Seed quality, and fatty acid and sugar contents of pepper seeds (*Capsicum annuum* L.) in relation to seed development and drying temperatures. Turk. J. Agric. For. 32, 529–536.

Folch J., Less M., Stanley G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Huang L.S., Grunwald C., 1990. Lipid and fatty acid changes during germination of alfalfa seeds. Phytochemistry 29, 1441–1445.

ISTA, 1996. International rules for seed testing rules, International Seed Testing Association. Zurich.

Kaymak H.C., 2012. The relationships between seed fatty acids profile and seed germination in cucurbit species. Žemdirbystė 99, 299–304.

Kaymak H.C., Guvenc I., Yarali F., Donmez M.F., 2009. The effects of bio-priming with PGPR on germination of radish (*Raphanus sativus* L.) seeds under saline conditions. Turk. J. Agric. For. 33, 173–179.

Krist S., Stuebiger G., Unterweger H., Bandion F., Buchbauer G., 2005. Analysis of volatile compounds and triglycerides of seed oils extracted from different poppy varieties (*Papaver somniferum* L.). J. Agric. Food Chem. 53, 8310–8316.

Mandal S., Yadav S., Singh R., Begum G., Suneja P., Singh M., 2002. Correlation studies on oil content and fatty acid profile of some *Cruciferous* species. Genet. Resour. Crop Ev. 49, 551–556.

Metcalfe L.D., Schmitz A.A., 1961. The rapid preparation of fatty acid esters for gas chromatographic analysis. Anal. Chem. 33, 363–364.

Nascimento W.M., Lima L.B., 2008. Eggplant seed priming to improve germination at low temperature. Rev. Bras. Sementes 30, 224–227.

O'Sullivan J., Bouw W.J., 1984. Pepper seed treatment for low-temperature germination. Can. J. Plant Sci. 64, 387–393.

Pérez-Gálvez A., Garrido-Fernández J., Mínguez-Mosquera M.I., Lozano-Ruiz M., Montero De Espinosa V., 1999. Fatty acid composition of two new pepper varieties (*Capsicum annuum* L. cv. Jaranda and Jariza): effect of drying process and nutritional aspects. J. Am. Oil Chem. Soc. 76, 205–208.

Steiner F., Pinto Júnior A.S., Zoz T., Guimarães V.F., Dranski J.A.L., Rheinheimer A.R., 2009. Germination of radish seeds under adverse temperatures. Brazilian J. Agr. Sci. 4, 430–434.

Swiader J.M., Ware G.W., McCollum J.P., 1992. Producing vegetable Crops. Interstate Publishers Inc., Danville, Illinois.

Taylor A.G., 1997. Seed storage, germination and quality. In: The Physiology of Vegetable Crops, Wien H.C. (ed.). CABI Publishing, New York, USA, pp. 1–37

Wang G.Y., Gu G.L., Zhang L.Y., 2008. Adverse temperature tolerance induction in cabbage seed at germination stage. Chinese J. Eco-Agric. 16, 1158–1162.

White J.M., 2000. Cabbage seed germination in two media under three temperatures. Proc. Fla. State Hort. Soc. 113, 260–261.

Wanasundara P.K.J.P.D., Wanasundara U.N., Shahidi F., 1999. Changes in flax (*Linum usitatissimum* L.) seed lipids during germination. J. Am. Oil Chem. Soc. 76, 41–48.

Xu G., Kafkafi U., 2003. Seasonal differences in mineral content distribution and leakage of sweet pepper seeds. Ann. App. Biol. 143, 45–52.

PROFILE KWASÓW TŁUSZCZOWYCH W NASIONACH: POTENCJALNE ZWIĄZKI MIĘDZY KIEŁKOWANIEM NASION W WARUNKACH STRESU TEMPERATUROWEGO U WYBRANYCH GATUNKÓW WARZYW

Streszczenie. Badanie podjęto w celu scharakteryzowania profilu kwasów tłuszczowych nasion papryki (*Capsicum annuum* L.), oberżyny (*Solanum melongena* L.), rzodkiewki (*Raphanus sativus* L.) i kapusty (*Brassica oleracea* L. var. capitata) oraz relacji między profilem kwasów tłuszczowych a kiełkowaniem nasion pod wpływem stresu temperaturowego w warunkach kontrolowanych. Testy kiełkowania przeprowadzono w czterech powtórzeniach po 50 nasion z każdego gatunku w niskiej, optymalnej i wysokiej temperaturze (5, 10, 15, 20, 25 30, 35 i 40°C). Procent kiełkowania papryki, oberżyny, rzodkiewki i kapusty wynosił odpowiednio: 1,28–72,10, 1,28–74,88, 22,51–88,72 i 1,28–74,94. Największe stężenie kwasu palmitynowego (C16:0), oleinowego (C18:1n-9) i linolowego (C18:2n-6) stwierdzono w nasionach papryki i oberżyny. Kwas stearynowy (C18:0) występował w stężeniu mniejszym niż 5%, a kwas mirystynowy, oleopalmitynowy, margarynowy, arachidowy, erukowy, behemowy i nerwonowy w jeszcze mniejszym (<1%). W nasionach rzodkiewki i kapust kwas erukowy (C22:1n-9) był głównym kwasem. Kwas oleinowy, linolowy, gadoleinowy i behemowy wystepował w mniejszym stężeniu, a w jeszcze mniejszym kwas mirystynowy, palmitynowy, oleopalmitynowy, arachidonowy i stearynowy (<1% i 5%). Proste współczynniki korelacji i stopniowa analiza regresji wielokrotnej wykazały, że w badanych gatunkach niskie lub wysokie stężenia kwasów tłuszczowych takich jak palmitynowy C16:0), oleopalmitynowy (C16:1n-7), margarynowy (C17:0), stearynowy (C18:0), oleinowy (C18:1n-9), linolowy (C18:2n-6), arachidowy (C20:0), gadoleinowy (C20:1n-9), arachidonowy (C20:4n-6), behemowy (C22:0), MUFA, n-6 PUFA oraz olej całkowity mogą odgrywać główną rolę w kiełkowaniu nasion w warunkach niskiej lub wysokiej temperatury.

Słowa kluczowe: kwasy tłuszczowe, papryka, oberżyna, rzodkiewka, kapusta, kiełkowanie nasion

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