

Genetic and environmental influences on fatty acid composition in different fenugreek genotypes

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ARTICLE INFO

Keywords:

Trigonella foenum-graecum
Genotype-environment Interaction
Fatty acid
Stability
Irrigation and rainfed

ABSTRACT

Fenugreek (*Trigonella foenum-graecum* L.) plays a crucial role in both the food and pharmaceutical industries due to its rich nutritional profile and medicinal properties, such as regulating blood sugar and reducing inflammation. This study offers a comprehensive analysis of the fatty acid composition in fenugreek seeds, revealing significant variability influenced by genetic and environmental factors. The analysis showed that linoleic acid ranged from 32.16 % to 45.96 %, linolenic acid from 17.3 % to 31.21 %, oleic acid from 11.40 % to 20.32 %, stearic acid from 3.78 % to 6.76 %, and palmitic acid from 8.74 % to 16.44 %. Both genotype and environmental conditions had a profound impact on these profiles, with notable variations arising from differences in water availability. The Israel and Kermanshah (Iran) genotypes recorded the highest linoleic acid levels under irrigated conditions, while the Kayseri (Turkey) genotype exhibited the highest linolenic and stearic acid values across both irrigated and non-irrigated environments. Additionally, the Ahvaz (Iran) and Ukraine genotypes excelled in oleic and palmitic acid concentrations. Principal component analysis (PCA) and Additive Main Effects and Multiplicative Interaction (AMMI) biplots further highlighted the complex genotype-environment interactions, providing essential insights for breeding fenugreek varieties optimized for diverse agro-climatic conditions.

1. Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is a time-honored herb used both medicinally and in cooking, celebrated for its wide range of therapeutic benefits and nutritional value. This leguminous plant, originally from the Mediterranean and now extensively grown in Asia and Africa, is highly prized for its seeds. Fenugreek grows naturally in the region extending from Iran to northern India and is cultivated in countries such as India, China, Egypt, Ethiopia, Morocco, Ukraine, Greece, and Turkey (Shahrajabian et al., 2021). In recent years, fenugreek cultivation in Turkey has steadily increased from 2020 to 2023, with the cultivated area expanding from 652.1 ha to 1074.6 ha. Correspondingly, production rose from 713 tons in 2020–1313 tons in 2023. This suggests an expanding demand for fenugreek or enhanced agricultural practices during this period (Tüik, 2024). Moreover, the increase in production is attributed to rising demand, improved agricultural techniques, and expanding export opportunities. Notably,

India leads global production, particularly in regions like Rajasthan and Gujarat, with Egypt and Ethiopia also making significant contributions (Narayana et al., 2022).

Fenugreek seeds are particularly valued for their rich nutritional composition. They are abundant in dietary fiber, proteins, vitamins, and essential fatty acids (Olaiya and Soetan, 2014; Ahmad et al., 2016; Syed et al., 2020). What makes them even more beneficial is their fatty acid profile, which plays a critical role in both human health and the plants physiological processes (Zandi et al., 2015). Approximately 7 % of the seeds composition is fixed oil, predominantly made up of linoleic, oleic, and linolenic acids (Leela and Shafeekh, 2008). From a human health perspective, linoleic and linolenic acids are essential fatty acids that contribute to heart health, anti-inflammatory processes, and overall well-being (Calder, 2015). With the growing interest in functional foods and natural health products, understanding the fatty acid profile of fenugreek seeds has become a priority for researchers and producers alike.

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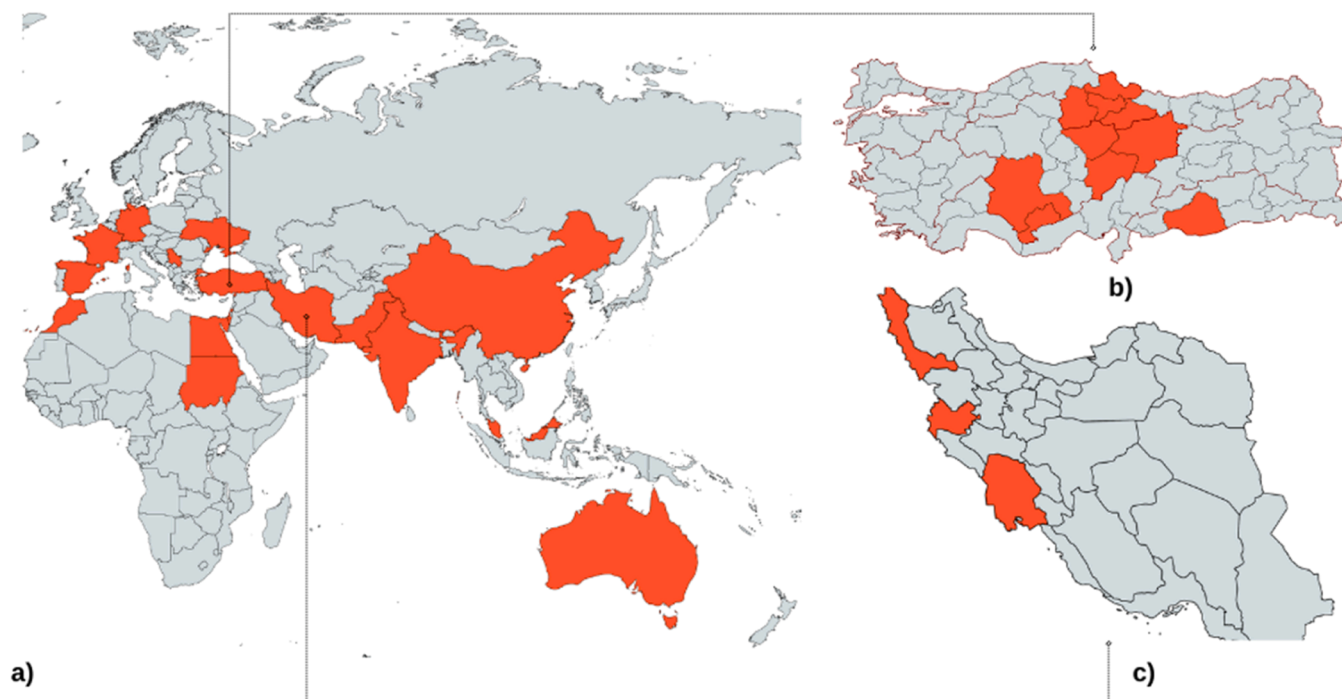


Fig. 1. Geographical distribution of fenugreek seed samples: a) All Regions, b) Turkey, c) Iran. * India; ZFTB0034, Pakistan; ZFTB0031, Serbia; ZFTB0022, Israel; ZFTB0023, Egypt; ZFTB0007, Malaysia; ZFTB0026, Ukraine; ZFTB0018, Germany; ZFTB0009, Morocco; ZFTB0017, China; ZFTB0019, Australia; ZFTB0015, Spain; ZFTB0016, South Sudan; ZFTB0012, France; ZFTB0014, Salmas/Iran; ZFTB0020, Urmia/Iran; ZFTB0001, Ahvaz/Iran; ZFTB0029, Kermanshah/Iran; ZFTB0010, Konya/Turkey; ZFTB0027, Samsun/Turkey; ZFTB0003, Yozgat/Turkey; ZFTB0002, Amasya/Turkey; ZFTB0030, Karaman/Turkey; ZFTB0028, Sivas/Turkey; ZFTB0005, Sanliurfa/Turkey; ZFTB0004, Corum/Turkey; ZFTB0006, Tokat/Turkey; ZFTB0024, Kayseri/Turkey; ZFTB0021, Berkem/Turkey; ZFTB0011, Guraslan/Turkey; ZFTB0013, Ciftci/Turkey; ZFTB0035.

Fenugreek oil, extracted from the seeds, is another product of significant importance. It is widely used in South Asian, Middle Eastern, and Mediterranean cuisines for its flavor and aroma. However, beyond its culinary uses, fenugreek oil offers health benefits due to its antioxidant, anti-inflammatory properties, and fatty acid content, which can support heart health, reduce cholesterol levels, and alleviate inflammation. In addition, it is also used for improving skin and hair health. Despite these advantages, there are precautions when using fenugreek oil, particularly its tendency to oxidize at high temperatures, forming harmful compounds. Therefore, it is advisable to use the oil at low temperatures and in moderation (Ahmad et al., 2016; Žuk-Gołaszewska and Wierzbowska, 2017).

In terms of medicinal applications, fenugreek oil has demonstrated efficacy in managing diabetes, improving hematological status, and mitigating renal toxicity, largely due to its immunomodulatory, insulin-stimulating, and antioxidant properties (Hamden et al., 2010). Interestingly, fenugreek oil has also been shown to enhance ovarian activity in mice, increasing both the quantity and quality of oocytes (Hassan et al., 2006). Furthermore, although fenugreek is generally suited for dry conditions, in cases of insufficient rainfall, irrigation becomes necessary to ensure optimal growth. In regions where rainfall is adequate, it can be utilized either as forage or as a medicinal herb within low-input agricultural systems, making it a versatile crop across various agro-climatic conditions.

One of the key factors affecting the fatty acid composition of fenugreek is its genetic variability. Different fenugreek genotypes exhibit significant variability in their fatty acid profiles. This variability is not only genetic but also influenced by environmental factors, making it a crucial area of study for breeders aiming to enhance fenugreek's nutritional qualities. Significant fluctuations in seed oil content have been observed among different genotypes grown under various environmental conditions, highlighting the importance of genotype-environment (GxE) interactions (Acharya et al., 2008; Lee, 2009). To

better understand these interactions, research efforts worldwide have focused on long-term crop breeding programs. For instance, the AMMI (Additive Main Effects and Multiplicative Interaction) model is used to analyze these interactions, offering comprehensive insights into both genotype and environmental factors (Wittkop et al., 2009; Hristov et al., 2010; Liersch et al., 2020; Hasaroeih et al., 2023).

The significance of fatty acids extends beyond their nutritional value; they are also essential components of lipid membranes in living cells, contributing to both structural and signaling processes (Ullah et al., 2022). However, environmental challenges such as drought stress can significantly impact the biosynthesis of fatty acids in plants. Water availability plays a crucial role in maintaining these metabolic processes, and its scarcity can lead to decreased photosynthesis and altered enzyme activities (Kapoor et al., 2020). Thus, breeding programs aiming to improve the nutritional quality and drought resilience of fenugreek must integrate insights from studies on environmental impacts. By identifying genotypes with stable fatty acid profiles under various irrigation and climatic conditions, breeders can develop fenugreek varieties that are not only nutritionally superior but also more adaptable to climate change.

In conclusion, for breeders and marketers, understanding the interplay between genotype and environment is crucial when optimizing fatty acid composition across different growing conditions. Numerous studies highlight the significant variation in the fatty acid content of fenugreek and other crops (Saxena et al., 2017; Bellaloui and Kassem, 2021; Zhang et al., 2023; Wang et al., 2024). However, there is not enough comprehensive information on how the fatty acid composition of this plant is affected by genetic and environmental factors. This study focuses on a diverse population of genotypes, representative of a broad geographic range, to examine these differences under dry and irrigated conditions, predicting that irrigation will significantly impact fatty acid levels, and genotypic diversity will lead to differences in fatty acid composition. By integrating both genotype and environmental

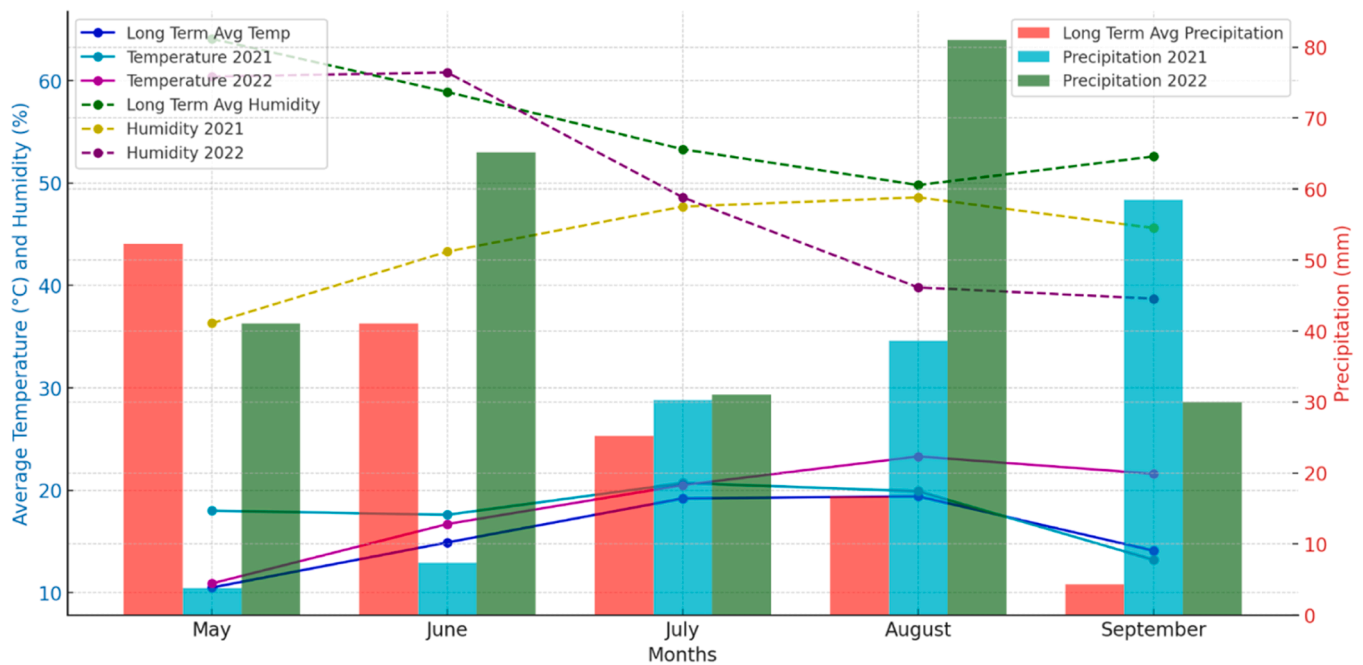


Fig. 2. Temperature, precipitation, and relative humidity data for the vegetation period in the research area, including long-term averages (1990–2020) and sowing and harvest times for the Years 2021 and 2022.

interactions with advanced statistical methods like principal component analysis and additive main effects and multiplicative interaction biplots, providing valuable insights for breeding programs focused on improving fenugreeks nutritional quality and adaptability across different environments. These hypotheses form the foundation for investigating the genetic and environmental influences in this study.

2. Material and methods

2.1. Plant materials

In this study, 31 different fenugreek seed genotypes were collected from various regions including India, Pakistan, Serbia, Israel, Egypt, Malaysia, Ukraine, Germany, Morocco, China, Australia, Spain, South Sudan, France, and specific areas within Iran (Salmas, Urmia, Ahvaz, Kermanshah) and Turkey (Konya, Samsun, Yozgat, Amasya, Karaman, Sivas, Sanliurfa, Corum, Tokat, Kayseri), as well as local cultivars (Berkem, Ciftci, Guraslan). Berkem, Ciftci, and Guraslan are also registered by the Seed Registration and Certification Center Directorate of Turkey. Each accession was assigned a unique number for identification. These regions and accession numbers* are shown in Fig. 1.

2.2. Site description

This study was conducted during two growing seasons in 2021 and 2022 at the experiment station of Ataturk University Research and Extension Center at Erzurum, Turkey (39.933 °N, 41.237 °E, 1789 m above sea level), to determine the oil content and fatty acid composition of 31 different fenugreek genotypes under both irrigated and dry conditions. Fig. 2 shows data on temperature, precipitation, and relative humidity for the vegetation period in the research area, considering the long-term averages (1990–2020) and the sowing and harvest times for the years 2021 and 2022. The data covers the months of May (May 6–31), June, July, August, and September (September 1–10).

As seen in Fig. 2, the 2021 growing season experienced significantly lower precipitation and humidity compared to the long-term average, particularly in May and June, which are critical periods for plant emergence and growth. The precipitation levels in May and June were

well below the long-term average. The average humidity in 2021 was also below the long-term average, resulting in a very dry season for the plants. Temperatures in 2021 were higher than average, contributing to very dry conditions and resulting in poor plant emergence and unreliable data, thus excluding 2021 data from the study. In contrast, 2022 experienced more favorable conditions with higher total precipitation (120.70 mm) and adequate rainfall in May and June, although it still faced dry periods from July onwards and higher-than-average temperatures, particularly in May, June, and July.

According to the average of the years, the soil properties of the experimental field have a clay-loam texture. The soil is poor in available phosphorus (77.3 kg ha⁻¹) and total nitrogen (0.857 %), but rich in available potassium (1633.3 kg ha⁻¹). The experimental field does not have a salinity problem (0.605 %) and is slightly alkaline (pH 7.455). The organic matter (1.29 %) and lime content (0.795 %) of the soil are low (Klute, 1986).

2.3. Experimental design and methodology

In both study years, fenugreek genotypes were sown on May 6 in rows spaced 30 cm apart using a plot drill (Pocta, Model CP-1 SR-1) with a sowing rate of 40 kg ha⁻¹. The plots were hand-thinned afterward to ensure uniformity. The experimental site, previously planted with spring wheat in both years, was moldboard-plowed in early October and then roller-harrowed.

Nitrogen (ammonium sulfate) and phosphorus (triple superphosphate) were the main fertilizers applied to all plots. The application rates were 40 kg N ha⁻¹ and 60 kg of P₂O₅ ha⁻¹. These fertilizers were broadcast on the plots and mixed into the soil using a disk harrow before sowing. Half the amount of fertilizer used in irrigated conditions was applied in dry conditions. This approach was chosen because, in dry conditions, the ability of plants to absorb and utilize nutrients efficiently is reduced due to limited water availability (Weih et al., 2018). Weed control was carried out by hand-hoeing during the growth period.

The experiment followed a randomized complete block design with three replications. Each experimental plot covers a total area of 6 m² (5 m in length × 1.2 m in width), consisting of four rows, each spaced 0.3 m apart. The research was designed separately for non-irrigated and

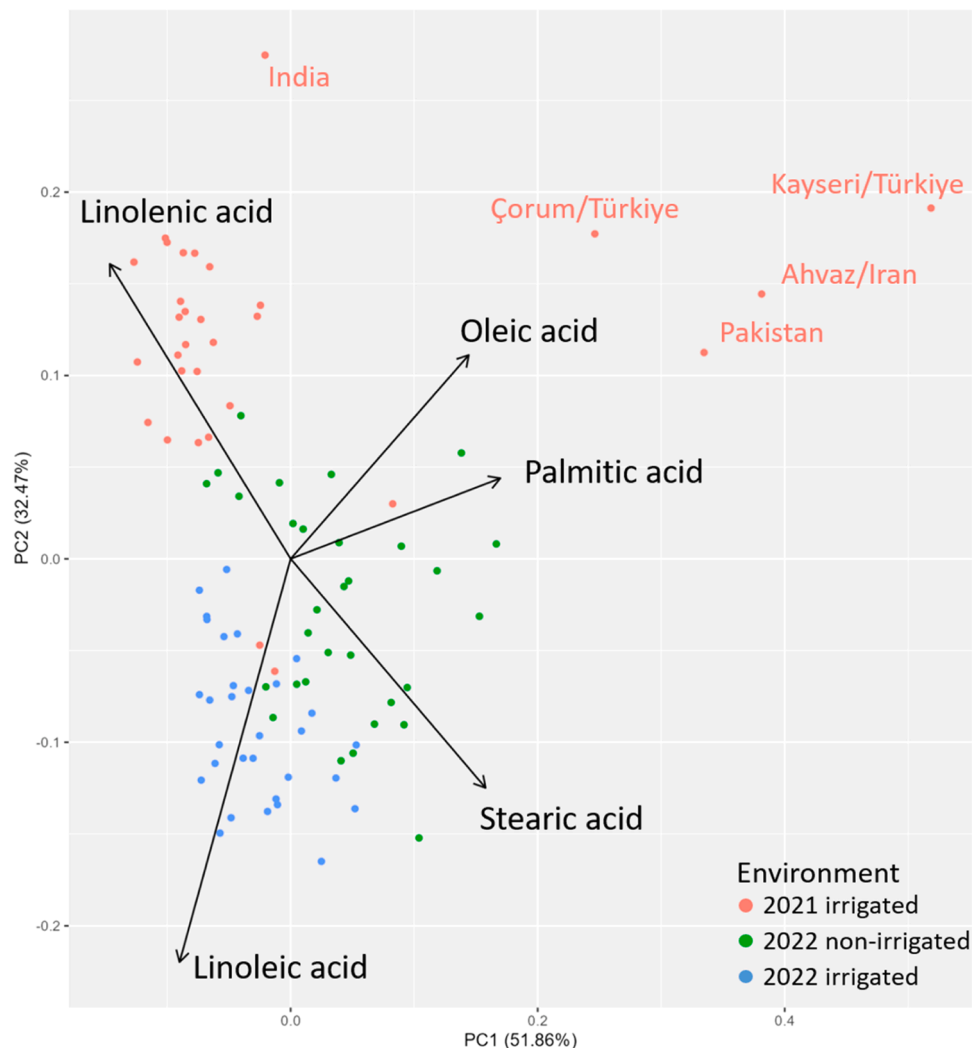


Fig. 3. Biplot from principal-component analysis (PCA) for linoleic acid, linolenic acid, oleic acid, stearic acid and palmitic acid in seeds from 31 fenugreek genotypes grown in different environments.

irrigated conditions. In the second year, no irrigation was performed until the pre-flowering stage due to sufficient rainfall. Irrigation was carried out five times in the first year and four times in the second year, as needed by the fenugreeks in irrigation conditions. Harvesting began after 50 % of the pods had reached harvest maturity. The period when the pods turned a yellowish-brown color was taken as the criterion for harvest maturity. During the harvest, one row from the edges of each plot and a 0.5 m section from the ends were considered as edge effects, while the remaining two central rows were cut with a sickle at ground level. The harvested plants were dried in a greenhouse environment, and after the drying process was completed, they were threshed to extract the seeds. The average vegetation period, considering all sources of variation, ranged from 107.45 to 122.08 days.

2.4. Obtaining the crude oils

A 3 g subsample of seed for each genotype replicate was used to determine seed oil concentration using a Soxhlet apparatus (Isolab, Eschau, Germany). The extraction solvent used was >95.0 % petroleum ether (Merck, Darmstadt, Germany). Following extraction, the products were collected and purified using a rotary evaporator (Heidolph Instruments GmbH & Co. KG, Bavyera, Germany) set at a constant temperature of 50°C. Afterward, the samples were placed under a fume hood for one hour to ensure that all petroleum ether remaining in the crude oil

completely evaporated into the environment. The oil content was found to range between 4.57 % and 8.61 % as an average of all sources of variation. Additionally, the samples were stored at +4°C for the determination of the fatty acid profile.

2.5. Fatty acid composition

The fatty acid composition of the extracted oil samples was analyzed following the derivatization of fatty acid methyl esters (Supelco 37 Component FAME Mix, Supelco Park, Bellefonte, PA, USA) with 2 N KOH (Merck, Darmstadt, Germany) in methanol (Merck, Darmstadt, Germany) at room temperature, according to the IUPAC Standard Method 2.301 (Dieffenbacher and Pocklington, 1992). The analysis of FAMES was performed using a GC (Agilent 7890 A, USA) equipped with an Agilent 5975 C mass selective detector and BPX90 GC Capillary Columns (100 m length, 0.25 mm ID, and 0.25 µm film, SGE Analytical Science). The injector temperature was set to 250°C. The oven temperature was initially held at 120°C for 1 min, then ramped to 250°C at a rate of 5.0°C per minute and held for 1 min. Helium was used as the carrier gas with a flow rate of 1 mL per minute and a split ratio of 1:10. Fatty acids were identified by comparing the retention times of sample peaks with those of authentic FAME standards, which were analyzed under the same conditions, and the results were expressed as a percentage of the total fatty acids. Over 98 % of the fatty acids were

identified, and the major fatty acids (linoleic acid, linolenic acid, stearic acid, palmitic acid and oleic acid) are presented.

2.6. Data Analysis

All the statistical analyses were carried out using RStudio software, version 1.2.5042. To detect significant differences between treatments, a two-way ANOVA was performed for each trait. When significant differences ($p < 0.05$) were found, multiple mean comparisons were conducted using the Tukey post-hoc test with the rstatix package. Pearson's correlation coefficients were calculated using the Hmisc package to investigate correlations among different traits with their mean values, and the results were visualized using the corrplot package. Additionally, principal-component analysis (PCA) was conducted with the ggfortify package to further explore the relationships among different genotypes and environment.

3. Results and discussion

3.1. Genotypic and environmental effect on fatty acids

The palmitic acid values ranged from 8.96 % to 16.44 % under irrigated conditions in 2021, from 8.74 % to 12.79 % under non-irrigated conditions in 2022, and from 8.88 % to 11.06 % under irrigated conditions in 2022 (Fig. 3). Considering the average values, no significant difference was found between the three environments (Table 2). Under irrigated conditions in 2021, the Kayseri/TR genotype (16.44 %) had the highest palmitic acid value, followed by the Ahvaz/IR (14.80 %) and Çorum/TR (13.61 %) genotypes, whereas the lowest palmitic acid value was observed in the Israel genotype (8.96 %). Under non-irrigated conditions in 2022, the highest palmitic acid value was observed in the Çorum/TR genotype (12.79 %), while the lowest was found in the Urmia/IR genotype (8.74 %). In the same year, under irrigated conditions, the highest palmitic acid value was observed in the Germany genotype (11.06 %), and the lowest in the Ahvaz/IR genotype (8.88 %). Notably, while the Ahvaz/IR genotype was among the genotypes with the highest palmitic acid content under irrigated conditions in 2021, it had the lowest palmitic acid content under irrigated conditions in 2022. The differences in palmitic acid content between 2021 and 2022 for the Ahvaz/IR genotype could be attributed to varying environmental factors such as temperature, rainfall, or soil conditions affecting fatty acid biosynthesis (Bellaloui and Kassem, 2021; Günenc et al., 2022).

The stearic acid value was found to be the lowest at 4.47 % under irrigated conditions in 2021. In 2021, under irrigated conditions, the highest stearic acid value (6.76 %) was observed in the Kayseri/TR genotype, which also had the highest value (6.33 %) under non-irrigated conditions in 2022. Under irrigated conditions in 2022, the Germany genotype had the highest stearic acid value (5.79 %).

The lowest average oleic acid content (12.18 %) was found under irrigated conditions in 2022, while the highest (14.28 %) was observed under irrigated conditions in 2021. Under irrigated conditions in 2021, the oleic acid content in oils extracted from fenugreek seeds ranged from 12.75 % (Spain) to 20.32 % (Kayseri/TR). In 2022, the highest oleic acid value under non-irrigated conditions was found in the Ahvaz/IR genotype (17.50 %), and under irrigated conditions, it was found in the Tokat/TR genotype (13.45 %). The lowest oleic acid values were observed in the Konya/TR genotype (11.68 %) under non-irrigated conditions in 2022 and in the Samsun/TR genotype (11.40 %) under irrigated conditions in the same year. The data indicate that the differences between the highest and lowest oleic acid values were more pronounced under non-irrigated conditions in 2021, suggesting that both genotypic and environmental factors play a crucial role in oleic acid content extracted from fenugreek seeds.

The average linoleic acid values varied significantly across three environments with the highest and lowest in 2022 irrigated (43.62 %)

Table 1

Mean comparison of the five fatty acids between three environments.

	2021 irrigated	2022 non-irrigated	2022 irrigated
Linoleic acid	39.03 ± 2.60 ^c	41.42 ± 1.57 ^b	43.62 ± 1.15 ^a
lowest/highest	Kayseri (32.16) Israel (45.15)	Çorum (36.94)/ Kermanshah (44.18)	Australia (40.81) Israel (45.96)
Linolenic acid	27.61 ± 3.83 ^a	23.39 ± 2.09 ^b	23.76 ± 1.42 ^b
lowest/highest	Kayseri (17.30) Ukrania (31.21)	Urmıye (19.02) Amasya (28.23)	Kermanshah (20.42) Urmıye (25.89)
Oleic acid	14.28 ± 1.77 ^a	13.82 ± 1.28 ^a	12.18 ± 0.51 ^b
lowest/highest	Spain (12.75) Kayseri (20.32)	Konya (11.68) Ahvaz (17.50)	Samsun (11.40) Tokat (13.45)
Stearic acid	4.47 ± 0.73 ^b	5.23 ± 0.61 ^a	5.03 ± 0.40 ^a
lowest/highest	Ukrania (3.78) Kayseri (6.76)	Konya (4.21) Kayseri (6.33)	France (4.27) Germany (5.79)
Palmitic acid	10.38 ± 1.78 ^a	10.52 ± 0.75 ^a	10.13 ± 0.45 ^a
lowest/highest	Israel (8.96) Kayseri (16.44)	Urmıye (8.74) Çorum (12.79)	Ahvaz (8.88) Germany (11.06)

Means (± standard deviation) between different environments marked by the same letters do not differ significantly (LSD post hoc test at $p < 0.05$).

and 2021 irrigated conditions (39.03 %), respectively (Table 1). In 2021 irrigated conditions, the highest linoleic acid value was observed in the Israel genotype at 45.15 %, while the lowest was in the Kayseri/TR genotype at 32.15 %. The low linoleic acid content in the Kayseri/TR genotype is likely due to its higher palmitic and oleic acid content. Similarly, in 2022 irrigated conditions, the Israel genotype again showed the highest value at 45.96 %. Under 2022 non-irrigated conditions, the Kermanshah/IR genotype exhibited the highest value at 44.18 % while the Çorum/TR genotype showed the lowest at 36.94 %. Overall, fenugreek plants grown under dry conditions had lower average linoleic acid values compared to those grown under irrigated conditions.

Another important fatty acid found in the composition of oils extracted from fenugreek seeds is linolenic acid. In our study, the highest average linolenic acid value (27.61 %) was found under 2021 irrigated conditions. The highest linolenic acid values were observed in the Ukraine genotype (31.21 %) under 2021 irrigated conditions, the Urmıye/IR genotype (25.89 %) under 2022 irrigated conditions, and the Amasya/TR genotype (28.23 %) under 2022 non-irrigated conditions. Conversely, the lowest linolenic acid values were found in the Kayseri/TR genotype (17.30 %) under 2021 irrigated conditions, the Kermanshah/IR genotype (20.42 %) under 2022 irrigated conditions, and the Urmia/IR genotype (19.02 %) under 2022 non-irrigated conditions.

Aljuhaimi et al., (2018) identified the major fatty acids in fenugreek seeds as linoleic, linolenic, oleic, palmitic, and stearic acids. The linoleic acid content ranged from 35.14 % (Saudi Arabia) to 41.04 % (Turkey). Linolenic acid content varied from 16.97 % (Turkey) to 25.66 % (Yemen), while oleic acid content ranged from 11.80 % (Yemen) to 19.93 % (Saudi Arabia). The palmitic acid content was between 10.06 % (Saudi Arabia) and 12.31 % (Turkey). Lastly, the stearic acid content varied from 2.9 % (Saudi Arabia) to 4.4 % (Turkey). Al-Jasas and Al-Jasser, (2012) reported that fenugreek seed oil contains 3.85 % palmitic acid, 8.29 % palmitoleic acid, 1.78 % stearic acid, 8.29 % oleic acid, 34.85 % linoleic acid, and 30.80 % linolenic acid. Another study indicated that fenugreek oil contains 11.0 % palmitic acid, 4.5 % stearic acid, 16.7 % oleic acid, 43.2 % linoleic acid, and 22.0 % linolenic acid

Table 2

ANOVA table in the form of mean square values for five fatty acids investigated in different environments (***: sig. < 0.001).

	Genotype	Environment	Genotype * Environment
Degree of freedom	30	2	60
Linoleic acid	13.2***	489.5***	9.7***
Linolenic acid	27.9***	509.5***	18.7***
Oleic acid	6.13***	112.94***	4.77***
Stearic acid	1.41***	14.40***	0.94***
Palmitic acid	4.49***	3.67***	3.85***

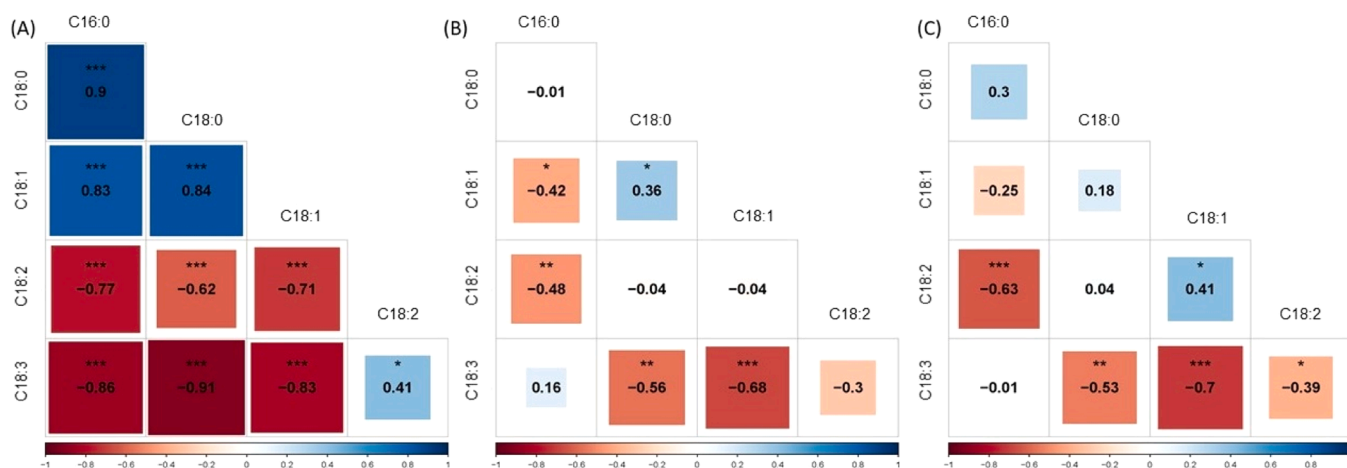


Fig. 4. Correlation among linoleic acid (C18: 2), linolenic acid (C18: 3), oleic acid (C18: 1), stearic acid (C18: 0) and palmitic acid (C16: 0) of fenugreek lines grown under (A) irrigated condition in 2021, (B) non-irrigated condition in 2022 and (C) irrigated condition in 2022.

(Suliman et al., 2008). In a study using the Guraslan/TR fenugreek cultivar, Beyzi, (2020) determined the palmitic acid value to be 9.00 %, stearic acid value to be 4.74 %, oleic acid value to be 13.05 %, linoleic acid value to be 43.65 %, and linolenic acid value to be 27.27 %. Saxena et al., (2017) reported the linoleic acid content in fenugreek seeds to range between 39.22 % and 50.58 %. Ali et al., (2012) identified linoleic acid as the main component of fenugreek seeds, with a linoleic acid content of 42.5 %.

ANOVA clearly showed that the fatty acid composition varied significantly ($p < 0.001$) among the evaluated genotypes under the different growing conditions (Table 2). The ANOVA results indicated that all five fatty acids (linoleic acid, linolenic acid, oleic acid, stearic acid, and palmitic acid) were significantly affected by the effects of genotype and environment and their interaction.

Genotypes showed considerable variation under both irrigated and dry conditions, as well as across different years. For instance, the genotype from India consistently showed high linoleic acid content under both irrigated conditions, highlighting its genetic potential to thrive with sufficient water supply. Similarly, Ahvaz/IR genotypes exhibited high oleic and stearic acid contents under irrigated conditions, indicating their ability to maximize fatty acid production when water is not a limiting factor. Under non-irrigated conditions in 2022, the data indicates a noticeable decline in fatty acid content across most genotypes, reflecting the stress response to water scarcity. For example, the genotype from Pakistan showed a significant reduction in linoleic acid content when not irrigated, underscoring its sensitivity to water stress. However, some genotypes, such as those from Corum/TR and India, maintained relatively high linolenic acid levels even under non-irrigated conditions, suggesting a degree of drought resilience. The yearly comparison between 2021 and 2022 also highlights the environmental interaction with genotypic performance. The 2021 irrigated conditions generally resulted in higher fatty acid content compared to 2022, even under similar irrigation regimes. This variation can be attributed to differences in climatic conditions, such as temperature, rainfall patterns, and soil quality between the two years. For instance, the higher linolenic acid content observed in 2021 irrigated conditions compared to 2022 suggests that factors beyond irrigation, such as seasonal climatic variations, play a significant role in fatty acid biosynthesis. Reduced enzyme activity and substrate availability under drought conditions can lower the levels of these fatty acids, particularly the unsaturated ones like linoleic and linolenic acids.

Fatty acid metabolic pathways are crucial for the biosynthesis of precursors that contribute to cuticular components and play a key role in plant responses to abiotic stress conditions (Kachroo and Kachroo, 2009). Irrigation appears to have a pronounced effect on the fatty acid

content across different genotypes. Adequate water availability is crucial for the optimal biosynthesis of these fatty acids in fenugreek seeds. In 2022, the content of linoleic and linolenic acids was higher under irrigated conditions, while a decrease was observed under dry conditions. Conversely, palmitic and oleic acid content increased under dry conditions. This difference was also found to be statistically significant (Table 2, Fig. 6). Additionally, when comparing 2022–2021 under irrigated conditions, the linoleic acid content increased while the oleic acid content decreased. Indeed, 2021 was significantly drier compared to 2022 (Fig. 2). Irrigation increases the level of linoleic acid while decreasing the level of oleic acid in plants. This effect is primarily related to the role of the FAD2 enzyme, environmental factors, and plant metabolism. The FAD2 enzyme, which is in the endoplasmic reticulum, converts oleic acid to linoleic acid. Irrigation enhances the activity of this enzyme, promoting the production of linoleic acid. Additionally, irrigation optimizes the plants temperature and light conditions, potentially increasing the expression of FAD2 genes. Under drought stress, plants accumulate oleic acid, but irrigation alleviates this stress, allowing the plant to convert more oleic acid to linoleic acid. Furthermore, irrigation improves the nutrient uptake and overall metabolic status of the plant, supporting the fatty acid desaturation processes. Hormone levels and gene expressions are also affected by irrigation, which increases FAD2 activity and thus linoleic acid production (Cao et al., 2013; Dar et al., 2017; Miao et al., 2019). Our results are consistent with the findings of Laribi et al. (2009), who studied *Carum carvi*, and Ullah et al. (2022), who focused on wheat, both of which reported an increase in oleic acid levels and a decrease in linoleic acid levels under drought stress. Baldini et al. (2002) suggested that this difference might be due to accelerated and earlier embryo development and the stimulation of enzymatic activities of fatty acid biosynthesis caused by water stress. Drought stress increases the saturation of fatty acids in plasma membrane lipids, leading to membrane stiffening, which plays a role in altering membrane fluidity under environmental conditions, and thus, palmitic and oleic acid levels increase as a means of coping with stress (López-Pérez et al., 2009). Significant decrease in the unsaturation level of plant membranes under stress, reducing the amounts of linoleic and linolenic acids while increasing palmitic and oleic acids (Singer et al., 2016). Based on the results of the present study, it was found that under drought stress, the amounts of palmitic and oleic acids increased, while linoleic and linolenic acids decreased, indicating a decrease in the degree of unsaturation of membranes under drought stress.

3.2. Relationships between environments and fatty acids

The biplot presented in the Fig. 3 illustrates the principal component

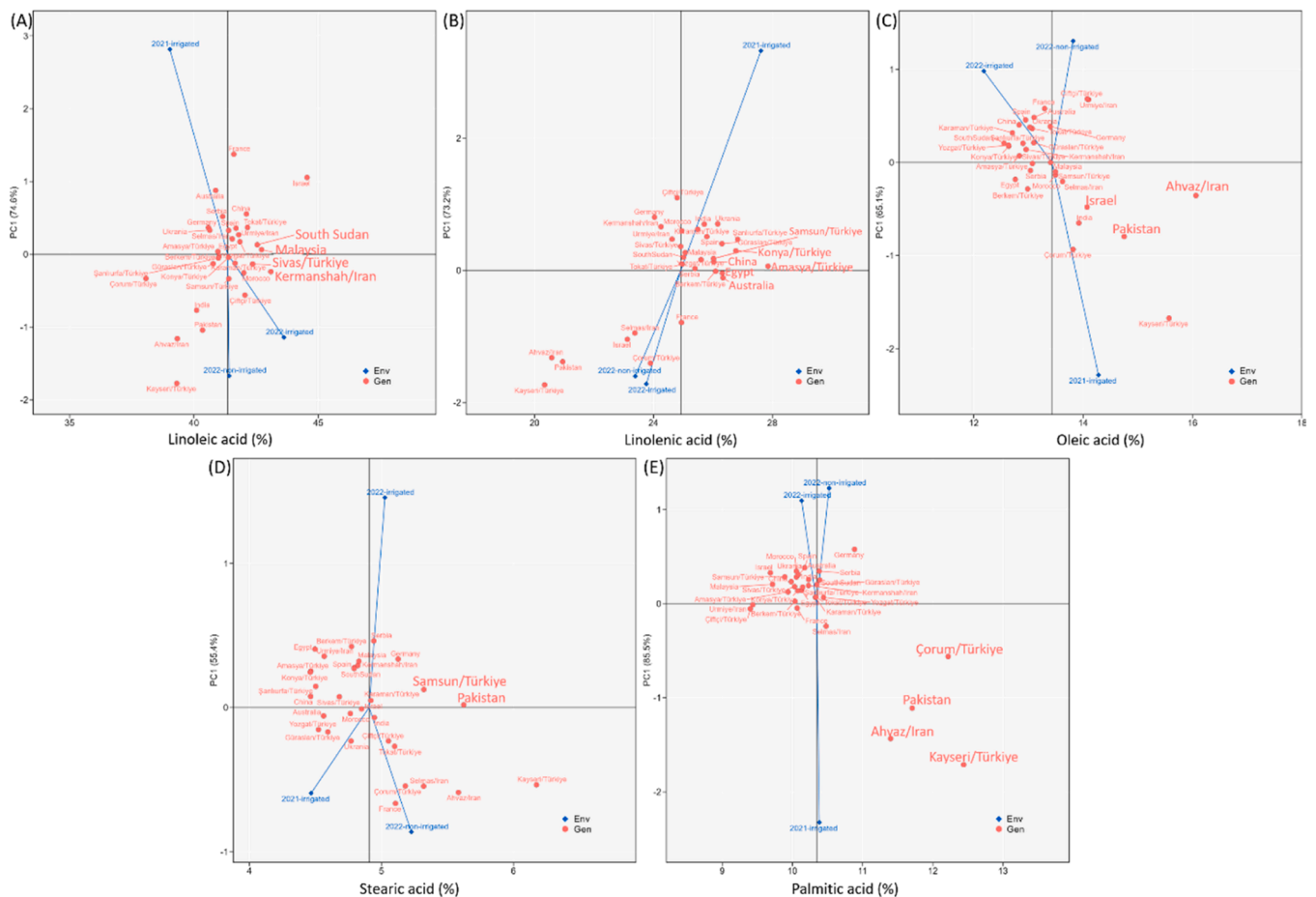


Fig. 5. Additive main effects and multiplicative interaction (AMMI) biplots showing (A) linoleic acid, (B) linolenic acid, (C) oleic acid, (D) stearic acid and (E) palmitic acid versus the first principal component score of 31 fenugreek lines grown in different environments including irrigated condition in 2021, non-irrigated condition in 2022 and irrigated condition in 2022. The vertical lines indicate the mean value of each fatty acid. Samples located closer to the horizontal axis (score 0 on PC1) had relatively higher stability across three growing conditions.

analysis (PCA) results for five fatty acids (linoleic acid, linolenic acid, oleic acid, stearic acid, and palmitic acid) in seeds from 31 fenugreek genotypes grown under different environmental conditions (2021 irrigated, 2022 non-irrigated, and 2022 irrigated). The PCA biplot displays the first two principal components, PC1 and PC2, which explain 51.86 % and 32.47 % of the total variation, respectively (Fig. 3). The biplot reveals a clear clustering pattern of genotypes based on their fatty acid profiles and environmental conditions. Genotypes grown under the 2021 irrigated condition are predominantly associated with higher linolenic acid content. Notably, genotypes from specific regions such as India, Çorum/TR, Kayseri/TR, Ahvaz/IR, and Pakistan exhibit unique fatty acid profiles with particularly high oleic acid and palmitic acid. Conversely, genotypes from the 2022 non-irrigated environment and 2022 irrigated environment are more dispersed, indicating large genotypic variations in oleic acid, palmitic acid, and stearic acid. This differentiation underscores the importance of tailored agricultural practices to optimize the nutritional quality of fenugreek seeds in diverse growing conditions.

3.3. Relationships between five fatty acids within each environment

The correlation between linoleic acid, linolenic acid, oleic acid, stearic acid, and palmitic acid under three different experimental conditions is shown in the Fig. 4(A) irrigated condition in 2021, (B) non-irrigated condition in 2022, and (C) irrigated condition in 2022. In 2021 irrigated conditions, strong positive correlations are observed among palmitic acid, stearic acid, and oleic acid, suggesting a similar

synthetic mechanism between them. Furthermore, all these three fatty acids are significantly and negatively correlated with both linoleic acid and linolenic acid, indicating a competitive relationship between them (Fig. 4A). In 2022, the five fatty acids show weakened correlations regardless of irrigation treatment (Fig. 4B and C). These correlation patterns underscore the influence of environmental conditions on the fatty acid composition in fenugreek seeds. Particularly, the patterns differ to a larger extent between two years than between irrigation treatments within the same year, suggesting a larger impact of climate change between years than the sole irrigation treatment. Fatty acids are synthesized through a shared pathway, making their concentrations interconnected. A strong negative correlation between oleic and linoleic acids was validated through regression and principal component analysis, consistent with the findings of Popa et al. (2017) and Ghaffari et al., (2023). Oleic acid is synthesized from stearic acid (C18:0) in plants by the stearoyl-CoA desaturase (SCD) enzyme (Tsai et al., 2019). Subsequently, oleic acid is converted into linoleic acid through the addition of a double bond by the FAD2 desaturase enzyme (Lou et al., 2014). During drought stress, the activity of this FAD2 desaturase enzyme decreases, which limits the conversion of oleic acid to linoleic acid. As a result, oleic acid accumulates in the plant, while linoleic acid levels may decrease (Shi et al., 2012; Dar et al., 2017).

3.4. Stability analysis on fatty acids of diverse genotypes

The AMMI (Additive Main Effects and Multiplicative Interaction) biplots illustrate the variation in fatty acid content - linoleic acid

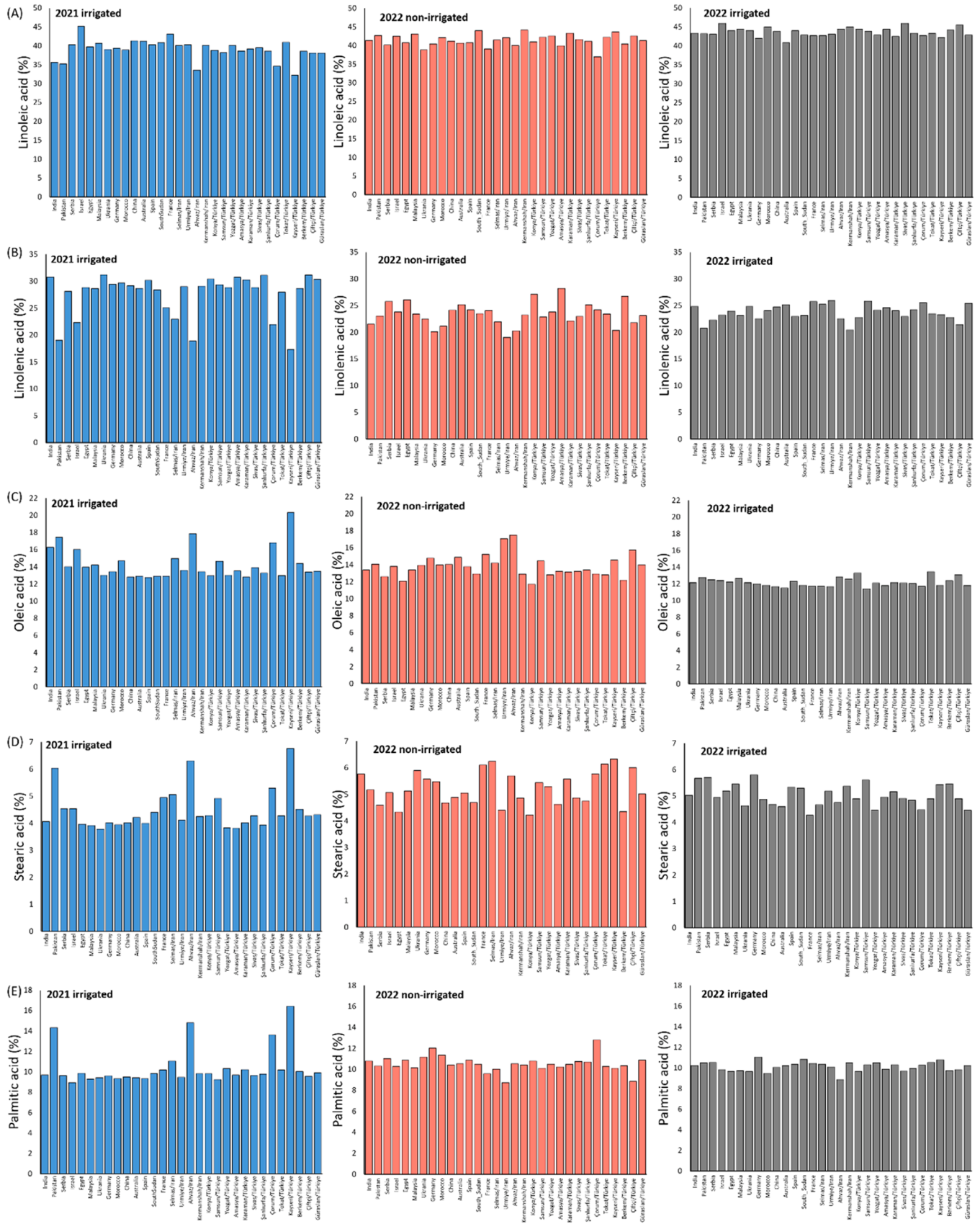


Fig. 6. Content of linoleic acid (A), linolenic acid (B), oleic acid (C), stearic acid (D) and palmitic acid (E) of each genotype grown under three environments.

(Fig. 5A), linolenic acid (Fig. 5B), oleic acid (Fig. 5C), stearic acid (Fig. 5D), and palmitic acid (Fig. 5E) across different fenugreek genotypes and environmental conditions. The biplots indicate how each genotype's fatty acid content varies with changes in environmental conditions. Basically, the environmental clustering in the biplots highlights the significant impacts of irrigation and year on fatty acid composition. For example, in the linolenic acid biplot, the 2021 irrigated environment is associated with higher linolenic acid levels, while the 2022 non-irrigated environment shows different clustering patterns. Similar trends are observed for oleic acid, stearic acid, and palmitic acid, where specific environmental conditions align with distinct fatty acid concentrations. These biplots provide a clear representation of how environmental factors such as irrigation significantly influence the fatty acid profiles of fenugreek genotypes.

The 2021-irrigated condition has the highest interaction with the content of linoleic acid (Fig. 5A), linolenic acid (Fig. 5B), oleic acid (Fig. 5C) and palmitic acid (Fig. 5E) of genotypes while the stearic acid content of genotypes shows the highest interaction with 2022 non-irrigated condition (Fig. 5D). In the biplots, genotypes positioned closer to the horizontal axis (PC1 score of 0) exhibit higher stability across different environments, indicating consistent fatty acid profiles regardless of environmental fluctuations. Among the genotypes with above-average values, South Sudan, Malaysia, Sivas/TR and Kermanshah/IR genotypes showed high and stable content of linoleic acid across three conditions (Fig. 5A). Konya/TR, Samsun/TR, Amasya/TR, China, Egypt, Australia and Berkem/TR genotypes showed high and stable content of linolenic acid (Fig. 5B). Ahvaz/IR, Pakistan and Israel genotypes showed high and stable content of oleic acid (Fig. 5C). Pakistan and Samsun/TR genotypes showed high and stable content of stearic acid (Fig. 5D). Several genotypes (Corum/TR, Pakistan, Ahvaz/IR and Kayseri/TR) showed high content of palmitic acid, but they were found with low stability across three conditions (Fig. 5E).

4. Conclusion

This study provides a comprehensive analysis of the fatty acid composition in fenugreek (*Trigonella foenum-graecum* L.) seeds, highlighting significant variability influenced by both genetic and environmental factors. Our findings indicate that irrigation significantly enhances the levels of essential fatty acids such as linoleic and linolenic acids while reducing the content of palmitic and oleic acids. These results underscore the critical role of water availability in the biosynthesis of fatty acids, suggesting that irrigation practices can be strategically optimized to improve the nutritional quality of fenugreek seeds. Moreover, genotypic diversity among fenugreek varieties plays a crucial role in determining fatty acid profiles, with some genotypes exhibiting more stable and favorable compositions under varying environmental conditions. By identifying these genotypes and implementing appropriate irrigation strategies, breeders can develop fenugreek varieties that offer enhanced nutritional benefits and adaptability to climate change. The study's principal component analysis and additive main effects and multiplicative interaction biplots further illustrate the complex interactions between genotype and environment, providing valuable insights for developing fenugreek varieties suited to diverse agro-climatic conditions. These findings contribute to the growing body of knowledge on functional foods and natural health products, paving the way for future studies focused on improving the quality and resilience of this valuable crop.

CRedit authorship contribution statement

Furkan Coban: Validation, Investigation, Resources, Writing – review & editing, Conceptualization. **Hakan Ozer:** Project administration, Validation, Investigation, Writing – review & editing. **Yuzhou Lan:** Validation, Formal analysis, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgements

This research was supported by the '1001 - The Scientific and Technological Research Projects Funding Program (No: TOVAG 2200003)' of TUBITAK (The Scientific and Technological Research Council of Turkey).

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