

RESEARCH ARTICLE

Pathway among fatty acid profile, seed germination, and vigor of watermelon cultivars

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ABSTRACT

Watermelon (*Citrullus lanatus*) seed is rich in oils and the fatty acid composition of watermelon seeds is a significant source of nutrition and income for especially Africa, the Middle East, and Asia countries. Although the fatty acid profile of watermelon seed is well documented, the underlying associations with seed traits have not yet been studied. Therefore, the current study aimed to determine both fatty acid profile of watermelon seeds and the relations between the fatty acid profile and seed/seedling characteristics. For this purpose, two common watermelon cultivars (Alaska F₁ and Galaktika) of seed were used to examine the first count, germination percentage, vigour, vigour index, seed coat ratio, 100-seed weight as well as fatty acid profile. Vegetative traits including seedling height, diameter, dry matter, and leaf area were also determined in these cultivars. As a result, there were significant differences among cultivars for vigour, vigour index, seedling height, seedling diameter, and fatty acids. In addition, linoleic (C18:2n-6) (68%), oleic (C18:1n-9) (15%), and palmitic acids (C16:0) (9.7%) were considerably highest in concentration in the watermelon seeds. The correlation analysis showed that palmitoleic acid (C16:1n7) and arachidic acid (C20:0) in watermelon seeds could play an important role in signalling seed and seedling traits. Also, seedling dry matter can be used indicator of a large number of fatty acids based on the correlation results. According to principal component analyses, among several fatty acids, C16:1n-7, C18:1n-9, and monounsaturated fatty acid (MUFA) were found close to each other. The path analyses indicated that MUFA, C16:1n-7, and C18:1n-9 could be used as markers to estimate the first count, germination rate, and vigour of watermelon seeds. To the best of our knowledge, this is the first research that aimed to determine seed and seedling traits of watermelon cultivars based on the fatty acid profile.

Keywords: *Citrullus lanatus*; Fatty acid; Germination; Seed; Vegetative traits

INTRODUCTION

Agricultural production is mainly dependent on the seed quality such as the purity, vigour, moisture content, genetic characteristics and germination ability (McDonald, 1999). The most important issue among them is seed vigour and it is known that seed vigour represents the yield potential in terms of the highest plant product in the oldest period under different environmental field conditions (Al-Maskri et al., 2004). It has been determined that lipid peroxidation takes place in the cell membranes of flour and the increase in the amounts of free fatty acids is the main indicator of the seed spoilage mechanism. One of the most important reasons for the deterioration of seeds is oxidation of fats. This oxidative degradation generally starts with unsaturated fatty acids in the cell membrane and the oxidation rate links to the ratio of unsaturation in the fatty acid molecule. In addition, the most important fatty acids in lipid degradation are linolenic acid, which is very rapidly affected by

enzymatic reactions or non-enzymatic metabolites. It has been reported that the higher the ratio of unsaturation, the higher the rate of degradation (Braccini et al., 2000).

Dietary fat is one of the most important macronutrients in the human diet. It is the most concentrated source of energy, acts as a medium of fat-soluble vitamins and essential fatty acids, and performs other important physiological functions in the organism. The biological importance of fat is mainly determined by the type and ratio of fatty acids. The presence of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), of which vegetable oils are good sources, seems particularly desirable (Bialek et al., 2017). Moreover, the fatty acid content is the most important determinant of the quality of edible oil. The oils with higher MUFAs are preferred for their shelf life extension and potential health benefits. Therefore, a high oleic/linoleic fatty acid concentration is a target trait in a breeding program

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(Bera et al., 2019). Besides MUFA and PUFA, saturated fatty acids (SFA) are considered to increase the level of serum low-density lipoprotein cholesterol in the blood (Johnson and Saikia, 2008). A high level of palmitic acid in the oil also boosts the risk of cardiovascular disease (Kratz et al., 2002). A higher proportion of linoleic acid results in off-flavours, rancidity, the short shelf life of the oil and its derivatives, making it undesirable for cooking purposes (WHO, 2003). In terms of health, MUFA has been desirable in lowering plasma cholesterol levels and reducing the risk of cardiovascular diseases (Vassiliou et al., 2009; Wang, 2009).

As aforementioned aspect, many studies have focused on the nutrition and health effects of fatty acids up to date. Specifically, limited information is available on the relationship between seed traits and fatty acids. In previous reports, the authors found that both seed germination and vigour are two vital seed traits that could be influenced by colour of seed and fatty acid content (Dogras et al. 1977; Kaymak, 2012; Kaymak, 2014a; Kaymak, 2014b, Kaymak, 2015). The fatty acid content of the seed has been related to germination and seed vigour in different crops. For example, in tomato, it has been found that seed germination at cold temperatures was significantly correlated with linolenic acid concentration of seed whereas negatively correlated with oleic acid concentration. In addition, Dogras et al. (1977) stated that there was a positive correlation between seed chilling resistance and unsaturation of the fatty acids in peas and broad bean seeds. The unsaturated and/or saturated fatty acid ratio of seed lipids in cotton seed exhibited a low correlation with germination percentage, while it was strongly correlated with emergence at low soil temperatures under field conditions (Bartkowski et al. 1977). It has been determined by many researchers that the oils stored in the seed are converted into carbohydrates and sugars in the cotyledon or endosperm. The lipase enzyme in the first step hydrolyses fats in the cotyledon or endosperm. Therefore, the conversion of fatty acids to sucrose is one of the first steps in germination (Kacar, 1989).

Watermelon (*Citrullus lanatus*) belongs to the *Cucurbitaceae* family and it is a worldwide cultivated vegetable valued for its high-water content, sweet flavour, and low-caloric value (Maoto et al., 2019). It is also a highly popular crop with great economic value and it is one of the world's most important vegetables for livelihood of millions. Global watermelon production is 102 million tons (FAOSTAT, 2020). China (60 m tons) is the world's largest watermelon producer and Turkey (3.5 m tons) is the second leading producer. Other leader countries are India (2.8 m tons), Iran (2.7 m tons), Algeria (2.2 m tons), and Brazil (2.1 m tons).

Watermelon seeds are used for subsistence oil production in various African countries and the Middle East (Sabahelkhier et al., 2011). Although the oil content of watermelon seeds vary according to the genotype, it is in the range of 10-35% (Ziyada and Elhussien, 2008; Mahla et al., 2018). Watermelon seed oil is a rich source of essential fatty acids, the amount of which varies depending on the extraction method and cultivar (Ouassor et al., 2020). Linoleic acid is the most abundant fatty acid in watermelon seed oil, regardless of the cultivar. Moreover, diverse bioactivities of the watermelon seed oil have been reported, including anti-inflammatory, antioxidant, antimicrobial, and cardioprotective activities (Eidangbe et al., 2010; Madhavi et al., 2012; Jorge et al., 2015; Bello et al., 2016; Thongtha et al., 2017). For this reason, watermelon seed oil can be a good alternative source of plant-derived oil for utilizing pharmaceutical applications (Biswas, 2017). In Turkey, especially in the eastern and southeastern regions, watermelon seeds are also consumed as snacks (Ok and Yilmaz, 2019).

As a result, when the research are evaluated in general, there are significant changes in seed quality depending on the oil content of the seeds and the distribution of fatty acids. However, there is a scarce study on the fatty acid and relationships between seed (first count, vigour, germination and others.) quality criteria's and seedling traits (height, diameter, dry matter etc.). Therefore, the purpose of the research presented in this paper is to understand how the fatty acid profile of watermelon cultivars affects the seed and seedling traits in particular. The current research also represents an attempt to study the correlation between some vegetative phenomena and seed traits in watermelon seeds from a different cultivar.

MATERIALS AND METHODS

Watermelon seeds (*Citrullus lanatus* L. cv. 'Alaska F₁') and (*Citrullus lanatus* L. cv. 'Galaktika') were used as the plant material. The 'Alaska F₁' seeds were obtained from Golden Seed Company (Izmir, Turkey) and the 'Galaktika' seeds were obtained from Bursa Seed Company (Bursa, Turkey).

These cultivars were chosen based on very strong plant structure, productivity, earliness, long shelf life, and high tolerance to anthracnose and fusarium. Standard germination tests were conducted using four replicates of 50 seeds from each species in Petri dishes in the dark. They were placed in a growth chamber for 14 days at 25 °C (ISTA, 1996). The seeds were incubated between two filter papers saturated with water containing Benlate 1 g L⁻¹ to prevent fungal growth. Visible-radicle protrusion was the criterion of germination (Kaymak et al., 2009).

The first count was verified on the 7th day (ISTA, 1996). Germinated seeds were recorded and discarded at 24-hour intervals during the 8 and 14 days (ISTA, 1996) and the results were expressed as the final germination percentage.

Measurement of germination parameters

The seed germination percentage was determined by following the equation (Maguire, 1962):

Germination percentage (GP) = (germinated seed number / total seed number) × 100

The seed vigour was estimated after twenty days (radicle longer than 2 mm). Vigour index (VI) was calculated using the following equations (Lu et al., 2015):

VI = (mean shoot length (cm) × GP (%))

We calculated seed coat ratio (SCR) depending on values of watermelon seed mass, seed coat mass, and seed kernel mass (Wu et al., 2019). Four samples of 100 seeds from each group were used to determine the mean seed weights (SW)(100-weights).

Apart from the germination experiment, seeds of the two cultivars were sown in a plastic seedling tray containing a mixture of peat and perlite (3:1 v/v) under plastic greenhouse conditions. Twenty seedlings of each cultivar, at 3–4 true leaf stages, were evaluated based on seedling height (cm), and seedling diameter (cm) using a millimetric ruler, the results were expressed in centimetres. For seedling dry matter, samples were dried in the oven at 70°C to a constant weight and seedling dry matter was given as a percentage. For leaf area, leaves were measured with a leaf area meter (CI-202 Portable laser leaf area meter by CID Bio-Science, USA).

Measurement of Fatty Acids Content

Lipid extraction of seeds of watermelon cultivars was made according to the method of Folch et al. (1957). Briefly, the samples were homogenized in chloroform/methanol (2:1 v/v) containing 0.01% (w/v) of butylated hydroxytoluene (“Sigma”, ≥99.0% (GC)) for 1 min. The obtained homogenate was carried out in ice and other mediums (filtration, incubation, etc.) at room temperature (20–22°C). Then, the solvent was evaporated under a stream of nitrogen and the lipid amount was determined gravimetrically. Fatty acid methyl esters (FAMES) were prepared from lipids as described in Metcalfe and Schmitz (1961). The crude lipid extract was saponified with sodium hydroxide (NaOH) in methanol and FAMES were prepared by transmethylation with boron trifluoride (BF₃) in methanol. FAMES were analysed with a gas chromatography (GC) (Agilent 6890 N), equipped with a

23-capillary column (60 × 0.25 mm, 0.25 μm) and flame ionization detector. Injector and detector temperature were set at 190°C for 35 min initially, then increased at 30°C per minute up to 220°C and held for 5 min. Hydrogen with a flow rate of 2 mL min⁻¹ with split ratio was 30:1 was used as the carrier gas. The individual fatty acids (FAs) were identified by comparing their retention times to those of a standard mix of FAs (“Supelco 37” component FAME mix, Cat. No. 47885-U) and quantified by comparing their peak (David et al., 2003).

Statistical analysis

This experiment was designed as randomized complete block design and each analysis was replicated four times. The data were subjected to one-way analysis of variance (ANOVA) in SPSS statistics software (Version 20.0, USA). Means were compared by using Duncan’s multiple range test (MSTAT-C software) at 0.05 error level. Data were presented as mean ± standard deviation (SD) of the mean. Additionally, correlation coefficients (r) among total oil, fatty acid profile, first count, germination percentage, vigour, vigour index, seed coat ratio, 100-seed weight, seedling height, seedling diameter, seedling dry matter, and leaf area were determined. Principal component analysis was performed to evaluate the relationships between fatty acids among watermelon cultivars. In addition, AMOS software was used for path analysis with a graphical method to present complex relationships among studied variables. A diagram, a pictorial representation of a model is transformed into a set of equations. The set of equations is solved simultaneously to test model fit and estimate parameters.

RESULTS AND DISCUSSIONS

The seed first count varied from 81% to 91%, and germination percentage from 86% to 91% among cultivars as shown in Table 1. The statistical evaluations revealed that neither the Alaska F₁ nor the Galaktika affected germination percentages, which was similar to that observed in watermelon cultivars by Radke et al. (2017). Although there is no significant difference among cultivars, Alaska F₁ showed a higher value in germination and this is the accepted optimum rate for watermelon (90-95%) (Paksoy et al., 2010). Vigour level has the potential to evaluate the physiological potential of seeds. As shown in Table 1, the highest values in vigour (96.7%) and vigour index (4.01%) belonged to seeds of the Alaska F₁. When comparing with the findings of other researchers, Matthews (1980), Silva and Vieira (2006) indicated that when germination occurs more rapidly, demonstrating higher germination values at the first count, may be considered more vigorous than those showing slower germination. This inconsistency may be a methodological

contrast and cultivar tendency. From Table 1, the seed coat ratio showed very similar values in cultivars. The 100 seed weight is an important indicator of seed size because it is usually a combination of seed length, seed width, seed hilum length, and seed thickness (Gong et al., 2022). Concerning 100-seed weight amongst cultivars, this attribute changed between 31.3 g to 42.5 g and the 100 seeds of Galaktika were at the highest weight (Table 1). In our results, seed weight and germination results showed different tendencies. Namely, Galaktika had a notably higher seed weight whereas it acted the same in terms of germination. These results were in agreement with those reported by Ocana Gil et al. (1991) and Kaura (2009). According to experience, all aforesaid differences may be resulted from cultivar characteristics. A similar effect has been noted in previous reports that the quality of watermelon seeds is dependent on the stage of seed maturation at harvest (Nerson and Paris, 1988; Nerson, 2002).

From Table 2, it is obvious that there were significant differences in seedling height and seedling dry matter among the Alaska F₁ and Galaktika cultivars. While the Alaska F₁ had the highest seedling height (4.37 cm), and a higher proportion of dry matter was determined in the Galaktika cultivar. It has been reported that seedling growth can be measured by seedling height and dry matter (Radke et al., 2017). The dry matter of seedlings has been associated with seed vigour because seeds provide a greater transfer of dry matter from their reserve tissues to the embryonic axis (Nakagawa, 1999). Leaf area measurements assume a great significance in plant growth studies in terms of guaranteeing the production of photo assimilates for the fruits and the plant (Rouphael et al., 2010; Melo

et al., 2014). In the current research, it was found that there were no significant differences in leaf area in tested cultivars. Seedling diameter depends more precisely on photosynthesis (Taiz and Zeiger, 2013). As seen in Table 2, the seedling diameter varied from 0.30 to 0.40 and we did not observe a statistical difference in this trait.

Oil content in the watermelon seeds is between 35% and 40% and the unsaturated fat in oil is 78%–86% prevalently linoleic acid (45%–73%) (Sarfaraz et al., 2020). As a matter of fact, watermelon seed oils have been widely studied depending on having a high content of linoleic acid (Ziyada and Elhussien 2008, Taiwo et al., 2008). Table 3 shows the fatty acid profile of the watermelon seeds. C16:0 (9.7%, on average), C18:1n-9 (15%, on average) and C18:2n-6 (68%, on average) are the main fatty acids present in seeds with a predominance of C18:2n-6, in agreement with the values found by Nolte and Loesecke (1939) (65.85%) and de Conto et al. (2011) (65.61%). For comparison, C18:2n-6, C18:1n-9, and C16:0 were previously reported to be in a range of 45.1–76.2%, 0.33–33.66%, and 4.30–16.2%, respectively (Biswas, 2017). Our results were in agreement with data from the previous literature evaluating the relative amounts of these three major fatty acids (Petchsomrit et al., 2020). The present results were also lower than those reported for C16:0 (15%), C18:0 (12%) and C18:1n-9 (21.15%) in four different watermelon seeds of cultivars (Raziq et al., 2012). However, the contents of C18:2n-6 was found to be higher than those reported (49%). In addition, our C16:0 and C18:1n-9 results are lower than Osaie et al. (2021), who found them in 2 seeds of watermelon cultivars as 16.65% and 22.01%, respectively. From the viewpoint of fatty acids, two noticeable differences were determined in cultivars based on statistical evaluations at 0.01 and 0.05% levels (Table 3). The Alaska F₁ had a greater rate in C16:1n-7 and C18:1n-9 than the Galaktika. On the other hand, the Galaktika showed reasonably higher levels in

Table 1: First count, germination percentage, vigour, vigour index, seed coat ratio, and 100-seed weight of two watermelon seeds

Seed traits	Abbreviations	Alaska F ₁	Galaktika
First count (%)	(FC)	91.7 ^{NS}	81.7
Germination percentage (%)	(GP)	91.7 ^{NS}	86.7
Vigour (%)	(V)	96.7 ^a	83.3 ^b
Vigour index	(VI)	4.01 ^a	2.84 ^b
Seed coat ratio (%)	(SCR)	44.0 ^{NS}	44.3
100-seed weight (g)	(SW)	31.3 ^b	42.5 ^a

NS: Non-significant. *Small letters show differences among cultivars in each row at 5% error level according to Duncan's multiple range test.

Table 2: Seedling height, plant diameter, plant dry matter, and leaf area of two watermelon cultivars

Seedling traits		Alaska F ₁	Galaktika
Seedling height (cm)	(SH)	4.37 ^{a*}	3.27 ^b
Seedling diameter (cm)	(SD)	0.40 ^{NS}	0.30
Seedling dry matter (%)	(SDM)	14.60 ^{b*}	16.50 ^a
Leaf area (cm ²)	(LA)	5.20 ^{NS}	5.12

NS: Non-significant. *Small letters show differences among cultivars in each row at 5% error level according to Duncan's multiple range test.

Table 3: Fatty acid profile of two watermelon seeds

Fatty acids (%)		Alaska F ₁	Galaktika
C14:0	Myristic acid	0.293±0.189 ^{NS}	0.372±0.119
C16:0	Palmitic acid	9.900±0.212 ^{NS}	9.487±0.233
C16:1n-7	Palmitoleic acid	0.099±0.001 ^{a1,2**}	0.054±0.001 ^b
C17:0	Margaric acid	0.086±0.005 ^{b*}	0.111±0.009 ^a
C18:0	Stearic acid	5.591±0.105 ^{b*}	6.746±0.591 ^a
C18:1n-9	Oleic acid	17.611±0.876 ^{a**}	12.411±0.639 ^b
C18:2n-6	Linoleic acid	66.022±1.342 ^{b**}	69.972±1.383 ^a
C20:0	Arachidic acid	0.254±0.006 ^{b**}	0.282±0.005 ^a
C22:1n-9	Erucic acid	0.145±0.066 ^{NS}	0.086±0.021
SFA	Total saturated	16.123±0.138 ^{NS}	16.996±0.708
MUFA	Monounsaturated	17.854±0.811 ^{a**}	12.550±0.659 ^b
n-6 PUFA	Polyunsaturated	66.022±0.949 ^{b*}	70.455±1.366 ^a
Total oil		22.972±1.036 ^{a*}	21.260±0.054 ^b

NS: Non-significant. ¹Small letters show differences among cultivars in each row ²*P = 0.05, ^{**}P = 0.01.

C18:2n-6 (69.972%) and C20:0 (0.282 %) rather than C17:0 (0.111%) and C18:0 (6.746%). El-Adawy and Taha (2001) found C18:2n-6 (59.61%), C18:1n-9 (18.07%) and C16:0 (11.3%). The results are comparable to the findings of Baboli and Kordi (2010): C18:2n-6 (68.3%), C18:1n-9 (13.3%), C16:0 (11.4%), and C18:0 (7%). Some differences in results can probably be explained based on the difference in watermelon cultivars used in each study. However, C14:0, C16:0, and C22:1n-9 contents of cultivars showed very similar values. Our results, considering saturated/unsaturated fatty acids, MUFA and total oil had higher values in the Alaska F₁ as compared to the Galaktika, whereas the highest n-6 PUFA content was in the Galaktika cultivar. As for SFA, it did not change remarkably among cultivars. Our all fatty acid values were close to the value of de Conto et al. (2011) and are in complete agreement with the report of Kaymak (2012) on watermelon.

To determine whether there is a linear relationship between the examined parameters, first of all, correlation analysis was performed. The results of correlation analyses among fatty acids and seeds with seedlings of watermelon examined are shown in Table 4. Pearson correlation test exhibited significant statistical correlations among watermelon cultivars either positive or negative (Table 4). It was clear that there was a strong correlation between C16:1n7 and SD. In addition, while C16:1n7 was highly negatively correlated with SDM, it was moderately positively correlated with FC, V, and SH. Furthermore, C17:0 was positively correlated with SW and SDM. It was also showed a negative correlation with FC and V. As for C18:0, it was negatively correlated with FC and V. C18:1n9 moderately positively correlated with SD, whereas it was negatively correlated with SDM and SW. Regarding C20:0, this parameter was only positively correlated with SDM. This was highly negatively correlated with SH and moderately correlated with GP, SD, and FC. A significant

negative correlation was observed between SFA and V. MUFA showed a strong positive correlation with a SD but a negative correlation with both SW and SDM. Our results are not consistent with Kaymak (2012), who found that C18:1n9, C18:2n6, and MUFA were significantly correlated with the germination percentage and first count. Likewise, our results do not support the earlier findings in soybean and cabbage seeds (Hailstones and Smith, 1988). Because it was stated that there was a relationship between unsaturated fatty acids and seed vigour.

As a result of the correlation analysis, it was revealed that there were many statistically significant relationships between the variables. Principal component analysis was performed to overcome this problem, as it would be difficult to accurately and clearly explain many statistically significant relationships and 3 parameters (C16:1n-7, C18:1n-9 and MUFA) which factor loadings were significant as a result of this analysis were determined (Fig. 1). In addition, path analysis method was applied to determine both direct and indirect effects of these determined parameters on dependent variables (Fig. 2). As a result of the path analysis, it was revealed that the RMSEA value was significant at the $P > 0.05$ level and the model fit indices values (GFI: 0.746 and CFI: 0.356) were compatible and acceptable. When the path coefficients are examined, it has been determined that the effect of MUFA on FC has the highest coefficient (1003.96) and positive effect in terms of contribution to the model. In a similar manner, MUFA also has a positive effect on GR and the Path coefficient was determined as 48.64. The effect of C16:1n-7 on FC was found to be significantly higher (663.07). Path coefficients of the positive effect of C18:1n-9 on FC and V were determined as 155.09 and 28.43, respectively. When the negative effects are examined, it was determined that MUFA had -53.75 path coefficients on V, C18:1n-9 had -29.38 on GR, and C16:1n-7 had -2.10 on V followed by -1.70 on GR.

Table 4: Relationships between fatty acids and seeds with seedlings of watermelon

	FC ¹ (%)	GP (%)	V (%)	SCR (%)	SW (g)	LA (cm ²)	SH (cm)	SD (cm)	SDM (%)
C14:0	0.071 ^{NS}	0.132 ^{NS}	0.003 ^{NS}	-0.659 ^{NS}	0.145 ^{NS}	-0.155 ^{NS}	-0.154 ^{NS}	-0.241 ^{NS}	0.177 ^{NS}
C16:0	0.414 ^{NS}	0.405 ^{NS}	0.385 ^{NS}	0.549 ^{NS}	-0.576 ^{NS}	0.104 ^{NS}	0.651 ^{NS}	0.681 ^{NS}	-0.611 ^{NS}
C16:1n7	0.945 [*]	0.891 ^{NS}	0.911 ^{*1,2}	0.439 ^{NS}	-0.987 ^{**}	0.370 ^{NS}	0.928 [*]	0.999 ^{**}	-0.994 ^{**}
C17:0	-0.978 [*]	-0.890 ^{NS}	-0.975 [*]	-0.287 ^{NS}	0.938 [*]	-0.491 ^{NS}	-0.777 ^{NS}	-0.878 ^{NS}	0.923 [*]
C18:0	-0.918 [*]	-0.773 ^{NS}	-0.978 [*]	-0.408 ^{NS}	0.894 ^{NS}	-0.655 ^{NS}	-0.642 ^{NS}	-0.806 ^{NS}	0.870 ^{NS}
C18:1n9	0.822 ^{NS}	0.770 ^{NS}	0.797 ^{NS}	0.541 ^{NS}	-0.916 [*]	0.331 ^{NS}	0.887 ^{NS}	0.959 [*]	-0.932 [*]
C18:2n6	-0.692 ^{NS}	-0.685 ^{NS}	-0.635 ^{NS}	-0.481 ^{NS}	0.804 ^{NS}	-0.163 ^{NS}	-0.859 ^{NS}	-0.883 ^{NS}	0.831 ^{NS}
C20:0	-0.901 [*]	-0.967 [*]	-0.752 ^{NS}	-0.069 ^{NS}	0.885 ^{NS}	0.006 ^{NS}	-0.999 ^{**}	-0.933 [*]	0.900 [*]
C22:1n9	0.618 ^{NS}	0.818 ^{NS}	0.361 ^{NS}	-0.568 ^{NS}	-0.474 ^{NS}	-0.474 ^{NS}	0.747 ^{NS}	0.519 ^{NS}	-0.485 ^{NS}
SFA	-0.816 ^{NS}	-0.650 ^{NS}	-0.906 [*]	-0.355 ^{NS}	0.768 ^{NS}	-0.701 ^{NS}	-0.466 ^{NS}	-0.650 ^{NS}	0.735 ^{NS}
MUFA	0.830 ^{NS}	0.783 ^{NS}	0.799 ^{NS}	0.525 ^{NS}	-0.920 [*]	0.319 ^{NS}	0.896 ^{NS}	0.963 [*]	-0.936 [*]
n-6 PUFA	-0.692 ^{NS}	-0.685 ^{NS}	-0.635 ^{NS}	-0.481 ^{NS}	0.804 ^{NS}	-0.163 ^{NS}	-0.859 ^{NS}	-0.883 ^{NS}	0.831 ^{NS}
Total oil	0.583 ^{NS}	0.410 ^{NS}	0.701 ^{NS}	0.883 ^{NS}	-0.750 ^{NS}	0.651 ^{NS}	0.548 ^{NS}	0.759 ^{NS}	-0.758 ^{NS}

NS: non-significant. ¹Small letters show differences among traits in each fatty acid. ²*P = 0.05, **P = 0.01.

¹FC: First count, GP: Germination percentage, V: Vigour, SCR: Seed coat ratio, SW: 100-seed weight, LA: Leaf area, SH: Seedling height, SD: Seedling diameter, SDM: Seedling dry matter.

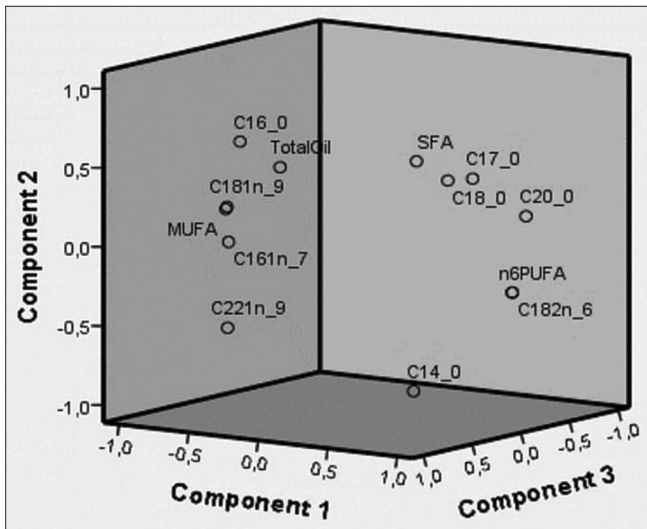


Fig 1. Principal component analysis showing the relationships fatty acids among watermelon cultivars

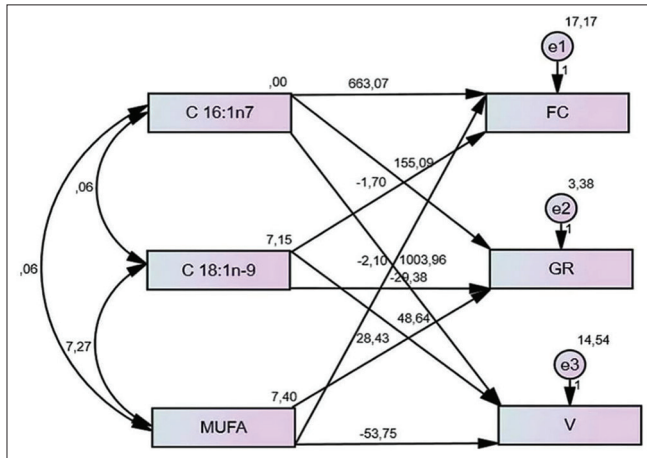


Fig 2. Path diagram of cultivars between fatty acids and first count (FC), germination rate (GR), vigour (V)

CONCLUSIONS

Based on the results, seed germination and vigour as well as fatty acid profiles of the two studied cultivars showed some significant differences. When compared to seed characteristics, the Alaska F₁ watermelon cultivar has high vigour and vigour index than Galaktika. Regarding seedling traits, seedling height and seedling diameter are at considerably higher levels in Alaska F₁ than in Galaktika. Moreover, fatty acid results indicate that both watermelon seeds of cultivars are rich sources of linoleic, oleic, and palmitic acids. The correlation test revealed that C16:1n7 and C20:0 fatty acids had significant relationships with most of the seed and seedling traits. In terms of seed and seedling traits, the seedling dry matter is correlated with a large number of fatty acids. Therefore, it is probable that the data for the C16:1n7 and C20:0 ratios could be used to determine the variations between other watermelon

cultivars. Apart from this, principal component analyses exhibited that C16:1n-7, C18:1n-9, and MUFA were much closer to each other than other variables. In addition to these findings, the path diagram showed the direct effect of MUFA, C16:1n-7, and C18:1n-9 fatty acids on the first count, germination rate, and vigour of watermelon seeds. This data enforces the idea that these three fatty acids could be an important contributor to some seedling traits of watermelon seeds. This may allow for even greater possibilities in estimating the response of seed and vegetative traits of any other species under different conditions.

AUTHORS CONTRIBUTIONS

Haluk Çağlar Kaymak: Conceptualization, methodology, validation, formal analysis, investigation, data curation, writing - review and editing, visualization, supervision.

Selen Akan: Methodology, validation, formal analysis, investigation, writing - original draft, review and editing.

Faika Yaralı Karakan: Methodology, validation, formal analysis, investigation, writing - original draft, review and editing.

REFERENCES

Al-Maskri, A. Y., M. M. Khan, M. J. Iqbal and M. Abbas. 2004. Germinability, vigour and electrical conductivity changes in acceleratedly aged watermelon (*Citrullus lanatus* T.) seeds. *J. Food Agric. Environ.* 2: 100-103.

Baboli, Z. M and A. A. S. Kordi. 2010. Characteristics and composition of watermelon seed oil and solvent extraction parameters effects. *J. Am. Oil Chem. Soc.* 87: 667-671.

Bartkowski, E. J., D. R. Buxton, R. H. Katterman and H. W. Kircher. 1977. Dry seed fatty acid composition and seedling emergence of Pima cotton at low soil temperatures. *Agron. J.* 69: 37-40.

Braccini, A. deL.E., Reis, M.S., Moreira, M.A., Sedyama, C.S and Scapim, C.A. 2000. Biochemical changes associated to soybean seeds osmoconditioning during storage. *Pesq. Agropec. Bras. Brasilia* 35: 433-447.

Bello, H. S., H. Y. Ismail, M. H. Goje and H. K. Mangga. 2016. Antimicrobial activity of *Citrullus lanatus* (watermelon) seeds on some selected bacteria. *J. Biotechnol. Res.* 2: 39-43.

Bera, S. K., J. H. Kamdar, S. V. Kasundra, S. V. Patel, M. D. Jasani and A. K. Maurya, P. Dash, A. B. Chandrashekar, K. Rani, N. Manivannan, P. Janila, M. K. Pandey, R. P. Vasanthi, K. L. Dobariya, T. Radhakrishnan and R. K. Varshney. 2019. Steady expression of high oleic acid in peanut bred by marker-assisted backcrossing for fatty acid desaturase mutant alleles and its effect on seed germination along with other seedling traits. *PLoS One.* 14: e0226252.

Bialek, A., M. Bialek, M. Jelinska and A. Tokarz. 2017. Fatty acid composition and oxidative characteristics of novel edible oils in Poland. *CyTA J. Food.* 15: 1-8.

Biswas, R. 2017. A comprehensive review on watermelon seed oil an

- underutilized product. *IOSR J. Pharm.* 7: 1-7.
- David, F., P. Sandra and P. L. Wylie. 2003. Improving the Analysis of Fatty Acid Methyl Esters Using Retention Time Locked Methods and Retention Time Databases. Agilent Technologies. Available from: <http://www.chem.agilent.com/Library/applications/5988-5871EN.pdf> [Last accessed 2021 Nov 28].
- de Conto, L. C., M. A. L. Gragnani, D. Maus, H. C. I. Ambiel, et al. 2011. Characterization of crude watermelon seed oil by two different extractions methods. *J. Am. Oil Chem. Soc.* 88: 1709-1714.
- Dogras, C. C., D. R. Dilley and R. C. Herner. 1977. Phospholipid biosynthesis and fatty acid content in relation to chilling injury during germination of seeds. *Plant Physiol.* 60: 897-902.
- Eidangbe, G. O., G. Ojeh, B. Idonije and O. Oluba. 2010. Palm oil and Egusi melon oil lower serum and liver lipid profile and improve antioxidant activity in rats fed a high fat diet. *J. Food Technol.* 8: 154-158.
- El-Adawy, T. A and K. M. Taha. 2001. Characteristics and composition of watermelon, pumpkin, and paprika seed oils and flours. *J. Agric. Food Chem.* 49: 1253-1259.
- FAOSTAT. 2020. Watermelon Production Quantities. Available from: <http://www.fao.org/faostat/en/#data/TP> [Last accessed on 2022 Feb 20].
- Folch, J., M. Less and G. H. S. Stanley. 1957. Simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226: 497-509.
- Gong, C., S. Zhao, D. Yang, X. Lu, M. Anees, N. He, H. Zhu, Y. Zhao and W. Liu. 2022. Genome-wide association analysis provides molecular insights into the natural variation of watermelon seed size. *Hortic. Res.* 9: uhab074.
- Hailstones, M. D. and M. T. Smith. 1988. Lipid peroxidation in relation to declining vigour in seeds of soya (*Glycine max* L.) and cabbage (*Brassica oleracea* L.). *J. Plant Physiol.* 133: 452-456.
- ISTA. 1996. International Rules for Seed Testing Rules. International Seed Testing Association, Zurich, Switzerland, pp. 179-180.
- Johnson, S. and N. Saikia. 2008. Fatty Acids Profile of Edible Oils and Fat in India, Centre for Science and Environment, New Delhi, pp. 1-48.
- Jorge, N., A. C. Silva and C. R. Malacrida. 2015. Physicochemical characterisation and radical-scavenging activity of *Cucurbitaceae* seed oils. *Nat. Prod. Res.* 295: 2313-2317.
- Kacar, B. 1989. Bitki Fizyolojisi. A.Ü. Ziraat fakültesi yayınları. No:1153. A.Ü. Basımevi. P. 424.
- Kaura, U. 2009. Seed Production, Viability and Germination of *Citrullus Lanatus* at the King Nehale Conservancy in Namibia. Master Thesis, University of Namibia, p. 99.
- Kaymak, H. C., I. Guvenc, F. Yarali and M. F. DonmezF. 2009. The effects of bio-priming with PGPR on germination of radish (*Raphanus sativus* L.) seeds under saline conditions. *Turk. J. Agric. For.* 33: 173-117.
- Kaymak, H. C. 2012. The relationships between seed fatty acids profile and seed germination in cucurbit species. *Zemdirbyste.* 99: 299-304.
- Kaymak, H. C. 2014a. Seed fatty acid profiles: Potential relations between seed germination under temperature stress in selected vegetable species. *Acta Sci. Pol.* 13: 119-133.
- Kaymak, H. C. 2014b. Potential effect of seed fatty acid profile of pepper (*Capsicum annum* L.) cultivars on germination at various temperatures. *Zemdirbyste.* 101: 321-326.
- Kaymak, H. C. 2015. Profile of (n-9) and (n-7) Isomers of monounsaturated fatty acids of radish (*Raphanus sativus* L.) seeds. *J. Am. Oil Chem. Soc.* 92: 345-351.
- Kratz, M., P. Cullen, F. Kannenberg, A. Kassner, M. Fobker, P. M. Abuja, G. Assmann and U. Wahrburg. 2002. Effects of dietary fatty acids on the composition and oxidizability of low-density lipoprotein. *Eur. J. Clin. Nutr.* 56: 72-81.
- Lu, M. M., De Silva, D. M., Peralta, E. K., Fajardo, A. N. and Peralta, M. M. 2015. Effects of nanosilica powder from rice hull ash on seed germination of tomato (*Lycopersicon esculentum*). *Appl. Res. Develop.* 5:11-22.
- Madhavi, P., M. Rao, K. Vakati, H. Rahman and M. C. Eswaraiyah. 2012. Evaluation of anti-inflammatory activity of *Citrullus lanatus* seed oil by *in-vivo* and *in-vitro* models. *Int. Res. J. Pharm. App. Sci.* 2: 104-108.
- Maguire, J. D. 1962. Speed of germination-aid in selection and evaluation for seedling emergence and vigor. *Crop Sci.* 2: 176-177.
- Mahla, H. R., S. S. Rathore, K. Venkatesan and R. Sharma. 2018. Analysis of fatty acid methyl esters and oxidative stability of seed purpose watermelon (*Citrullus lanatus*) genotypes for edible oil. *J. Food Sci. Technol.* 55: 1552-1561.
- Maluf, W. R and E. C. Tigchelaar. 1982. Relationship between fatty acid composition and low-temperature seed germination in tomato. *J. Am. Soc. Hortic. Sci.* 107: 620-623.
- Maoto, M. M., D. Beswa and A. I. O. Jideani. 2019. Watermelon as a potential fruit snack. *Int. J. Food Prop.* 22: 355-370.
- Matthews, S. 1980. Controlled deterioration: A new vigour test for crop seeds. In: P. D. Habbleshwait (Ed.), *Seed Production*. Butterworths, London, pp. 647-660.
- McDonald, M. B. 1999. Seed deterioration: Physiology, repair and assessment. *Seed Sci. Tech.* 27: 177-237.
- Melo, D. M., Charlo, H. C. O., Castoldi, R and Braz, L. T. 2014. Growth dynamic of 'Fantasy' net melon cultivated in substrate under protected cultivation. *Rev Biotm.* 27:19-29.
- Metcalfe, L. D. and A. A. Schmitz. 1961. The rapid preparation of fatty acid esters for gas chromatographic analysis. *Anal. Chem.* 33: 363-364.
- Nakagawa, J. 1999. Testes de vigor baseados no desempenho das plântulas. In: F. C. Krzyzanowski, R. D. Vieira and J. B. F. Neto (Ed.), *Vigor de Sementes: Conceitos e Testes*. ABRATES, Londrina, pp. 2.1-2.24.
- Nerson, H. and H. S. Paris. 1988. Effects of fruit age, fermentation and storage on germination of cucurbit seeds. *Sci. Hortic.* 35: 15-26.
- Nerson, H. 2002. Effects of seed maturity, extraction practices and storage duration on germinability in watermelon. *Sci. Hortic.* 93: 245-256.
- Nolte, A. J. and H. W. Von Loesecke. 1939. Characteristics and composition of watermelon seed oil (Cuban Queen Variety). *J. Am. Chem. Soc.* 61: 889-891.
- Ocana Gil, M., C. P. Werner and T. C. Crowther. 1991. Seed quality effects in bulb onions (*Allium cepa* L.). *Ann. Appl. Biol.* 119: 663-669.
- Ok, S and E. Yilmaz. 2019. The Pretreatment of the seeds affects the quality and physicochemical characteristics of watermelon oil and its by-products. *J. Am. Oil Chem. Soc.* 96: 453-466.
- Osaie, B. A., S. Liu, S. Amanullah, P. Gao, C. Fan, Y. Wan, S. Pei and F. Luan. 2021. Assessment on seed oil percentage and physicochemical properties of watermelon (*Citrullus lanatus*). *Riv. Ital. Delle Sostanze. Grasse.* 98: 217-222.
- Ouassor, I., Y. Aqil, W. Belmaghraoui and S. E. Hajjaji. 2020. Characterization of two Moroccan watermelon seeds oil varieties by three different extraction methods. *OCL.* 27: 1-8.
- Paksoy, M., C. Aydin, O. Türkmen and M. Seymen. 2010. Modeling of some physical properties of watermelon (*Citrullus lanatus*

- (Thunb.) Mansf.) seeds depending on moisture contents and mineral compositions. Pak. J. Bot. 42: 2775-2783.
- Petchsomrit, A., M. I. McDermott, S. Chanroj and W. Choksawangkarn. 2020. Watermelon seeds and peels: Fatty acid composition and cosmeceutical potential. OCL. 27: 1-9.
- Radke, A. K., V. N. Soares, P. E. R. Eberhardt, A. B. N. Martins, L. W. Dias, F. Xavier and F. A. Villela. 2017. Comparison of tests for the evaluation of watermelon seed vigor. Aust. J. Basic Appl. Sci. 11: 150-156.
- Raziq, S., F. Anwar, Z. Mahmood, S. A. Shahid and R. Nadeem. 2012. Characterization of seed oils from different varieties of watermelon [*Citrullus lanatus* (Thunb.)] from Pakistan. Grasas Y Aceites. 63: 365-372.
- Rouphael, Y., A. H. Mouneimne, C. M. Rivera, M. Cardarelli, A. Marucci and G. Colla. 2010. Allometric models for non-destructive leaf area estimation in grafted and ungrafted watermelon (*Citrullus lanatus* Thunb.) J. Food Agric. Environ. 8: 161-165.
- Sabahelkhier, M. K., K. E. A. Ishag and A. K. Sabir Ali. 2011. Fatty acid profile, ash composition and oil characteristics of seeds of watermelon grown in Sudan. Br. J. Sci. 1: 76-80.
- Sarfraz, A., G. Abullais, C. Osh and Y. K. Vijay. 2020. Watermelon seed oil: Its extraction, analytical studies, modification and utilization in cosmetic industries. Int. Res. J. Eng. Technol. 7: 2862-2865.
- Silva, J. B. and R. D. Vieira. 2006. Avaliação do potencial fisiológico de sementes de beterraba. Rev. Bras. Sement. 28: 128-134.
- Taiwo, A. A., M. O. Agbotoba, J. A. Oyedepo, O. A. Shobo, I. Oluwadare and M. O. Olawunmi. 2008. Effects of drying methods on properties of water melon (*Citrullus lanatus*) seed oil. Afr. J. Food Agr. Nutr. Dev. 8: 1684-5374.
- Taiz, L. and E. Zeiger. 2013. Fisiologia Vegetal. 5th ed. ArtMed, Porto Alegre.
- Thongtha, S., P. Sawai and K. Srisook. 2017. A comparative study on antioxidant and nitric oxide-inducing activity of some watermelon cultivars grown in Thailand. Burapha Sci. J. 22: 14-22.
- Vassiliou, E. K., A. Gonzalez, C. Garcia, J. H. Tadros, G. Chakraborty J. H. and Toney. 2009. Oleic acid and peanut oil high in oleic acid reverse the inhibitory effect of insulin production of the inflammatory cytokine TNFalpha both *in vitro* and *in vivo* systems. Lipids Health Dis. 8: 1-10.
- Wang, C. T. 2009. Peanut production, trade and utilization Peanut Science and Technology Bulletin, National Peanut Agric. Indus Res Syst. 1: 8-32.
- WHO. 2003. Diet, Nutrition and the Prevention of Chronic Diseases, WHO Technical Report Series 916, Report of a Joint WHO/FAO Expert Consultation, World Health Organization, Geneva.
- Wu, L. M., S. C. Chen and B. Wang. 2019. An allometry between seed kernel and seed coat shows greater investment in physical defense in small seeds. Am. J. Bot. 106: 371-376.
- Ziyada, A. K. and S. A. Elhussien. 2008. Physical and chemical characteristics of *Citrullus lanatus* var colocynthoide seed oil. J. Phys. Sci. 19: 69-75.