

Morphological and Phytochemical Assessment of Six Fennel Genotypes from Different Origins Using GC-MS Profiling

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This field experiment evaluated six fennel genotypes from different geographical locations (Jordan, Germany, Italy, Pakistan, and Iraq) for their agronomic performance and chemical profiles using GC-MS with alcoholic and aqueous extraction methods. Significant variation was observed in most agronomic attributes except branch number per plant. The Hannan genotype excelled in seed weight (10.90 g) and total yield (279.20 g/m²), making it ideal for commercial use, while the Amigo genotype was superior in umbel yield (48.22 umbel/plant). Chemical profiles showed considerable variation due to genetic background and extraction methods, with aqueous extracts containing more polar compounds and methanolic extracts containing more non-polar compounds such as terpenoids and esters. The Di Firenze genotype had the highest number of compounds (32) in its methanolic extract, while the Amigo genotype had the fewest compounds. Cluster analysis revealed high genetic variability between genotypes, especially with methanolic extraction, indicating the role of genetic diversity in metabolite variation. The findings suggest the Hannan genotype is suitable for seed production and the Amigo for umbel production. Further research is needed to explore genotype stability across environments and to investigate genetic mechanisms underlying metabolite biosynthesis.

Key words: chemical profiling, active compounds, solvent extraction, GC-MS analysis, fennel genotypes

Fennel (*Foeniculum vulgare* Mill) is a cross-pollinated herb belonging to the Apiaceae family. The Mediterranean shores are believed to be the origin of the herb. Fennel is a well-known aromatic medicinal plant rich in valuable active compounds used for human consumption, cosmetics, and pharmaceutical purposes; however, the essential oil is the main active

substance found in its fruits (Rather *et al.* 2016). The fennel content of essential oil is about 2–4%; however, it appears in different proportions in various plant parts (Gross *et al.* 2009). Several constituents such as fenchone, estragole, trans-anethole, and limonene are dominant compounds in fennel essential oil, while myrcene, pinene, and camphor

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are present in lower concentrations (Cosge *et al.* 2008) are dominant compounds of fennel essential oil, while myrcene, pinene, and camphor appear in lower concentrations (He & Huang 2011). Like other medicinal plants, components of the essential oil from fennel's fruit are highly affected by many factors, especially environmental conditions, management practices, and plant genotype.

Undoubtedly, the extraction method affects the type and/or concentration of the phytochemicals obtained. Moreover, active ingredients may be effectively altered due to the source of the plant material and plant parts (Cosge *et al.* 2008). Most medicinal and aromatic plants require regular agronomic and chemical evaluations based on the latest developments in their genetic material, along with the extraction techniques used (Cosge *et al.* 2008; He & Huang 2011). In this context, recent reports indicate that different fennel genotypes exhibit significant quantitative and qualitative differences, reflecting variations in their active compounds (Al-Mehemdi *et al.* 2020). Hosseini *et al.* (2021) also observed high variability in the active components of fennel caused by genetic differences among twenty genotypes, with results consistent with those of Ziydan *et al.* (2024). Akbari-Kharaji *et al.* (2020) noted that fennel genotypes, based on their physiological components, possess strong genetic potential for growth even in harsh environmental conditions, which is reflected in their chemical composition. Therefore, plant breeders are aiming to develop or adapt new genotypes that suit wide range of agricultural regions. Growth factors such as photosynthetic photon flux density and rhizosphere temperature can influence active compounds, thereby enhancing phytochemical accumulation (Nguyen *et al.* 2019). The time to maturity has been shown to positively affect fennel's essential oil content, with medium and late-maturity genotypes exhibiting the highest oil yields (Bahmani *et al.* 2015). Additionally, phytochemicals in fennel are linked to certain agronomic traits; for example, Pank *et al.* (2011) confirmed that the volatile oil components in fennel are highly correlated with fruit size. They therefore emphasised the importance of the thousand-seed weight characteristic and its relationship to volatile oil yield in fennel, noting that these traits are quantitatively inherited with high heritability.

Likewise, Singh *et al.* (2014) observed a positive genetic correlation between stem diameter and the t-anethole compound, and between the diameter of the main stem and seed yield in the fennel.

Highlighting the importance of the genetic variation of fennel genotypes and growing environment in the chemical composition of phytochemicals of fennel is critical for plant breeding programs and medicinal applications. To understand the impact of genetic diversity and extraction methods on the phenotypic performance and chemical composition of fennel genotypes, the present study conducted a comparative evaluation of six fennel genotypes from different geographical regions using alcoholic and aqueous extractions coupled with GC-MS profiling.

MATERIAL AND METHODS

Genetic Material and Experiment Implementation

The experiment included six bitter and sweet fennel genotypes (Table 1). The study aimed to investigate the seed chemical content and some phenotypic traits. Seeds of the aforementioned fennel genotypes were supplied by the Centre for Desert Studies at the University of Anbar, where the experiment was carried out. Two extraction methods were used: Alcoholic and aqueous. The randomised complete block design (RCBD) was used to implement this experiment, with three replications of each treatment. The area of the experimental units is 2 × 2 m.

Phenotypic traits were recorded according to the combined evaluation of phenotypic and agronomic traits, including plant height, number of branches, number of umbels per plant, umbel length, seeds per umbel, 1,000 seed weight, and yield at harvest (Lopes *et al.* 2010).

Preparation of Extracts

Harvested fruits were stored for 14 days at room temperature, and 30 g of each genotype's fruits were ground using an electrical grinder (Hisense, Germany). Alcoholic and aqueous extractions of each genotype were prepared as follows:

A weight of 25 g of each ground seed was put in a volumetric beaker containing 125 mL of methyl alcohol (Chem-Lab NV, Zedelgem, Belgium) and

then kept on a shaker for 24 hours at room temperature (25 ± 2). Then, the samples were filtered using a Buchner funnel with suction pressure. To concentrate the extraction into a slurry, each extracted sample was placed on a magnetic hotplate stirrer (2,500 rpm) (Stuart, BioCote, UK) at 40°C for 90 minutes. The aqueous extraction was prepared following the same procedure as in the methyl alcohol extraction; however, distilled water was used as the solvent. Produced extractions were stored in the fridge at 4°C before further analysis using GC-MS.

GC-MS Conditions

GC-MS analysis performed using GC-MS-QP2010 plus instrument (Shimadzu, Kyoto, Japan) equipped with auto-injector and 5 mm capillary column (DB) of $30 \times 0.25 \mu\text{m}$ dimension with $0.25 \mu\text{m}$ film thickness. Helium was used as the carrier gas, and the column velocity flow rate was adjusted to 1.15 [mL/min] , controlled through electronic pressure. A 70-eV ionisation system was applied for mass spectroscopic analysis with a scanning time of 0.5 sec and a scanning mass of 40 to 1,000 $[\text{m/z}]$. The oven was heated based on programming of the primary temperature, which was established at 80°C (isothermal for 2 min) to be gradually elevated at 10°C per min up to 200°C . Then, 5°C per min up to 280°C was terminated in an isothermal 35 min. All samples were injected, depending on the split mode, at 250°C . Two mass spectral databases, National Institute of Standards and Technology (NIST14), and Wiley 10th/NIST 2014 mass spectral library (W10N14) were adopted to characterise the isolated

components via retention time and mass spectra, as mentioned in Dawood *et al.* (2020).

Statistical Analysis

The results of phenotypic assessment were statistically analysed using Genstat software (v.12) according to the experimental design. The significant differences between treatment means were compared against the least significant difference (*LSD*) at the probability level of 0.05. The phenology tree was constructed from the binary matrix data, which were made according to presence (1)/absence (0) using Hierarchical Cluster Analysis (HCA), Multivariate Statistical Packages (MVSP v. 3.22) based on Jaccard's Coefficient. The radar analysis and the classification of the phytochemical compounds' plots were constructed using R Packages (V.4.4.3) [(install.packages(„fmsb“) and install.packages („scales“) and ggplots2 package respectively] (Olivoto & Lúcio 2020).

RESULTS

Phenotypic Assessment

The data presented in Table 2 clearly compare the six fennel genotypes in terms of the phenotypic traits. The genotype Amigo was significantly superior in plant height [146.89 cm] over all other genotypes except Sahoo. While Hannan genotypes showed the highest number of branches per plant, the fewest branches were recorded for the Shuayhae genotype. The Amigo genotype showed the highest number of umbels per plant, compared to 23.33

T a b l e 1

Fennel genotypes information supplied by the Centre of Desert Studies at the University of Anbar

Genotype	Origin	Introduction date	Method of development	Breeding country	Notes
Amigo	Italian	2010	–	Italy	Bitter fennel
Di Firenze	Italian	2010	–	Italy	
Hannan	Jordanian	2006	Recurrent selection	Iraq	Sweet fennel
Sahoo	Germany	2013	Recurrent selection	Iraq	Bitter fennel
Sajjet	Pakistani	unknown	Recurrent selection	Iraq	Sweet fennel
Shuayshae	Local variety	unknown	Irradiation (Co60)	Iraq	

[umbel/plant] recorded by the Shuayhae genotype. Also, the Amigo genotype recorded the highest number of umbellate per umbel. However, the Sahoo genotype recorded the lowest number of umbellate per umbel, followed by the Hannan genotype. Although the Sahoo genotype was marked with the lowest number of umbellate per plant, it showed the highest number of seeds per umbellate, in contrast with the Amigo genotype, which gave the lowest

number of seeds per umbellate with 20.72 [seeds per umbellate] (Table 2). Hannan exhibited the highest thousand-seed weight compared to the Amigo genotype, which gave the lowest trait mean. Due to the superior performance of the Hannan genotype in key agronomic traits (number of branches per plant) and yield attributes (seed weight), this genotype showed the maximal yield of 297.20 [g/m²] in comparison, the Sahoo genotype acted differently

T a b l e 2

Phenotypic traits of the six fennel genotypes (Mean $\pm$ SE, n = 3)

Genotypes	Height [cm]	Branch [Branch/plant]	Umbel [Umbel/plant]	Umbellate [Umbellate/umbel]	Seed [Seed/umbellate]	*TSW [g]	Yield [g/m ²]
Amigo	146.89 $\pm$ 2.71 ^a	3.00 $\pm$ 0.17	48.22 $\pm$ 1.05 ^a	77.72 $\pm$ 1.60 ^a	20.72 $\pm$ 0.04 ^{cd}	5.29 $\pm$ 0.31 ^b	197.88 $\pm$ 1.61 ^b
Di Firenze	128.89 $\pm$ 1.09 ^b	3.06 $\pm$ 0.18	41.86 $\pm$ 0.68 ^c	33.61 $\pm$ 0.37 ^d	26.72 $\pm$ 0.39 ^b	10.54 $\pm$ 0.03 ^a	159.77 $\pm$ 0.56 ^c
Hannan	125.00 $\pm$ 1.69 ^b	6.83 $\pm$ 0.39	40.78 $\pm$ 0.05 ^c	24.63 $\pm$ 0.31 ^e	21.78 $\pm$ 0.10 ^c	10.9 $\pm$ 0.63 ^a	297.20 $\pm$ 2.15 ^a
Sahoo	144.33 $\pm$ 0.25 ^a	4.22 $\pm$ 0.24	43.95 $\pm$ 0.23 ^b	23.72 $\pm$ 0.36 ^e	34.42 $\pm$ 0.26 ^a	6.74 $\pm$ 0.39 ^c	53.04 $\pm$ 0.18 ^f
Sajjet	101.50 $\pm$ 0.09 ^d	3.61 $\pm$ 0.21	34.61 $\pm$ 0.31 ^d	50.76 $\pm$ 0.04 ^c	19.65 $\pm$ 0.56 ^d	9.41 $\pm$ 0.54 ^b	176.08 $\pm$ 1.38 ^d
Shuayshae	107.72 $\pm$ 0.45 ^c	2.89 $\pm$ 0.17	23.33 $\pm$ 0.19 ^e	56.39 $\pm$ 3.66 ^b	20.19 $\pm$ 0.01 ^d	7.47 $\pm$ 0.43 ^c	185.06 $\pm$ 0.86 ^c
<i>LSD</i> _{0.05}	3.50	N.S	1.80	1.77	1.43	0.85	2.85

*TSW – thousand seeds weight; *LSD* – least significant difference; N.S – non-significant. Means followed by the same letter are not significantly different at the ($p < 0.05$).

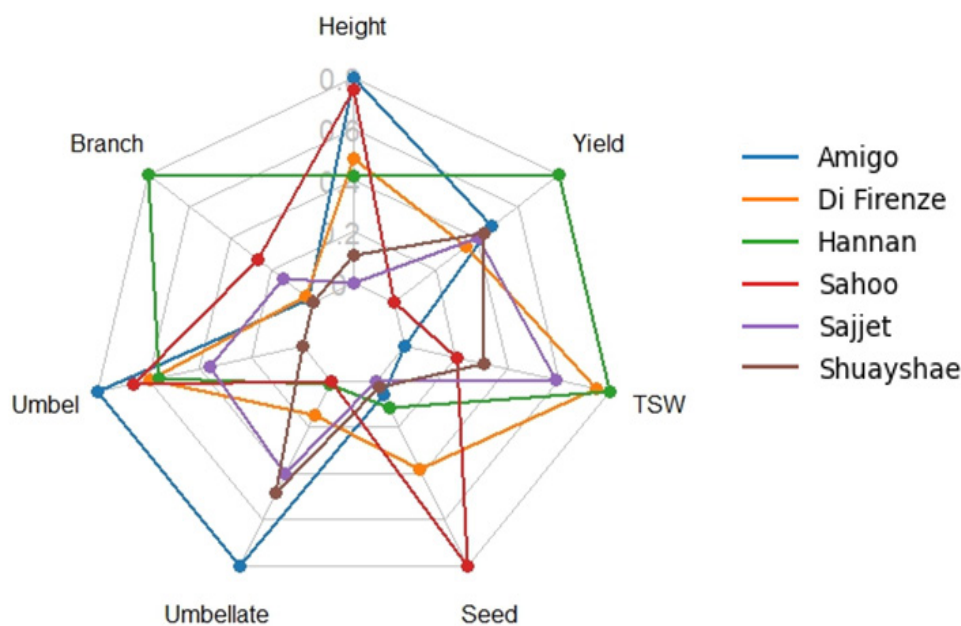


Figure 1. Radar plot of phenotypic traits of six fennel genotypes constructed by R packages. TSW – thousand seeds weight.

by revealing the lowest yield of 53.04 [g/m²]. Although the Amigo genotype has the most umbels per plant and most umbellate per umbel, its yield was lower due to fewer seeds per umbellate and lower thousand-seed weight. Sahoo genotype has the most seeds in umbellates, but its yield was the lowest, probably due to the lower number of umbellate per umbel and lower thousand-seed weight. Therefore, the Hannan genotype could be the best choice for seed production. The Amigo genotype can be used when the umbel and umbellate are the targets. Radar plot analysis (Figure 1) indicated that the outer genotypes at the edge are superior in the trait, while genotypes in the inner circles give less superiority. In this content, the Hannan genotype is located at the edge at the end of the thousand-seed weight and yield. This confirms the above-mentioned data, which showed that the Hannan genotype sacrifices phenotypic traits such as plant height and umbellate density for thousand-seed weight and total yield. It demonstrated that the Hannan genotype could be an ideotype for other fennel genotypes.

The hierarchical relationship among the six fennel genotypes included in this study was shown based on their yield (Figure 2). Based on the significant variation in the total yield, Figure 2 groups the genotypes into three clusters. The first cluster included Di Firenze, Amigo, Sajjet, and Shuayshae, which indicates that they have similar yields. This homogeneous group showed moderate yield performance.

The second cluster consisted of only the Hannan genotype, suggesting that this genotype has a distinct pattern of yield that is higher than that of genotypes in other groups. The last cluster consisted of one genotype, namely Sahoo, indicating that this genotype has quite different yield performance in comparison with other genotypes included in this study.

GC-MS Analysis of Fennel Seed Extracts

GC-MS analysis of the alcoholic and aqueous extracts showed different phytochemical groups. Mainly, the phytochemical groups were Alkaloids, Fatty acids, Esters, Terpenes, phenolic, Ketones, alcohols, Ethers, Nitro Compounds, Alkenes/Alkanes, Lactones, Aldehydes, Sulfur-containing Compounds and Phthalates (Figure 3).

The chemical profiles of the six fennel genotypes varied due to their genetic background and extraction methods. This variation influences the nutritional value and pharmaceutical usage of the fennel. Each fennel genotype possesses a unique chemical compound as a signature driven by the genetic variation in biosynthetic pathways. Di Firenze (Italian genotype) was at the top of the other studied genotypes by giving the largest number of phytoconstituents in both methanolic and aqueous extraction (32 and 22, respectively). In contrast, Sahoo (German genotype) produced the least number of phytochemicals for both alcoholic and aqueous extract (6 and 18, respectively). On the other hand, the local cultivated genotype, Shuayshae, gave 10 and 12

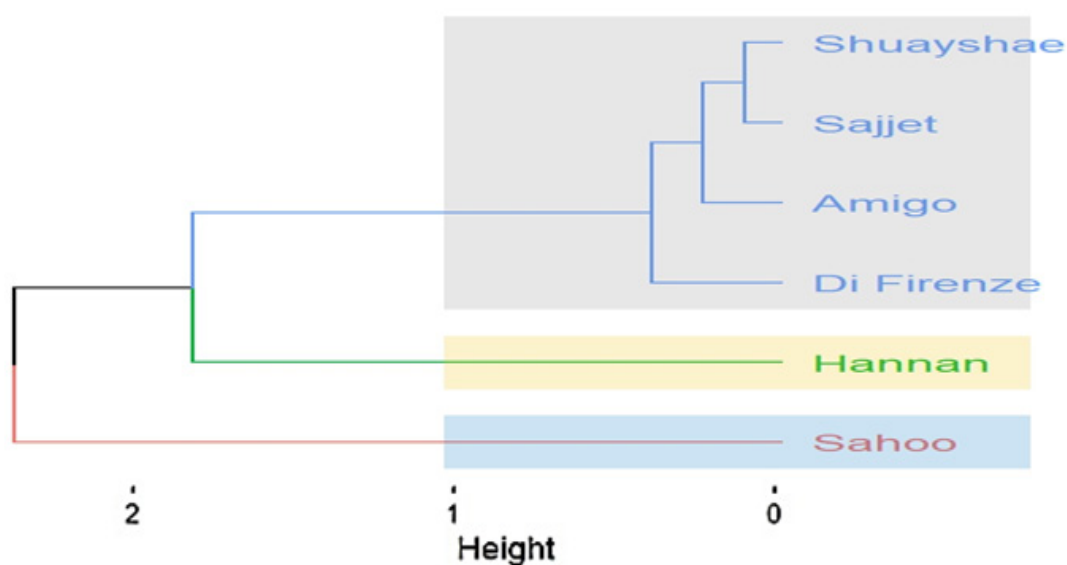


Figure 2. Yield-based dendrogram of six fennel genotypes using the R package.

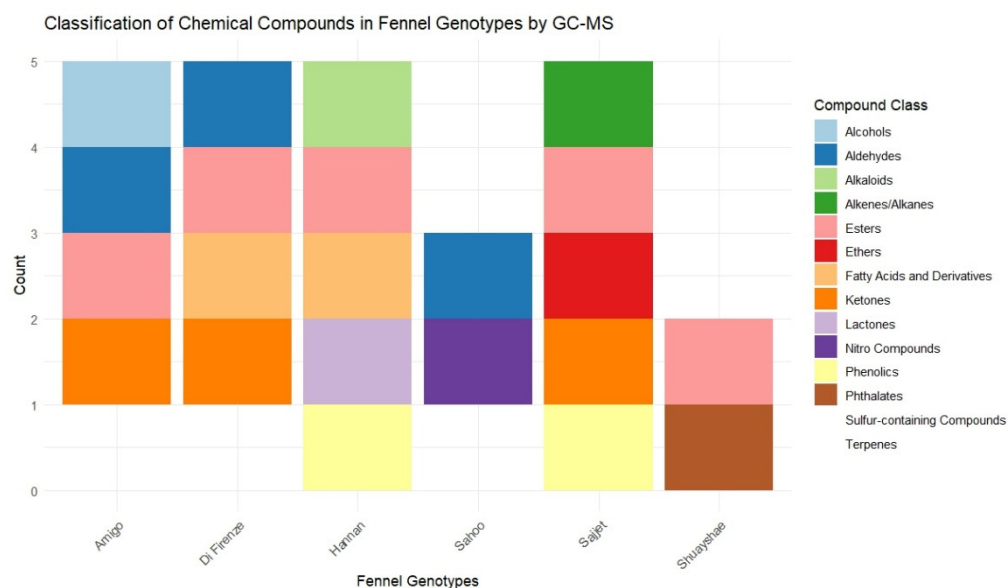


Figure 3. Classification of the chemical compounds in the six fennel genotypes using R package.

active compounds for the methanolic and aqueous extract, respectively.

The Aqueous extraction showed that the Amigo genotype contains methyl ester (hexadecanoic acid methyl ester) and bicyclic compounds (bicycle [3.3.1] nonane-2,7-dione). At the same time, Di Firenze contains terpenoids such as (3-chloro-phenyl) (furan-2-ylmeth) and aldehydes such as 2,4-decadienal. The Hannan genotype was dominated by the high seed-associated compounds, such as methyl tetradecanoate and fatty acids, such as methyl 14-methylhexadecanoate. Nitro compounds such as 1-nitro-2-acetamido-1,2-dideoxy-d-glucitol were found mainly in the Sahoo genotype but were deficient in esters. In comparison, the Sajjet genotype featured cyclic ethers such as 1,3-dioxolane,2-(6-octynyl)-and Furan derivatives such as 5-pentyl-2(5H)-furanone. Finally, Shuayshae contained alkenes such as Z-5-nonadecene and epoxy fatty acids such as trans-4,5-epoxy-(E)-2-decenal when the aqueous method was used in extraction (Supplementary Data).

When chemicals were extracted by the methanolic method, Amigo was rich in sesquiterpenes such as 2,6,10-dodecatrien-1-ol and aromatic alcohols such as phenyl ethyl alcohol. Di Firenze was rich in methyl esters of unsaturated fatty acids, such as cis-11-eicosenoic acid methyl ester and halogenated compounds. Hannan genotype was characterised

with aliphatic hydrocarbons such as heptadecane and lactones such as heptadecanolide, when the alcoholic extraction method was employed. Scarce sulphur-containing compounds (3-buten-1-ylethynylsulfide) and aldehydes were observed in Sahoo genotypes. Sajjet genotype was dominant in phenolic derivatives such as ethyl 3-hydroxybenzoate and cyclic ketones. Shuayshae also contained epoxy esters specifically methyl 9,10-epoxyoctadecanoate and phthalates (diethylphthalate) (Supplementary Data). The Hannan genotype's seed weight and high yield aligned with its content in lipid-soluble compounds such as fatty acid esters. Sajjet and Di Firenze genotypes produced various secondary metabolites, which probably can be advantageous in adapting to environmental stresses. Nitro compounds availability in the Sahoo genotype suggests a unique biosynthesis pathway that might relate to pest resistance. The availability of nitro compounds in Sahoo and phthalates in Shuayshae suggests that those genotypes might have specific defence mechanisms to adapt to the environment.

Using either alcoholic or aqueous methods in extraction significantly altered the chemical profile of fennel genotypes due to the variation in the polarity and solubility of water and alcohol. Due to the polarity of water, aqueous methods mainly target polar compounds such as glycosides, phenolic acids, and water-soluble terpenoids. On the other hand,

the alcoholic method mainly targeted non-polar to moderately polar compounds such as terpenoids, aromatic hydrocarbons, and esters. In conclusion, the diversity of chemical profiling among fennel genotypes was shaped by the genetic variation and the method used in extraction. Understanding those reasons would help target a suitable genotype or extraction method to isolate the required bio compounds and optimise their various applications (Supplementary Data).

Phylogeny Tree of Fennel Genotypes According to Chemical Profiling

Based on the common chemical compounds extracted from fennel genotypes, their similarity was

measured according to Jaccard's coefficient. When the alcoholic extract method was used, the similarity was low, which suggests more significant variability in methanol-soluble compounds. Figure 4 and Table 3 show the relationship between fennel genotypes. The genotypes indicated similarity near-zero or zero, explaining that there is no shared metabolite composition. Sajjet genotype was closer to Amigo and Di Firenze, as the similarity indices were 0.029 and 0.059, respectively. Meanwhile, the Shushyae, Sahoo, and Hannan genotypes met zero similarity, indicating that genetic backgrounds affected the chemical compositions when they were alcohol-extracted (Figure 4 and Table 3).

When the aqueous extract method was used, the

T a b l e 3

Jaccard similarity index of chemical profiling by methanolic extract for the six fennel genotypes

Genotypes	Amigo	Di Firenze	Hannan	Sahoo	Sajjet	Shuayshae
Amigo	1.000					
Di Firenze	0.000	1.000				
Hannan	0.000	0.000	1.000			
Sahoo	0.000	0.000	0.000	1.000		
Sajjet	0.029	0.059	0.000	0.000	1.000	
Shuayshae	0.000	0.000	0.000	0.000	0.000	1.000

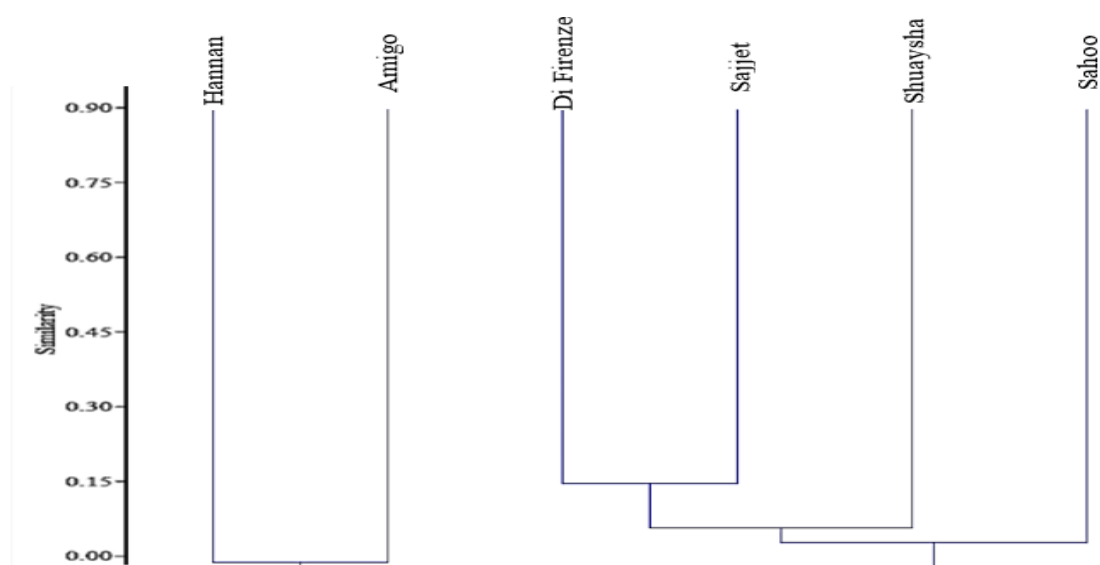


Figure 4. Phylogenetic tree of the six fennel genotypes generated by MVSP (v.3.22) based on Jaccard's Coefficient using the chemical profiling extracted by methanol.

T a b l e 4

Jaccard similarity index of chemical profiling by aqueous extract for the six fennel genotypes

Genotypes	Amigo	Di Firenze	Hannan	Sahoo	Sajjet	Shuayshae
Amigo	1.000					
Di Firenze	0.000	1.000				
Hannan	0.026	0.000	1.000			
Sahoo	0.000	0.065	0.028	1.000		
Sajjet	0.000	0.196	0.024	0.105	1.000	
Shuayshae	0.000	0.075	0.033	0.034	0.125	1.000

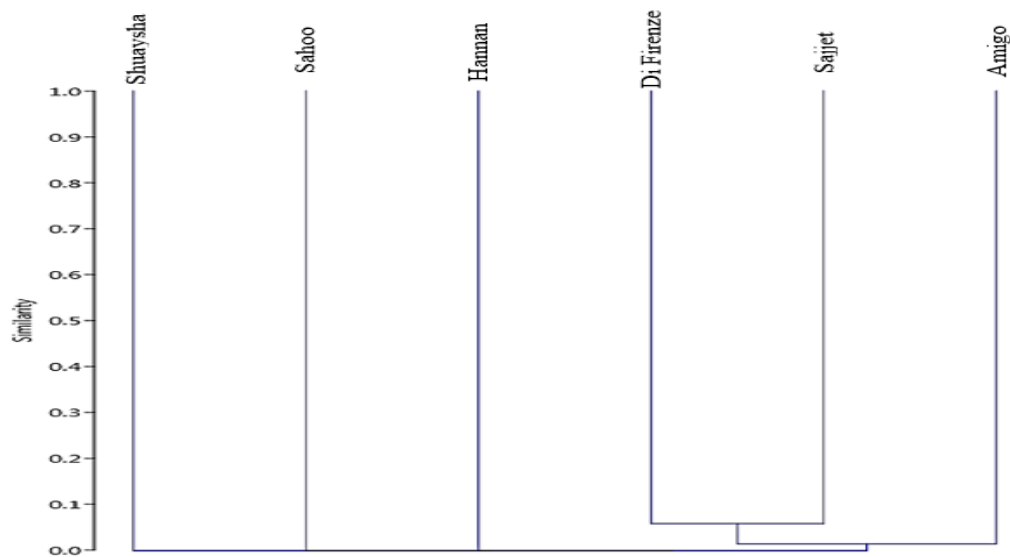


Figure 5. Phylogenetic tree of the six fennel genotypes generated by MVSP (v.3.22) based on Jaccard's Coefficient using the chemical profiling extracted by water.

chemical profile of the six fennel genotypes differed from their profiles when the alcoholic methods were used. The phylogenetic trees (Figures 4 and 5) confirmed this variability between the two methods. The phylogenetic tree in Figure 5 also showed two main clusters. The first cluster included Sahoo, Shuashyae, Sajjet and Di Firenze, while the second cluster included only Hannan and Amigo. The highest similarity index was recorded between Di Firenze and Sajjet genotypes (0.195) (Table 4), indicating that alcoholic methods effectively extracted almost the same chemical compounds. Amigo and Hannan genotypes showed very low similarity with the remaining fennel genotypes under the current investigation, indicating the unique chemical compounds soluble in water.

DISCUSSION

Current investigation findings highlighted the apparent effect of genetic background on the fennel plant morphology and chemical composition. The observed variation in yield, seed weight, and chemical composition between genotypes under study was consistent with previous studies that focused on the role of genetic variation in the plant performance and secondary metabolites production (Singh *et al.* 2014; Al-Mehemdi *et al.* 2020). Therefore, the Hannan genotype emerged as the best candidate for yield as it produced the highest seed weight [10.90 g] and total yield per unit [297.20 g/m²]. This dominance can be attributed to the plant vigour, branching architecture, and effective resource use, which led to

seed development or umbel density observed in the Amigo genotype. A robust growth with greater photosynthesis capacity enables more assimilates to be forwarded to the reproductive stage. That was clear in the Hannan genotype, which showed a higher number of branches per plant, and this likely provided a greater number to the reproductive sites, eventually improving seed setting. On the other hand, the Amigo genotype showed greater umbel density and greater umbellate number per umbel; however, those traits are often related to greater flower production, but this did not help in the highest yield, as the increased number of florets was accompanied by smaller seeds. Hannan and Amigo genotypes followed different strategies in yielding. The seed size and resource concentration illustrate the yield in the Hannan genotype, while the number of umbellate in Amigo explains its yield. Those phenotypic variations are important in outlining the objectives of breeding programs, whether cultivating them for high yield or phytochemical diversity.

Morphological and phytochemical variations among the six fennel genotypes play a vital role in defining the breeding programs' aims, determining whether they will be used for agronomic or pharmacological purposes (Bilia *et al.* 2000). Furthermore, the noted chemical diversity among these genotypes is a key factor in defining each genotype's potential bioactivity. Current investigations revealed many compounds that are already known to have antimicrobial and antioxidant properties (Rather *et al.* 2016). Specifically, terpenoids and aromatic alcohols, which were predominant in methanolic extract in genotypes such as Amigo and Di Firenze, were reported to have strong antimicrobial activity and may limit pathogen proliferation (Diao *et al.* 2013). The Amigo genotype was marked with a high content of phenyl ethyl alcohol, a compound widely recognised for its antibacterial efficacy (Kalemba & Kunicka 2003). Furthermore, compounds such as methyl tetradecanoate and cis-11-eicosenoic acid methyl ester, which were obtained from Hannan and Di Firenze genotypes, represent promising candidates for antioxidant biological activities. Such compounds offer potent, natural alternatives for good preservation due to their capacity to hinder oxidation, while also enhancing the shelf life and stability of pharmaceutical formulations (Tiwari *et al.* 2011;

Saxena *et al.* 2013). In general, extraction methods have a profound impact on the range of recovered chemicals, hence their biological activity. Polar bioactive compounds, such as glycosides and phenolic acids, were dominant in the aqueous extracts across all genotypes and were probably responsible for the observed anti-inflammatory and antioxidant effects. Conversely, non-polar compounds such as esters, terpenes, and aromatic alcohols were prevalent in the methanolic extract, which may have antifungal and antibacterial effects. Collectively, these findings of the current study highlight the potential of improving fennel genotypes with certain phytochemicals. Raal *et al.* (2012) stated significant differences in trans-anethole and fenchone content across fennel samples from Estonia, Norway and Austria, reflecting geographic influences on chemical composition. European Spanish genotypes also showed distinct essential oil chemotypes depending on location. Likewise, investigations of Italian wild fennel demonstrated morphological and chemical diversity linked to genotype and environment. These comparisons support the view that genetic background and environment cooperatively shape the biochemical profiles of fennel across diverse regions.

Significant differences were observed between the two extraction methods. The used solvents targeted distinct chemical classes. The aqueous method revealed polar compounds such as glycosides and phenolic acids, whereas the methanolic method produced non-polar metabolites such as terpenoids and esters. However, lipid-soluble compounds, e.g., docosanoic acid methyl ester (58.48%), were prominent in the aqueous extracts, while sesquiterpenes and aromatic alcohols were dominant in the methanolic extract. Based on this variability, selecting suitable extraction methods is especially important when a desired bioactive compound is the target. The richness of Hannan genotype seed with fatty acid esters in alcoholic extract relates to the high seed weight, indicating that lipid-rich seed might enhance its production (Pank *et al.* 2011), who correlate fatty acid content with seed size.

Phylogeny analysis exposed that genetic divergence shaped the genotype-specific chemical signature. The very low Jaccard similarity between the genotype Sajjet and the two genotypes Amigo and Di Firenze (0.029 and 0.059, respectively) in-

icates that the Sajjet genotype shares almost no non-polar metabolites in its methanolic extract with Amigo and Di Firenze, respectively. This level of similarity values reflects a high degree of chemical divergence, signifying that each genotype has a largely diverse profile of non-polar compounds, e.g. esters, terpenoids, and aromatic alcohols. In contrast, an aqueous extract revealed a moderate similarity of 0.195 between Sajjet and Di Firenze, suggesting that polar compounds might be related to environmental adaptation or potentially tied to conserved biosynthetic pathways. The secondary metabolites might influence the agronomic traits. Therefore, agronomically, fennel genotypes performed differently, and their chemical profiles varied (Toxopeus & Lubberts 1994; Elsahookie 2007). Based on their genetic background, genotypes may allocate resources differently, some favouring yield, while others favouring vegetative growth or stress resistance (Pank *et al.* 2011). However, the plant's chemical composition significantly affects the field performance; therefore, the highly yielding Hannan genotype contains more methylated compounds and alkaloids. Meanwhile, the moderate-yielding genotypes have balanced levels of secondary metabolites (e.g., Di Firenze and Sajjet). The clustering pattern based on yield (Figure 2) aligned partially with the phytochemical diversity shown in Figures 4 and 5, respectively. Hence, the Hannan genotype is characterised by high yield and the Sahoo genotype is characterised by relatively low yield (Table 2) and also has relatively varied phytochemical markers. Meanwhile, genotypes appeared in one cluster (Figure 2) (Di Firenze, Amigo and Sajjet) and showed relatively similar phytochemical compounds, mainly when the aqueous extraction method was used.

At last, it is worth mentioning that the current investigations' findings were specific to environmental conditions, seasons and a limited number of genetic materials. The observation on the fennel genotypes' performance, chemical content diversity, and possible application in breeding programs cannot be generalised unless further trials are conducted in a wide range of environments. Thus, further investigations, including a large number of genetic materials and different geographic locations are important to ensure the stability of introduced fennel genotypes.

CONCLUSIONS

The current investigation demonstrated that fennel genotypes showed diversity in morphology and phytochemical diversity, which was driven mainly by genetic variation and extraction methods. The Hannan genotype is the best choice for commercial use due to its superiority in seed weight and total yield per unit making it the most important candidate for seed production. Meanwhile, Amigo and Di Firenze genotypes exhibited a distinct chemical composition, especially with valuable metabolites, highlighting their capability for ornamental umbel production and specific metabolic extraction, respectively. Additionally, the low genetic similarity of metabolic extract between certain genotypes explains the differences in non-polar metabolites. The diversity in phytochemicals is beneficial for breeding programs. Further investigations are required to uncover the stability of those genotypes across various environments. Also, molecular markers could be used to explore the genetic mechanisms underlying metabolite biosynthesis.

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