RESEARCH ARTICLE

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Unveiling the phytochemical variability of fatty acids in world marigold (*Calendula officinalis* L.) germplasm affected by genotype

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Abstract

Marigold is an annual herbaceous medicinal and aromatic plant, native to the Mediterranean region. Although marigold flowers have attracted considerable attention, the noteworthy characteristics of marigold seeds have often been overlooked. The industrial sector holds keen interest in marigold due to the presence of calendic acid in its seeds. Moreover, calendic acid exhibits promising anti-cancer properties, adding to the growing interest in the medicinal potential of this plant. In this study, a total of 31 marigold genotype seeds from fifteen different countries were used as experimental material. The observed seed oil content exhibited a range of values spanning from 6.00% to 20.33%, with a mean value of 11.59%. GC/MS analysis was conducted to evaluate the chemical variability associated with genotypic changes. Notably, the main fatty acids observed in the oil of these genotypes were α-calendic acid (ranging from 6.91% to 51.42%), linoleic acid (ranging from 30.50% to 48.25%), oleic acid (ranging from 8.26% to 22.50%), and palmitic acid (ranging from 3.86% to 9.28%). Particularly noteworthy is the emergence of genotypes PI 420376, PI 545694, PI 545701, PI 578109, PI 597588, PI 597591, and PI 597594, boasting calendic acid content exceeding 50%. Furthermore, the values of calendic acid exhibit significant variation across countries. The range extends from the United Kingdom, displaying one of the lower values, to Ontario, Canada, which represents countries with notably higher values. Consequently, there exists a necessity to enhance the proportion of calendic acid within marigold through strategic plant breeding techniques. This can be achieved through the selection and development of marigold cultivars with higher calendic acid contents.

Keywords: Calendic acid, Genotype, Fatty acid profiles, Plant genetic diversity, Polyunsaturated fatty acids

INTRODUCTION

Marigold (*Calendula officinalis* L.), a member of the Compositae family, is an oilseed plant indigenous to the Mediterranean region (Krol et al., 2016). It is a versatile plant, valued for its beauty, therapeutic properties, and culinary uses. While its flowers have attracted considerable attention, the often overlooked, marigold seeds possess a number of impressive properties. The oil is obtained from the seeds of the plant is valued for its potential health benefits. It contains polyunsaturated fatty acids like linoleic acid and calendic acid and it finds extensive usage in skincare items due to its hydrating and revitalizing characteristics, making it highly sought after (Ahmad and Ahsan, 2020). It has the potential to support skin health, facilitate the recovery process for wounds and minor burns, minimize the visibility of scars, and enhance the overall complexion (Orchard and van Vuuren, 2019).

Within this highly adaptable plant lies an exceptional fatty acid recognized as calendic acid, which has garnered considerable attention (Olennikov et al., 2022). Calendic acid, also referred to as trans,trans,cis-8,10,12-octadecatrienoic acid, is a unique polyunsaturated fatty acid (Avato and Tava, 2022). It is characterized by its distinctive molecular structure, containing three double bonds along its carbon chain. This structural feature sets calendic acid apart from other fatty acids and contributes to its notable properties and potential health benefits. Calendic acid and linoleic acid are both fatty acids that belong to the omega-6 family. They share a structural similarity but differ in terms of the number and position of double bonds along their carbon chain (Crombie and Holloway, 1985). Calendic acid and linoleic acid both have 18 carbon atoms in their chain. Linoleic acid is a polyunsaturated fatty acid with two double bonds, located at positions 9 and 12 along the carbon chain. Linoleic acid serves as the precursor for the synthesis of various other omega-6 fatty acids, including calendic acid (Cahoon et al., 2001). Calendic acid stands out among various fatty acids essential for important bodily functions due to its notable cytotoxic effects (Verma et al., 2018). This cytotoxic action primarily operates through lipid peroxidation and the reduction of lcf1 gene expression, which plays a role in encoding long-chain fatty acyl-CoA synthetase (Suzuki et al., 2001; Dulf et al., 2013; Garaiova et al., 2023). Moreover, it might inhibit tumour growth, induce apoptosis (programmed cell death) in cancer cells, and modulate inflammatory responses (Garaiova et al., 2023). Both α-calendic acid and β-calendic acid have exhibited anti-cancer properties in laboratory-based in vitro studies (Yuan et al., 2014; Dubey et al., 2019).

Germplasm collections serve as vital repositories of plant genetic diversity (Çelik et al., 2023; Nadeem et al., 2020; Nadeem et al., 2021; Yılmaz et al., 2021). Various institutions, botanical gardens, and research organizations have initiated efforts to collect, conserve, and document marigold germplasm from different regions. These collections ensure the preservation of valuable genetic resources, allowing breeders and researchers to access diverse genetic material for future breeding programs and scientific studies (Barut et al., 2020). To understand the genetic diversity present within marigold germplasm, breeders and researchers conduct comprehensive phenotypic and genetic characterization studies. Through these studies, breeders attain valuable knowledge regarding the diversity of traits and identify promising genotypes displaying sought-after attributes (Kurt et al., 2020; Güneş and Tonçer, 2023).

The industrial sector has shown a keen interest in marigold due to the discovery that its seeds contain approximately 60% calendic acid (Cromack and Smith, 1998). Marigold seed holds significant importance due to its rich composition of calendic acid and the potential for increasing its content through plant breeding. Moreover, it is important to investigate genetic diversity, apply breeding techniques, and understand the basic mechanisms that regulate fatty acid biosynthesis (Dulf et al., 2013). This knowledge can be applied to other plants and contribute to broader research and development efforts in plant breeding, lipid metabolism, and functional crop improvement. The objective of this work is to elucidate the role of genetic factors, specifically the genotype of marigold plants, in the modulation of differences observed in the fatty acid profiles.

MATERIALS AND METHODS

Seed samples

The field study was conducted at the experimental area of the Department of Field Crops at Çukurova University, located in Adana (37°00'55.20" N, 35°21'25.80" E), Türkiye, during the 2021 cultivation period. In this study, a total of 31 marigold (*Calendula officinalis* L.) genotype seeds were used as an experiment material (Table 1). These genotypes were obtained from the United States Department of Agriculture (USDA). The area generally experiences a Mediterranean climate, characterized by warm and arid summers, along with temperate and rainy winters. The soil composition at the location consisted of clay-loam texture, containing a minimal amount of organic material (1.11%). The soil was tilled using a field cultivator. In situations where rainfall was lacking, irrigation was carried out on a weekly basis using sprinklers after the sowing. Weed management was executed using a hoe. Throughout the experiment, no pesticides were applied. For plant fertilization, N and P_2O_5 were administered to the plots at a dosage of 25 kg/ha in the form of diammonium phosphate (DAP) (18–46–0). However, potassium was not applied during the research due to the soil's ample potassium content.

Oil extraction and preparation of fatty acid methyl esters (FAME)

Marigold seeds were isolated and milled. A 5 g sample was mixed with 117 ml of n-hexane, and subsequently, seed oil extraction was performed using an ultrasonic bath set at 55 °C for a duration of 45 minutes. Following this, the n-hexane was removed from the extract through evaporation at 70 °C using a rotary evaporator. The remaining oil was then quantified at the Department of Field Crops, Faculty of Agriculture, Çukurova University. To analyze the composition of oil fatty acids, the oil underwent methylation to produce fatty acid methyl esters (Stefanoudaki et al., 1999). Prior to analysis, the fatty acids were transformed into methyl esters by agitating a mixture of 0.5 ml of oil and 5 mL of hexane for 5 minutes. Subsequently, 0.5 mL of 2 N methanolic potassium hydroxide was added to the solution and shaken for an additional 5 minutes, followed by centrifugation for 5 minutes.

Table 1. List of investigated marigold germplasm

No	Genotype	Name	Origin	Improvement status
1	Ames 24244	NU 40517	England, United Kingdom	Uncertain
2	PI 279690	Orange Shaggy	England, United Kingdom	Cultivated
3	PI 293762	A 23846	Former, Soviet Union	Cultivated
4	PI 420253	34	Portugal	Cultivated
5	PI 420375	35	Spain	Cultivated
6	PI 420376	44	Spain	Cultivated
7	PI 506435	-	Ukraine	Cultivated
8	PI 535879	'Promyk'	Poland	Cultivar
9	PI 545694	86-3A	India	Cultivated
10	PI 545699	NU 40598	England, United Kingdom	Cultivated
11	PI 545701	NU 52322	Illinois, United States	Cultivated
12	PI 560148	31	Finland	Cultivated
13	PI 578105	Ames 19025	Kazakhstan	Cultivated
14	PI 578106	Ames 19026	Alma-Ata, Kazakhstan	Cultivated
15	PI 578107	880608	Germany	Cultivated
16	PI 578109	883077	Algeria	Uncertain
17	PI 597588	NU 40010	Maryland, United States	Cultivated
18	PI 597589	'Ball's Orange'	England, United Kingdom	Cultivar
19	PI 597591	'Radio'	England, United Kingdom	Cultivar
20	PI 597592	'Pacific Beauty Lemon'	Maryland, United States	Cultivated
21	PI 597593	NU 45275	Former Serbia and Montenegro	Cultivated
22	PI 597594	NU 48884	Ontario, Canada	Cultivated
23	PI 600911	'Orange Gitana'	Netherlands	Cultivar
24	PI 603111	'Orange Baby'	New York, United States	Cultivar
25	PI 607418	CAL 44/89	Algeria	Uncertain
26	PI 613018	'Orange Sunshine'	England, United Kingdom	Cultivar
27	PI 613019	'Pacific Beauty Cream'	England, United Kingdom	Cultivar
28	PI 613020	W-F Formula Blend	California, United States	Cultivated
29	PI 618688	NU 40508	England, United Kingdom	Cultivar
30	PI 662007	Orange King Improved	England, United Kingdom	Cultivar
31	PI 675148	Beauty Mixed	Illinois, United States	Cultivar

Gas chromatography ± mass spectrometry (GC/MS) analysis

GC-MS analyses were carried out in the Department of Biology at Kahramanmaras Sutcu Imam University. After the oil was extracted, 1 µl of the esterified sample was injected to the GC-MS device. Qualification of the oil was assessed using an Agilent 5975C Mass Spectrometer coupled with an Agilent GC-6890II series. The GC was equipped with an HP-88 capillary column (100 m x 250 µm m x 0.20 µm film thickness) and He was used as carrier gas with a flow rate of 0.8 mL/min. The GC oven temperature was programmed as follows: 170 °C (1 min), 230 °C at 15 °C/min and then kept at 230 °C at 20 min. The injector temperature was 250 °C. The mass spectrometer was operating in EI mode at 70 eV. The split ratio was 20:1, and the mass range analyzed 35-400m/z with a scan speed of 1000 amu/s. To identify the compounds, the Wiley7n.1, Famdbwax.L, and Famedb23.L libraries were utilized.

Statistical analysis

Statistical software JMP® (version 14.0, SAS Institute Inc., Cary, NC, 1989-2019) was used to conduct principal components on correlations and constellation plot analysis. In order to construct the heat map, Flourish studio was used. The Metan package within the R Studio software was utilized to calculate correlations among the fatty acids employing the Pearson coefficient.

RESULTS AND DISCUSSION

The biovariability of fatty acids

The seed oil content and fatty acid composition of the seeds of 31 different marigold genotypes were analyzed and the results are presented in Table 2. The seed oil content ranged from 6.00% to 20.33% with a mean of 11.59%. Variations in marigold seed oil content have also been observed in the works of other researchers; 13.60% to 21.70% (Dulf et al., 2013), 14.88% to 19.76% (Krol et

al., 2016), 15.81% to 20.10% (Król and Paszko, 2017), 13.30% to 15.40% (Zarrinabadi et al., 2019). Król and Paszko (2017) reported that marigold plants cultivated in Mediterranean climates possess notably lower amounts of oil compared to those cultivated in temperate climates. However, despite the prevailing Mediterranean climate in our region, the ratios of seed oil align closely with the values reported in the literature. The chemical composition of marigold oil varied according to genotypes. The representative GC-MS chromatogram of the fatty acids is provided in Figure 1. The heatmap for the fatty acids of marigold samples is presented in Figure 2. Ten compounds were found, representing 90.47% to 100% of the total seed oils. The main fatty acids in the seed oil of these genotypes were α-calendic acid, linoleic acid, oleic acid, and palmitic acid, respectively.

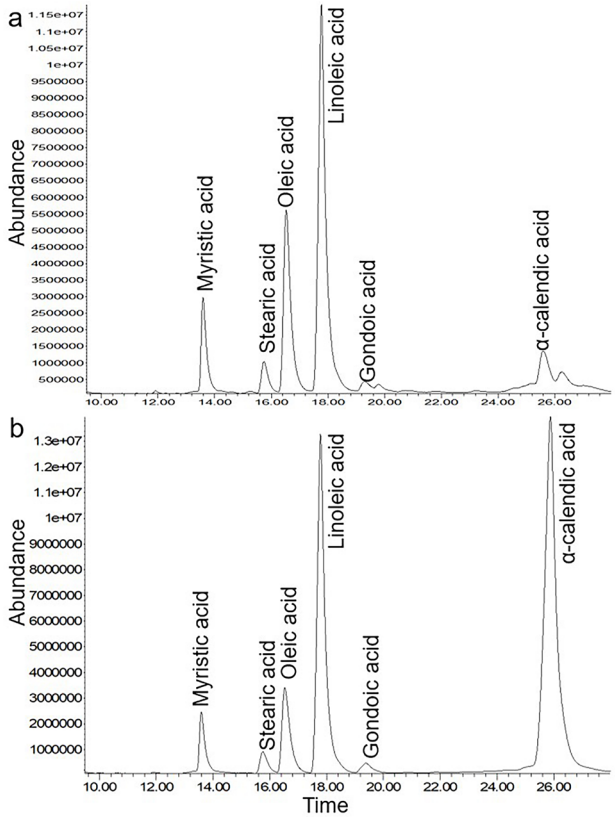

Figure 1. GC/MS chromatogram of the different genotypes a: Ames 24244, b: PI 597594.

The α-calendic acid was the highest polyunsaturated fatty acid (PUFA) in the genotypes. The α-calendic acid ranged from 6.91% to 51.42% with a mean of 40.12%. Our results showed that the highest α-calendic acid was found in PI 597594, while the lowest percentage was found in Ames 24244. When contrasting the α-calendic acid levels in marigold with previous studies, diverse results were observed; 59.00% (Feder et al., 2009), 51.40% to 57.60% (Dulf et al., 2013), 38.79% to 53.43% (Krol et al., 2016), 40.10% to 51.44% (Król and Paszko, 2017), 42.92% to 50.98% (Rahimi et al., 2020), 45.95% to 46.27% (Salama and Sabry, 2023). There is a broad range of calendic acid values based on the countries. The range extends from UK (6.91%), which represents one of the lowest values, to Ontario, Canada (51.42%), which is among the countries with the highest values. There seems to be diversity in the calendic acid content even within genotypes from the same geographical region. For example, Ames 24244 and PI 545699, both originating from England, United Kingdom, have distinct calendic acid values of 6.91% and 47.97% respectively. This suggests that even within close geographical regions, plants might possess varying calendic acid levels due to growing conditions, soil structure, or other factors. Additionally, there is variability in calendic acid values across countries from different continents. For instance, plants from geographically distant areas like India (51.27%) and Canada (51.42%) showcase similar elevated calendic acid values. This highlights the possibility of achieving comparable outcomes in diverse geographical locations, attributed to the intricate interplay of plant genetic structures, cultivation methodologies, and environmental factors. Through a process known as elongation and desaturation, linoleic acid can be converted into calendic acid by introducing an additional double bond at position 8 (Cao et al., 2013). Different genotypes may have distinct genetic traits that influence the production and accumulation of calendic acid in their seeds. Moreover, environmental conditions, such as temperature, sunlight, soil composition, and moisture levels, can influence the biosynthesis and accumulation of calendic acid in seeds. Furthermore, the content of calendic acid can vary during different stages of plant development. It is possible that certain marigold genotypes have higher levels of calendic acid at specific growth stages, which may contribute to the observed differences in content among genotypes. In addition, variations in the activity or expression of enzymes involved in biosynthetic pathways can affect the production and accumulation of calendic acid. Differences in the regulation of these pathways among marigold genotypes can contribute to variations in calendic acid content. Understanding these factors and their interplay is crucial for researchers and breeders aiming to develop marigold genotypes with desired calendic acid profiles.

The linoleic acid was found in high quantities for all genotypes. The results indicate that the contents of linoleic acid were detected between 30.50% to 48.25% with a mean of 35.98%. Various outcomes concerning the linoleic acid content in marigold have also been documented by researchers; 28% (Feder et al., 2009), 28.50% to 31.90% (Dulf et al., 2013), 30.70% to 36.63% (Krol et al., 2016), 31.99% to 36.52% (Król and Paszko, 2017), 21.20% to 28.01% (Rahimi et al., 2020), 26.56% to 26.81% (Salama and Sabry, 2023). According to Özgül-Yücel's (2005) findings, the fixed oil derived from marigold seeds in Turkey is distinguished by its elevated levels of

linoleic acid and relatively low amounts of calendic acid. The predominant factor influencing the ratio of calendic acid is believed to be primarily associated with the timing of harvest (Barut et al., 2022).

The oleic acid was the highest monounsaturated fatty acid (MUFA) in the genotypes. The oleic acid ranged from 8.26% to 22.50% with a mean of 12.91%. Diverse results based on the oleic acid content of marigold were also reported by the researchers; %4 (Feder et al., 2009), 3.64% to 5.78% (Krol et al., 2016), 2.78% to 6.08% (Rahimi et al., 2020), 5.87% to 6.72% (Salama and Sabry, 2023). On the other hand, palmitic acid was the highest saturated fatty acid (SFA) in the genotypes. The palmitic acid ranged from 3.86% to 9.28% with a mean of 5.20%. Upon comparing the palmitic acid levels in marigold with previous investigations, varying outcomes were

Table 2. Fatty acid profile of marigold genotypes

Fatty acids		**C 14:0**	**C 16:0**	**C 16:1**	**C 17:0**	**C 18:0**	**C 18:1**	**C 18:2**	**C 20:1**	**C 22:0**	**C 18:3n6**	**Total**	**MUFAs**	**PUFAs**	**SFAs**
RT (min)		**11.879**	**13.679**	**14.479**	**14.679**	**15.679**	**16.479**	**17.879**	**19.279**	**21.879**	**25.679**				
Genotype	**Seed oil cont. (%)**	**Relative peak area (%)**													
Ames 24244	6.00	0.16	8.55	nd	nd	3.53	21.82	47.63	1.67	0.20	6.91	90.47	23.49	54.54	12.44
PI 279690	8.00	0.16	5.15	nd	nd	2.23	13.38	35.64	1.21	0.03	40.03	97.83	14.59	75.67	7.57
PI 293762	11.67	0.06	3.97	0.11	nd	2.31	12.10	33.34	1.51	0.01	45.55	98.95	13.71	78.89	6.35
PI 420253	15.00	0.10	4.73	nd	nd	2.09	11.00	33.45	1.19	0.04	47.32	99.91	12.18	80.77	6.96
PI 420375	6.00	0.13	8.96	nd	nd	4.07	17.87	44.66	1.77	0.21	17.26	94.93	19.64	61.92	13.38
PI 420376	16.00	0.11	4.18	0.06	nd	1.96	8.68	31.71	1.32	0.01	51.31	99.33	10.05	83.01	6.27
PI 506435	9.33	0.11	5.55	0.27	nd	2.44	15.57	34.90	1.23	0.11	39.62	99.81	17.08	74.52	8.21
PI 535879	16.00	0.08	4.10	nd	nd	2.24	14.34	33.59	1.30	0.06	43.99	99.71	15.65	77.58	6.49
PI 545694	14.00	0.07	4.26	0.17	nd	2.07	8.89	31.21	1.42	0.49	51.27	99.85	10.47	82.48	6.90
PI 545699	12.67	0.14	4.26	nd	nd	2.12	11.29	32.65	1.31	0.04	47.97	99.78	12.60	80.62	6.56
PI 545701	11.33	0.11	4.34	0.18	nd	1.94	11.47	30.50	1.12	nd	50.04	99.71	12.78	80.54	6.39
PI 560148	12.00	-	3.99	nd	nd	1.90	9.79	34.65	3.46	nd	45.98	99.77	13.25	80.63	5.89
PI 578105	10.00	0.09	5.44	nd	nd	2.27	14.56	35.48	1.24	0.14	31.92	91.14	15.80	67.40	7.94
PI 578106	6.00	0.10	5.76	nd	nd	2.50	10.97	34.19	1.59	0.22	43.09	98.42	12.56	77.28	8.58
PI 578107	8.67	0.15	8.48	0.14	0.22	3.79	22.50	48.25	1.86	0.21	11.64	97.23	24.50	59.88	12.85
PI 578109	9.33	0.07	4.42	nd	nd	2.20	9.40	31.79	1.30	0.11	50.66	99.95	10.70	82.45	6.80
PI 597588	20.33	0.08	4.13	0.01	nd	2.03	8.77	32.50	1.27	0.04	51.12	99.95	10.05	83.62	6.28
PI 597589	10.00	0.09	5.24	nd	nd	1.65	10.28	33.46	1.49	0.05	47.46	99.72	11.77	80.92	7.03
PI 597591	12.33	0.08	3.86	nd	nd	1.91	8.26	33.24	1.41	0.02	50.20	98.97	9.67	83.44	5.85
PI 597592	11.00	0.05	4.21	nd	nd	2.08	15.92	33.39	1.65	0.09	42.09	99.47	17.56	75.48	6.42
PI 597593	8.67	0.11	6.70	nd	nd	2.99	16.84	44.56	1.58	0.05	24.84	97.68	18.42	69.40	9.86
PI 597594	17.33	0.07	4.19	nd	nd	1.99	8.43	32.06	1.34	0.01	51.42	99.50	9.77	83.48	6.26
PI 600911	10.33	0.09	5.14	nd	nd	2.71	18.96	40.97	1.31	0.03	29.38	98.58	20.27	70.35	7.97
PI 603111	13.67	0.07	4.09	nd	nd	1.88	11.32	34.90	1.48	nd	46.01	99.76	12.79	80.92	6.05
PI 607418	12.00	0.07	4.03	nd	nd	1.89	11.88	32.94	1.49	nd	45.97	98.27	13.37	78.92	5.99
PI 613018	8.67	0.12	6.42	nd	nd	3.20	14.70	43.81	1.34	0.14	23.70	93.42	16.04	67.51	9.87
PI 613019	18.00	0.06	4.21	nd	nd	2.00	14.30	34.51	1.34	0.02	43.56	100.00	15.64	78.07	6.29
PI 613020	13.00	0.07	4.28	nd	nd	1.63	10.24	32.54	1.43	0.02	48.47	98.69	11.67	81.01	6.01
PI 618688	12.00	0.07	4.75	nd	nd	2.14	12.22	33.91	1.45	0.12	44.79	99.45	13.66	78.70	7.09
PI 662007	7.33	0.18	9.28	nd	0.20	3.31	14.23	46.23	2.07	0.21	21.21	96.92	16.30	67.44	13.18
PI 675148	12.67	0.07	4.37	nd	nd	2.00	10.38	32.61	1.21	0.08	48.91	99.64	11.59	81.52	6.52
Min	6.00	0.05	3.86	0.01	0.20	1.63	8.26	30.50	1.12	0.01	6.91	90.47	9.67	54.54	5.85
Mean	11.59	0.10	5.20	0.13	0.21	2.36	12.91	35.98	1.49	0.10	40.12	98.28	14.44	76.09	7.75
Max	20.33	0.18	9.28	0.27	0.22	4.07	22.50	48.25	3.46	0.49	51.42	100.00	24.50	83.62	13.38

(C14:0 (Myristic acid), C 16:0 (Palmitic acid), C 16:1 (Palmitoleic acid), C 17:0 (Heptadecanoic acid), C 18:0 (Stearic acid), C 18:1 (Oleic acid), C 18:2 (Linoleic acid), C 20:1 (Gondoic acid), C 22:0 (Behenic acid), C 18:3n6 (α-calendic acid)), nd: not detected.

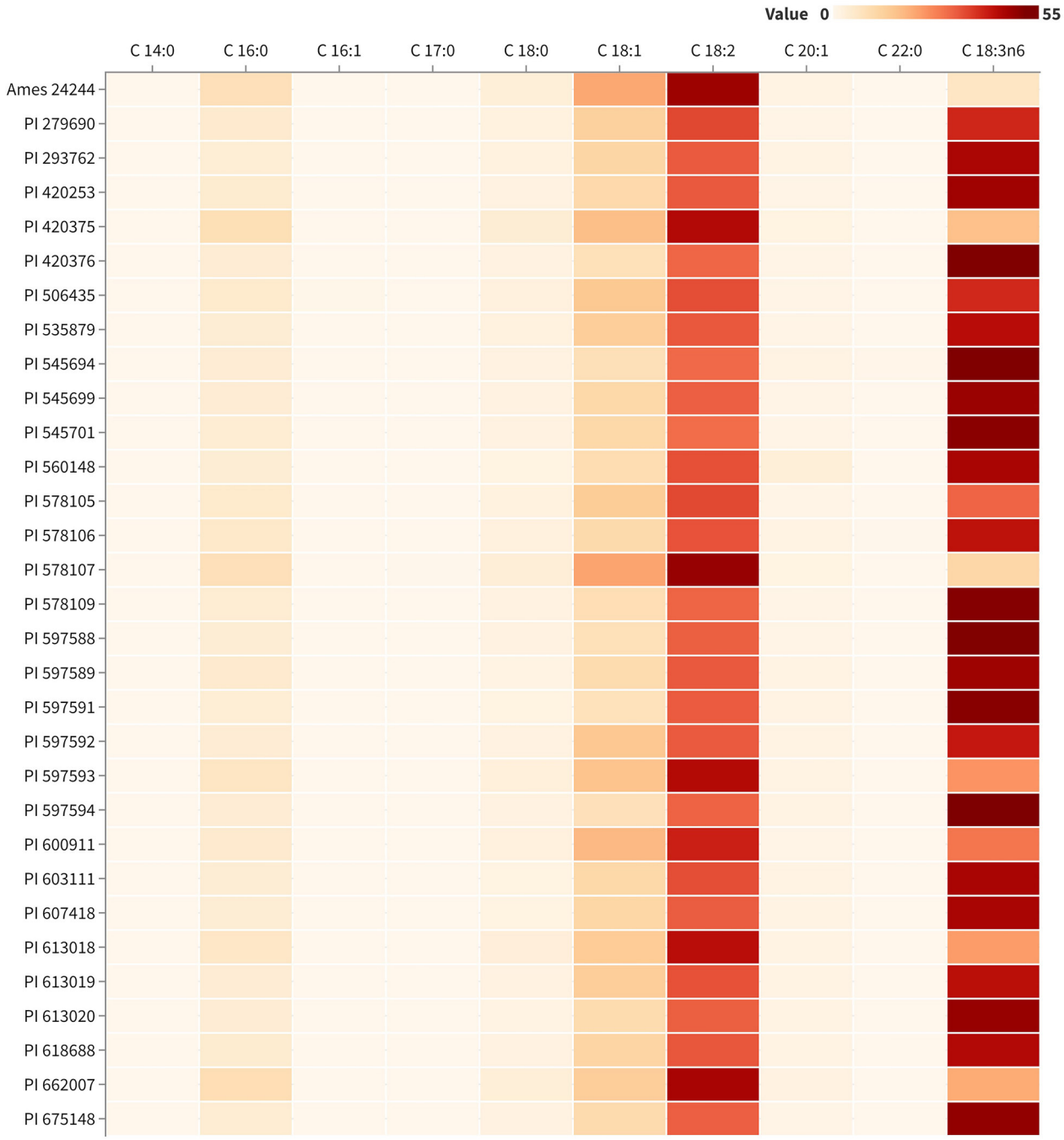

Figure 2. Heatmap of fatty acid profiles for different marigold genotypes (Value=%).

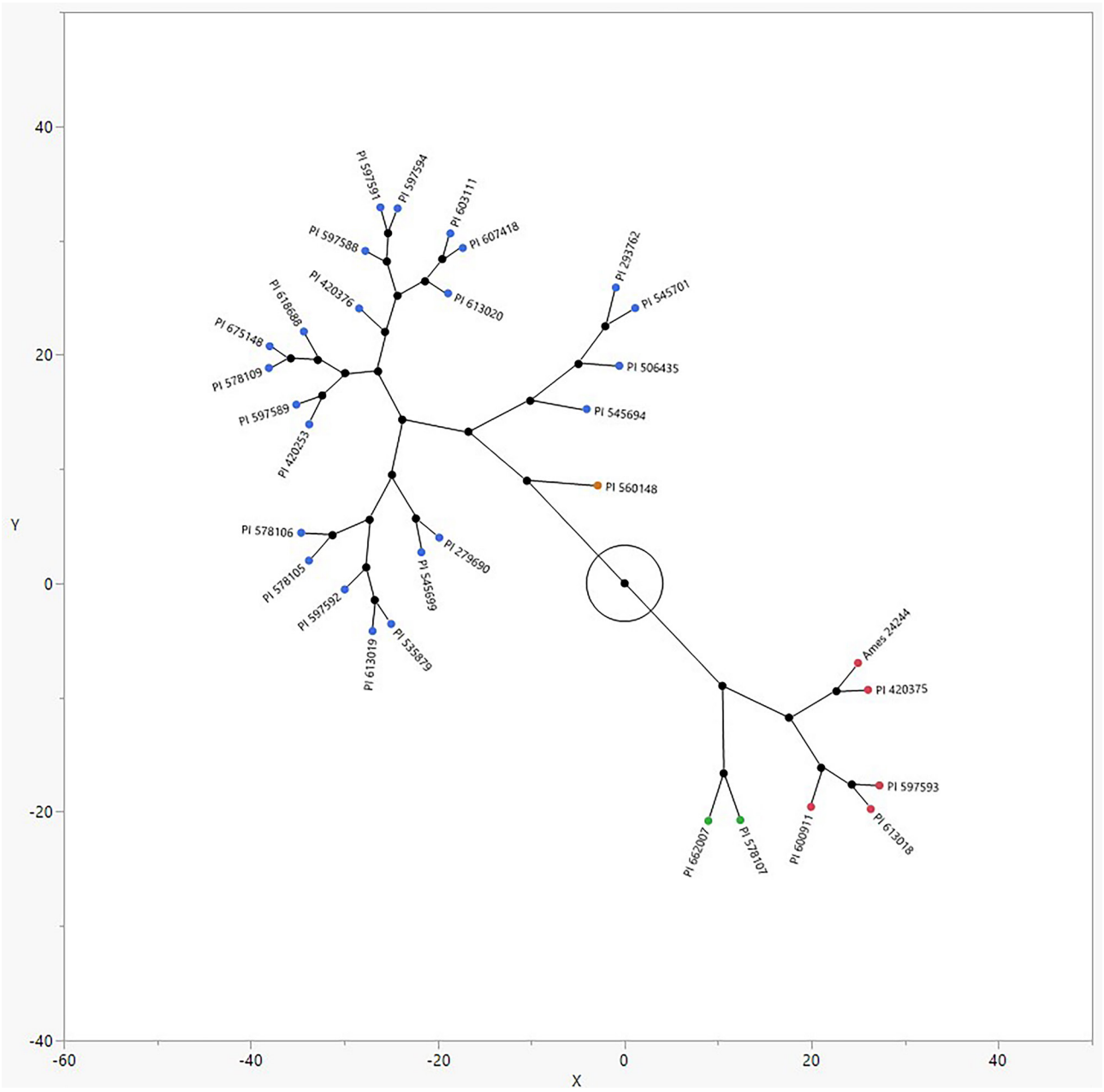

Figure 3. Constellation plot analysis of marigold genotypes.

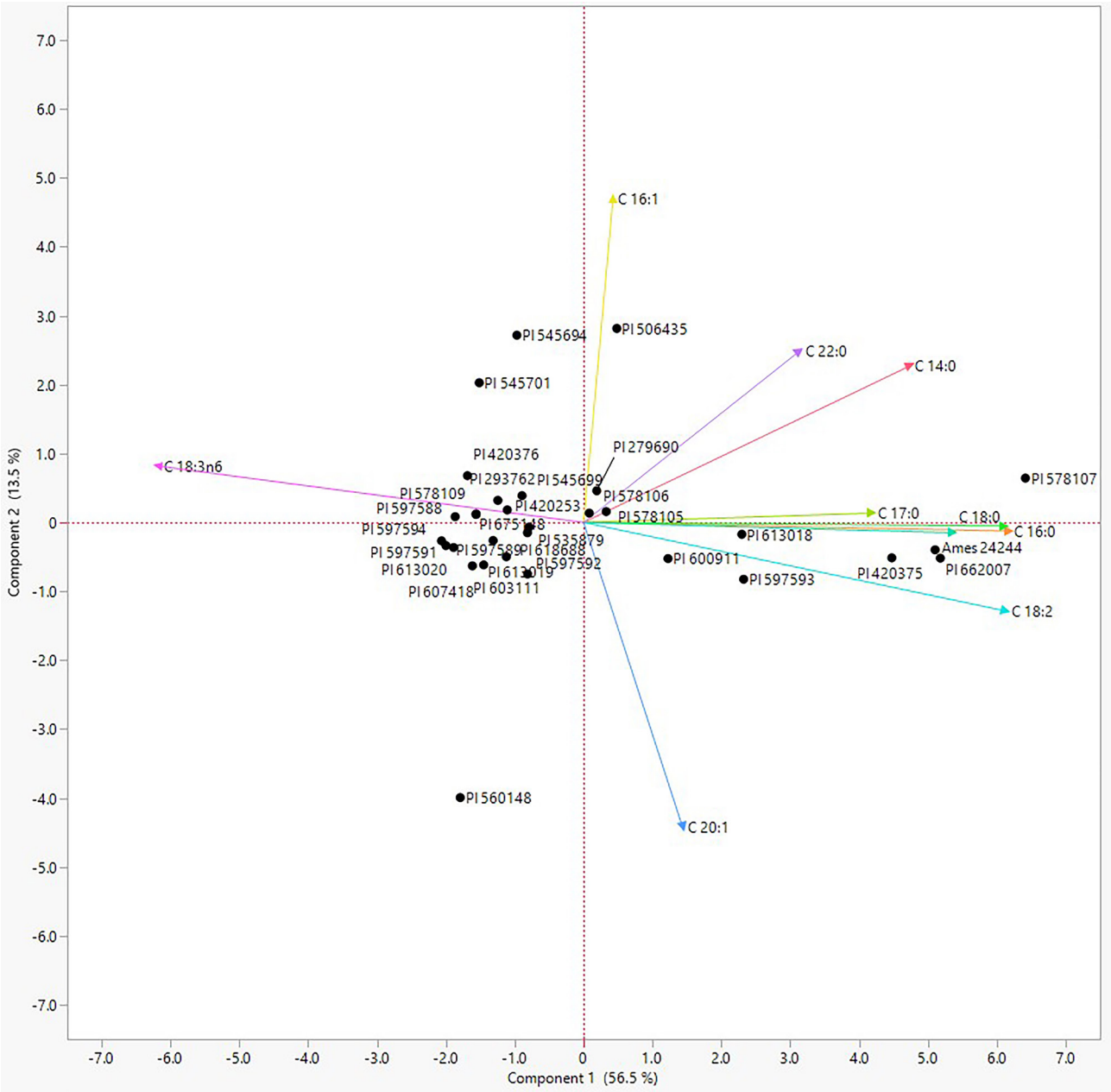

Figure 4. Principal component analysis performed on correlations among genotypes based on fatty acids. (C14:0 (Myristic acid), C 16:0 (Palmitic acid), C 16:1 (Palmitoleic acid), C 17:0 (Heptadecanoic acid), C 18:0 (Stearic acid), C 18:1 (Oleic acid), C 18:2 (Linoleic acid), C 20:1 (Gondoic acid), C 22:0 (Behenic acid), C 18:3n6 (α-calendic acid)).

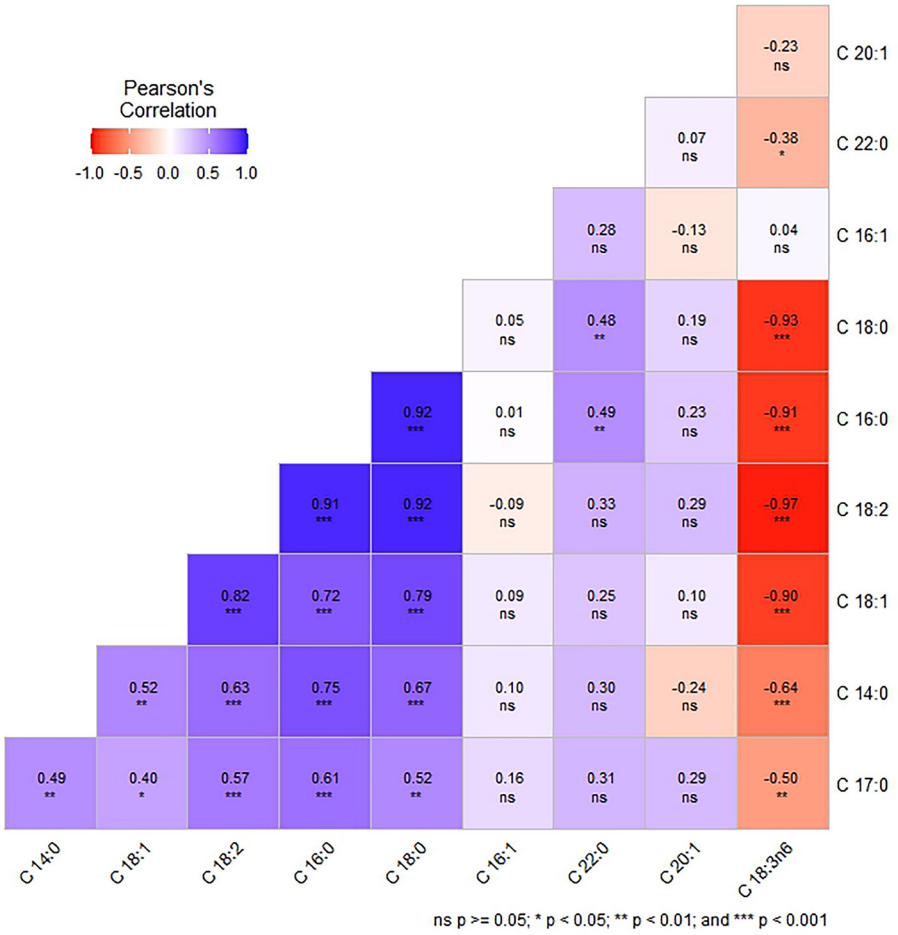

Figure 5. Correlation analysis among the fatty acids (C14:0 (Myristic acid), C 16:0 (Palmitic acid), C 16:1 (Palmitoleic acid), C 17:0 (Heptadecanoic acid), C 18:0 (Stearic acid), C 18:1 (Oleic acid), C 18:2 (Linoleic acid), C 20:1 (Gondoic acid), C 22:0 (Behenic acid), C 18:3n6 (α-calendic acid)).

observed; 3.43% to 5.39% (Krol et al., 2016), 3.77% to 5.65% (Król and Paszko, 2017), 13.12% to 18.62% (Rahimi et al., 2020), 5.23% to 5.32% (Salama and Sabry, 2023). In addition, it was determined that the oil composition of marigold contains a small amount of myristic acid, palmitoleic acid, heptadecanoic acid, stearic acid, gondoic acid, and behenic acid. SFAs level of studied marigold genotypes ranged from 5.85% to 13.38% with a mean of 7.75%. MUFAs level of studied marigold genotypes ranged from 9.67% to 24.50% with a mean of 14.44%. PUFAs level of studied marigold genotypes ranged from 54.54% to 83.62% with a mean of 76.09%. Dulf et al. (2013) reported SFAs level 6.39 to 7.34%, MUFAs level 5.09 to 6.99%, and PUFAs level 60.4 to 66.4%.

Constellation plot, principal component biplot, and correlation analysis of the genotypes based on the fatty acids

To explore the genetic diversity of marigold genotypes, a constellation plot analysis was performed (Figure 3). The utilization of a constellation plot provided optimal outcomes in discriminating the genetic diversity among marigold genotypes. This plot was divided into two main groups: A and B. These main groups were also divided into two subgroups: A1, A2, B1, and B2. Most of the marigold genotypes (23 genotypes-blue color) were located in Group A1. Subgroup A2 had one genotype (orange color). Five genotypes were located in Subgroup B1 (red color), and two genotypes were found in subgroup B2 (green color). The analysis of genotype and fatty acid composition revealed associations as depicted in Figure 4. PC1, the primary principal component, accounted for 56.5% of the standardized dataset's variability, while PC2, the secondary principal component, explained 13.5% of the variation. Together, these two components encompassed a total of 70% of the overall variation. Correlation analysis was performed to observe the relationship between fatty acids (Figure 5). The correlation graph indicates that calendic acid was negatively correlated with myristic acid, palmitic acid, heptadecanoic acid, stearic acid, oleic acid, linoleic acid, and behenic acid. The graph depicting correlations reveals that there is a negative correlation between calendic acid and several other fatty acids including myristic acid, palmitic acid, heptadecanoic acid, stearic

acid, oleic acid, linoleic acid, and behenic acid. This suggests that as the level of calendic acid increases, the levels of these other fatty acids tend to decrease.

CONCLUSION

Through the analysis of fatty acids in marigold seeds using GC/MS, this study revealed significant chemical variability among different genotypes from various countries. The main fatty acids identified in the seed oil included α-calendic acid, linoleic acid, oleic acid, and palmitic acid. There exists a broad spectrum of calendic acid contents among genotypes collected from 15 countries. The range spans from the UK, registering one of the lowest contents, to Ontario, Canada, which stands as one of the nations exhibiting the highest contents. Furthermore, there is variability in calendic acid contents across countries located on different continents. As an instance, plants from geographically distant regions such as India and Canada display comparably high calendic acid contents. This underscores how analogous outcomes can arise across diverse geographical locations, owing to the intricate interplay of plant genetic structures, cultivation methodologies, and environmental factors. To meet the growing demand for calendic acid, it is crucial to enhance its production through plant breeding methods. This can be achieved by selectively breeding marigold cultivars with higher levels of calendic acid. This research lays the foundation for further exploration and advancement in the field of marigold cultivation and calendic acid production. The findings will contribute to a deeper understanding of the phytochemical diversity within marigold and will have implications for future breeding programs aimed at developing marigold cultivars with specific fatty acid profiles to meet various market demands and enhance its health-promoting properties.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declared that for this research article, they have no actual, potential, or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. In addition, all the authors verify that the Text, Figures, and Tables are original and have not been published.

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Not applicable.

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