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ORIGINAL PAPER

Phenolic and nutrient profiling of pearl millet seeds from Southern Tunisia: insights into a nutritious staple crop

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Abstract

Pearl millet is a climate-resilient crop suitable for arid regions due to its ability to tolerate drought and severe climatic conditions. It is a nutritious grain with potential health-promoting properties that can contribute to the world food security and livelihoods for small-scale farmers. In Tunisia, this crop has historically possessed a diverse and rich germplasm, but it now faces the threat of genetic erosion. The preservation programs for these species have been infrequent and have not attributed the plant its due value, resulting in a period of neglect and lack of proper monitoring. To rejuvenate this field, efforts are underway to revitalize research and development initiatives, as part of these efforts, seeds collected from the southern Tunisian regions of Medenine and Djerba were sown in the experimental plot of the Arid Zones Institute for 2 years. Subsequently, the harvested grains were processed and subjected to biochemical analyses. The aim of this study was to assess the profiles of phenolic compounds, sugar content, fatty acids, and mineral composition in ten pearl millet genotypes. An LC–MS analysis allowed for the identification of various phenolic acids in millet grains, with quinic acid, p-coumaric acid, and caffeic acid emerging as the predominant phenolic acids. Furthermore, two flavonoid compounds were identified, quercetin and apigenin. The analysis of sugar contents by LC–MS showed an abundance of glucose and fructose, followed by maltose and sucrose. Mean values ranged between 323.98 mg/kg for G4 and 2163.86 mg/kg for G8. The minerals K, Mg, Na, and Ca were detected in interestedly high quantities. GC–MS analysis showed the detection of seven fatty acids. Arachidic acid (0.53–2.23%) and linolenic acid (1.01–2.23%) were present in relatively abundant amounts. Linoleic acid and linoleic acid were the most frequently occurring fatty acids. When examining the variability among the assessed attributes, heatmap analysis revealed correlations between each trait and the clustered genotypes. Our findings indicate that pearl millet serves as a rich source of diverse bioactive molecules, making it an interesting material for potential industrial applications.

Keywords *Pennisetum glaucum* L. · Phenolic compounds · Minerals · Fatty acids · Sugar content

Introduction

The world faces the challenge of increasing agricultural output to meet the nutritional needs of an expected global population of 9 billion, with a particular emphasis on Africa, which is projected to have 2.5 billion people (d'Amour et al. 2016; Röös et al. 2017). To achieve sustainable food and nutrition security for the growing population, a 60–110% increase in global agricultural production is essential within the same timeframe. Rice, wheat, and maize are the principal crops that sustain the world, jointly accounting for 60 percent of the overall energy consumption of the global population. Pearl millet is ranked as the sixth-largest cereal globally, contributing to 1.3% of total cereal production (de Assis et al. 2018; Satyavathi et al. 2021).

Sharma et al. (2021) reported that pearl millet is originated and domesticated in the wilds of West Africa over 4000 years ago, spreading later worldwide with over 30 million ha, the majority of the pearl millet cultivation is being done in Africa (> 18 million ha) and Asia (> 10 million

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ha) (Raheem et al. 2021). Millet is primarily cultivated in arid and semi-arid regions of Africa and India for human consumption and, to a lesser extent, for fodder and building materials (Anitha et al. 2021; Hassan et al. 2021). The demand for pearl millet is growing among health-conscious consumers. Its gluten-free nature makes it ideal for individuals with gluten intolerance, enhancing its potential in the health food industry (Gupta et al. 2022). Pearl millet is resilient to high temperatures and drought conditions. Its deep root system improves soil structure, reduces erosion, and adds organic matter, contributing to soil fertility. In addition, it supports biodiversity and reduces pest and disease incidence in intercropping systems (Akrimi and Hajlaoui 2021; Nelson et al. 2022). In addition, pearl millet requires fewer inputs like fertilizers and pesticides compared to other cereals (Nelson et al. 2022). The increasing demand of millet production opens opportunities for developing value-added products such as millet flour, snacks, and beverages, enhancing profitability for producers and processors (Kumari et al. 2019; Mirzababaee et al. 2022). For smallholder farmers, cultivating pearl millet provides an additional income stream, reduces economic risk, and improves livelihood resilience (Rouamba et al. 2021; Kargwal, Yadvika et al. 2023). Besides, the use of pearl millet as a feedstock for ethanol production has been investigated, highlighting its potential as a bioenergy source (Kargwal, Yadvika et al. 2023). This field of research has been very trendy lately. Researchers have been exploring the energy potential of sugarcane using advanced techniques, artificial intelligence is also being exploited in the bioindustry (Saha et al. 2018; Gaudio et al. 2021). In Tunisia, the dairy sector plays a strategic role in the country's agriculture, emphasizing the importance of sustainable agricultural practices; actually increasing pearl millet production can have positive environmental effects due to its low water and input requirements (Houissa et al. 2019; Triki et al. 2023). The most commonly cultivated millet variety in Tunisia is pearl millet (*Pennisetum glaucum*), and it holds considerable potential as an alternative to sorghum. This annual cereal is grown in the southern part of the country as a summer crop covering approximately 3000 hectares (Radhouane 2008; Triki et al. 2023). Pearl millet is cultivated in southern part of Tunisia due to its resilience to high temperatures and drought conditions, and unlike wheat and rice, it require moderate climates and are less tolerant to extreme drought and heat (Radhouane 2007, 2008). In regions with limited water resources and where irrigation is necessary like southern Tunisia, large-scale millet farming can exacerbate water stress, particularly if not managed sustainably. Second, while millet can contribute positively to soil health due to its deep root system, improper farming practices such as excessive tillage or inadequate crop rotation can lead to soil degradation, erosion, and fertility loss (Chary et al. 2020; Kane-Potaka et al. 2021). Furthermore, monoculture farming practices, common in large-scale millet cultivation, pose threats to biodiversity by reducing natural habitats, diminishing species diversity, and increasing susceptibility to pest and disease outbreaks. Sustainable agricultural approaches can be implemented. These may include crop rotation: alternating millet cultivation with other crops in a rotation system can help improve soil health, reduce pest and disease pressure, and enhance biodiversity (Lauriault et al. 2023; Victor et al. 2023).

Pearl millet grains possess very high nutritional values, carbohydrates, and a higher fat content (soluble and insoluble), resulting in greater energy, fibers, increased protein content, antioxidants and iron, and zinc in comparison with other major cereals (Gupta et al. 2022; Samtiya et al. 2023); in fact, wheat and rice often contain lower levels of fiber and certain micronutrients compared to pearl millet, and corn, although high in energy content, lacks the protein quality and micronutrient density found in pearl millet. Hence, by any nutritional parameter, millets are miles ahead of rice and wheat (Uppal et al. 2015; Slama et al. 2020; Gupta et al. 2022). The polyphenol content of millet is associated with several health benefits (Jideani et al. 2014; Olatoye et al. 2023; Triki et al. 2023). Phenolic acids act as antioxidants by donating hydrogen or electrons, while carotenoids function as antioxidants by reducing singlet oxygen and free radicals (Zhang and Liu 2015). Numerous studies have highlighted millet as an economical source of protein and energy. Millet is also rich in vitamins B and A, calcium, iron, and zinc. It contains essential minerals such as potassium, phosphorus, magnesium, zinc, copper, and manganese (Gupta et al. 2022; Pei et al. 2022). Millets, being rich in nutrients and devoid of gluten compared to other cereals, are an apt selection for manufacturing gluten-free food and beverages. These encompass a range of products such as couscous, flatbreads, doughs, porridges, gruels, non-alcoholic beverages, and even beers, primarily targeted at individuals with gluten sensitivity. (Amadou et al. 2011; Gupta et al. 2022; Samtiya et al. 2023).

Efforts have been made to develop iron- and zinc-biofortified pearl millet to enhance its nutritional value (Wrigley et al. 2004; Samtiya et al. 2023). Furthermore, it is recognized as a valuable source of phenolic acids with potent antioxidant and anti-proliferative properties. Millet phenolics are present in either free or conjugated forms and can be classified into two categories: hydroxycinnamic and hydroxybenzoic acids. Hydroxybenzoic acids, derivatives of benzoic acid, include vanillic, p-hydroxybenzoic, gallic, and syringic acid. On the other hand, hydroxycinnamic acids consist of caffeic, coumaric, and ferulic acid, featuring a C6-C3 structure (Manach et al. 2004; Triki et al. 2023). Today, there is a growing interest in these compounds due to their potential health benefits (Falcinelli et al. 2018; Slama

et al. 2020). Since synthetic antioxidants might reveal toxic side effects, are costly to produce and less effective than plant natural antioxidants, identifying more natural antioxidant sources constitutes a real interest for researchers (Houissa et al. 2019; Samtiya et al. 2023).

Research on pearl millet in southern Tunisia has been halted for about 20 years (Triki et al. 2023). Since then, this genetic heritage has been at risk of genetic erosion. In addition, there are virtually no varieties adapted to the conditions of southern Tunisia listed in the national catalog of cereals of Tunisia. It is within this context that the present research work is integrated, aiming to valorize pearl millet as a neglected genetic resource. Besides, to the best of our knowledge, there is little research on the assessment of the phytochemical profile of seeds. In this study, phytochemical investigation on the seeds of *Pennisetum glaucum* was carried out to assess the fatty acids and phenolic profiles, mineral composition, and nutritional potential of pearl millet cultivars collected from arid land farmers.

Table 1 Genotypes and origin distribution of *Pennisetum glaucum*

Code	Origin	Panicle form
G1	Djerba	Fusiform
G2	Djerba	Fusiform
G3	Djerba	Candle
G4	Djerba	Cylindrical
G5	Djerba	Fusiform
G6	Medenine	Cylindrical
G7	Medenine	Oblong
G8	Medenine	Conical
G9	Medenine	Cylindrical
G10	Medenine	Fusiform

Materials and methods

Plant material

Ten genotypes of *Pennisetum glaucum* L. cultivated in the field of the Arid Regions Institute of Medenine are selected to assess the present study (Table 1), five of them were collected from a littoral zone, Djerba and five from a continental zone, Medenine. The experimental station is situated in the southeastern region of Tunisia, experiencing an arid climate characterized by cold winters and hot summers (Fig. 1), with an annual rainfall not surpassing 150 mm, the temperature fluctuates significantly, exceeding 30 °C. Seeds were planted for 2 successive years in May 2019 and May 2020.

The trial was conducted in a randomized complete block design (RcBD) with three replications, the plots were immediately irrigated after sowing and then subsequently irrigated twice weekly with equal quantities during the trial period. Harvesting dates were determined based on the color change indicative of maturity, leading to the collection of seeds in August 2019 and August 2020. Following the fruit drying process, the seeds were manually extracted, ground into a fine powder, and subsequently stored at 4 °C for analysis.

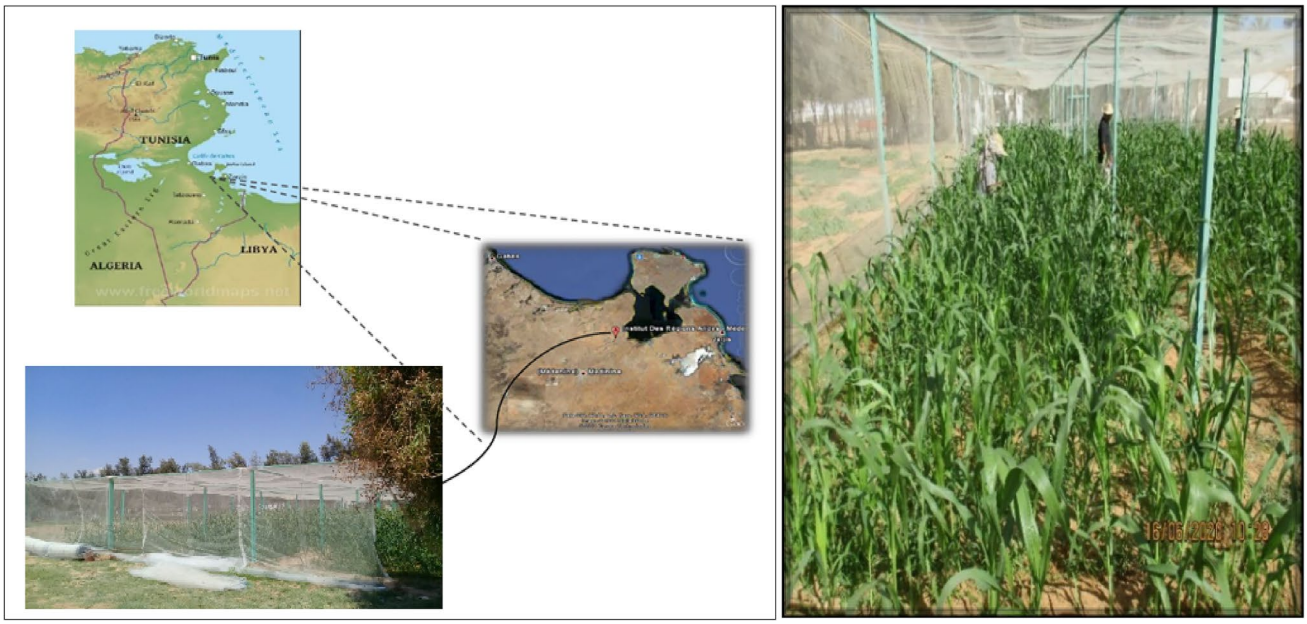

Fig. 1 Collection zone and experimental plot of studied *Pennisetum glaucum* genotypes

Mineral contents

Mineral analysis was performed according to the method described by Al-Showiman (1990). 1 g of pearl millet seed plant material was calcined in a muffle furnace at 550 °C for 5 h. The resulting ash was then dissolved in 5 mL of 20% hydrochloric acid and the final volume of the solution was adjusted to 50 mL with distilled water in a volumetric flask. Finally, separate analyses were performed for each mineral element using an atomic absorption photometer (Shimadzu A 6800, Kyoto, Japan).

Sugar contents

A 10 g seed sample was refluxed with 100 ml of high purity water–ethanol (80/20, v/v) for 3 h. The remaining filtrate was evaporated to dryness under reduced pressure. Each sample was then diluted to 50 ml with ultrapure water. The obtained solution was centrifuged at 6000 rpm for 20 min and filtered through a 0.45 µm membrane. The sugar composition was studied using HPLC at room temperature. A Eurospher NH2 column was used (pore size:100 Å, particle size: 7 mm, inside diameter: 250 mm 4.6 mm) (for those with knowledge, Germany). Prior to use, solvents were filtered through a 0.45 µm membrane filter and degassed for 15 min with a model SM 25E-MT ultrasonic bath cleaner (Branson Ultrasonics Corporation, Danbury, CT). The mobile phase used was acetonitrile and ultrapure water (80/20, v/v). The liquid chromatography was coupled to a refractive index detector K-2301 from Knauer (Germany). The flow rate and injection volume during the experiment were 1 ml/min and 2 ml/min, respectively (Booi 1992).

Fatty acids content

The fatty acid composition of *Pennisetum glaucum* seeds was determined as methyl esters by gas chromatography (GC–MS: QP2010Ultra/SHIMADZU/SUPEL COWAXTM10/FUSED SILICA capillary column 30mx 0.25 mm film thickness). After saponification, a solution of 0.1 g of oil and 2 ml of hexane is mixed with 0.2 ml of 2N methanolic potassium hydroxide solution. Peak fatty acid levels were identified according to the analytical methods described in the European Economic Community Regulations (EEC, 1991). Many fatty acids, including C14:0 myristic acid; C16:0 palmitic acid; C16:1 palmitoleic acid; C17:0 heptadecanoic acid; C17:1 heptadecenoic acid; C18:0 stearic acid; C18:1 oleic acid; C18:2 linoleic acid; C18:3 linolenic acid and C20:0 arachidic acid. Each fatty acid was expressed as a percentage of total peak area relative to standard compounds.

Preparations of extracts from seed parts

The extraction of the free phenolic compounds was performed by maceration at room temperature as reported by Xiang et al. (2019a, b) with minor modifications. 1 g of each ecotype granule was suspended in 10 ml of each sample. The mixture was homogenized with agitation in an ULTRA-TURRAX® (IKA T 25 digital ULTRA-TURRAX®) for 5 min and 24 h, followed by centrifugation at 3000 rpm for 30 min (Gyrozen, Korea), and the resulting supernatant was centrifuged on a rotary device and evaporated in a vacuum evaporator (Cole-Parmer Rotary Evaporator System, USA). The methanolic extracts were lyophilized using a lyophilizer (Bioblock Scientific Christ ALPHA 1–2, Illkirch Cedex, France) and stored at 4 °C in sealed dark vials until analysis.

HPLC analysis of phenolic compounds

20 mg of each plant extract was dissolved in 1 mL of ethanol, and the mixture was then filtered through a 0.45 µm membrane filter and injected into an HPLC column. LC–ESI–MS analysis was performed using an LC–MS 2020 quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an electrospray ionization (ESI) source operating in negative ionization mode. The mass spectrometer was coupled online with an ultrafast liquid chromatography system consisting of an LC-20CE XR binary pump system, an SIL-20AC XR autosampler, a CTO-20AC column oven, and a DGU-20A 3 R degasser (Shimadzu, Kyoto, Japan). Analyses were performed using an Aquasil C18 guard column (10 mm × 3 mm, 3 µm, thermo Electron) and an Aquasil C18 column thermo Electron, Dreieich, Germany (150 mm × 3 mm, 3 µm). Mobile phase consisted of A (0.1% formic acid in water, v/v) and B (0.1% formic acid in methanol, v/v), linear gradient solution: 0–45 min, 10–100% B; 45–55 min, 100% B. Equilibration time is controlled at 5 min between each run. The injection volume was 5 µl, the mobile phase flow rate was 0.4 ml/min, and the column temperature was fixed at 40 °C. Spectra were monitored in SIM (selected ion monitoring) mode and processed with Shimadzu LabSolutions LC–MS software. The mass spectrometer was run in negative ion mode, capillary voltage was−3.5 V, nebulizer gas flow was 1.5 L/min, dry gas flow was 12 L/min, DL temperature (dissolution line) was 250 °C, the block source temperature was 400 °C, the voltage detector was of 1.2 V, and the full-scan spectra was in the range 50–2000 m/z (Bedford, MA, USA) Phenolic compounds present in different samples were identified by comparing retention times and spectra those of standard compounds. Chemical standards of the highest purity (≥ 99.0%) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Results are expressed in mg/100 g plant material.

Statistical analyses

The seed samples of the studied plant were analyzed in triplicate. The data obtained were then processed using SPSS software (version 20.0) to perform statistical analyses. Comparison tests were used to assess significant differences between genotype populations, with a significance level set at $p < 0.05$. Results were presented as mean- ± standard deviation (SD). Multivariate analyses (Heatmap and PCA) were assessed using XLSTAT 2019 based on Pearson correlations to identify the sources of the major part of the variability and to classify the assessed genotypes regarding their attributes.

Results and discussion

Mineral composition

The minerals identified in millet genotypes were potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), copper (Cu), iron (Fe), zinc (Zn), and manganese (Mn) (Table 2). The mineral analysis revealed that millet grains provide an excellent source of these essential elements. Significant variations ($p < 0.05$) were clearly observed among studied populations (Table 2). The diversity in mineral content observed among local millet populations underscores their ability to adapt to specific climatic conditions, highlighting the importance of considering these environmental factors in the selection and management of plant varieties. Genotype G6, originating from Medenine, exhibited the highest values for the three mineral compositions (K, Mg, Cu in mg/100 g). In addition, genotypes from Medenine (continental origin) showed relatively higher iron contents (Fe) compared to genotypes collected from Djerba. Our results corroborate several other studies that have highlighted the nutritional profile of millets (Table 2). Our results demonstrate that millet contains at least two to three times more calcium than rice, aligning with Malik (2015) findings. Recent researches recommend the consumption of millet due to its health benefits, particularly its mineral richness; indeed, the nutrient content of millets is comparable to, and sometimes it surpasses, that of traditional cereals. Their rich concentrations of calcium, iron, and zinc make them valuable additions to both human and animal dietary regimes (Bashir et al. 2014; Hassan et al. 2021; Satyavathi et al. 2021; Triki et al. 2022a, b). Elevated magnesium content in pearl millet grains has been associated with a reduction in migraine attacks and relief from severe breathing problems in individuals with asthma. Furthermore, it helps the cardiovascular system by lowering blood pressure, which in turn helps in reducing the chances of heart attack or stroke (Ratnavathi and Tonapi 2022). Furthermore, its iron bioavailability contributes to increasing hemoglobin levels and preventing anemia (Malik 2015; Kulkarni et al. 2021). Regarding iron content, pearl millet and little millet are so rich that rice is not even in the competition. Recent studies demonstrated that pearl millet has a large amount of phosphorus which is very essential for bone growth and development as well as for development of ATP which is the energy currency of our body. Also, many researches showed that pearl millet contains high amount of calcium and has at least twice the amount of this nutriment compared to rice (Kulkarni et al. 2021; Kumar et al. 2022; Satyavathi et al. 2022).

While increasing the mineral content of pearl millet can enhance its nutritional value and address micronutrient deficiencies, it is essential to manage these changes carefully to avoid potential health risks, mainly mineral imbalance and digestive issues (Malik 2015; Nandini et al. 2021). In fact, high levels of certain minerals, such as iron or calcium, can inhibit the absorption of other essential nutrients like zinc, magnesium, and copper, potentially leading to deficiencies. Excessive intake of specific minerals can cause toxicity. For instance, too much iron can result in conditions like hemochromatosis, while excessive calcium can lead to

Table 2 Mineral composition of different *Pennisetum glaucum* L. grains (mg/100 g of dry weight)

Populations	Na (mg/100 g)	K (mg/100 g)	Ca (mg/100 g)	Mg (mg/100 g)	Cu (mg/100 g)	Zn ((mg/100 g)	Fe (mg/100 g)	Mn (mg/100 g)
G1	$129{,}08^{a} \pm 26{,}97$	$1752^{ab} \pm 494$	$411{,}79^{ab} \pm 122{,}89$	$602{,}66^{ab} \pm 171{,}30$	$30{,}18^{a} \pm 6{,}8$	$13{,}26^{c} \pm 0{,}8$	$10{,}11^{de} \pm 1{,}06$	$1{,}30^{ab} \pm 0{,}36$
G2	$48{,}13^{cd} \pm 8{,}9$	$1513{,}16^{ab} \pm 192{,}54$	$190{,}24^{bc} \pm 24{,}17$	$465{,}00^{abc} \pm 30{,}05$	$19{,}88^{b} \pm 2{,}16$	$13{,}26^{c} \pm 0{,}54$	$17{,}22^{cd} \pm 2{,}29$	$0{,}61^{d} \pm 0{,}22$
G3	$45{,}21^{cd} \pm 3{,}47$	$1239{,}16^{b} \pm 228{,}07$	$179{,}05^{bc} \pm 52{,}2$	$415{,}50^{bc} \pm 88{,}79$	$20{,}74^{b} \pm 3{,}77$	$7{,}74^{d} \pm 0{,}51$	$8{,}005^{e} \pm 4{,}19$	$0{,}54^{de} \pm 0{,}41$
G4	$88{,}82^{abc} \pm 15{,}31$	$1298{,}83^{b} \pm 687{,}16$	$340{,}05^{abc} \pm 250{,}86$	$494{,}50^{abc} \pm 248{,}54$	$17{,}75^{b} \pm 0{,}6$	$7{,}74^{d} \pm 0{,}69$	$4{,}56^{e} \pm 3{,}01$	$1{,}07^{b} \pm 0{,}19$
G5	$97{,}37^{ab} \pm 28{,}01$	$1567{,}83^{ab} \pm 40{,}12$	$392{,}95^{ab} \pm 70{,}68$	$635{,}66^{ab} \pm 20{,}49$	$16{,}96^{b} \pm 0{,}11$	$7{,}74^{d} \pm 0{,}11$	$5{,}50^{e} \pm 0{,}45$	$0{,}99^{bc} \pm 0{,}075$
G6	$95{,}62^{ab} \pm 18{,}67$	$2082{,}50^{a} \pm 194{,}11$	$364{,}09^{abc} \pm 37{,}22$	$696{,}50^{a} \pm 110{,}57$	$32{,}76^{a} \pm 2{,}55$	$34{,}07^{b} \pm 2{,}28$	$51{,}46^{a} \pm 5$	$1{,}55^{a} \pm 0{,}05$
G7	$35{,}26^{d} \pm 24{,}28$	$1188{,}50^{b} \pm 285{,}81$	$152{,}23^{c} \pm 42{,}46$	$304{,}50^{c} \pm 36{,}75$	$19{,}83^{b} \pm 1{,}84$	$40{,}97^{a} \pm 0{,}063$	$48{,}02^{a} \pm 0{,}84$	$1{,}08^{b} \pm 0{,}04$
G8	$53{,}51^{bcd} \pm 38{,}26$	$1626{,}83^{ab} \pm 302{,}83$	$294{,}21^{abc} \pm 112{,}89$	$629{,}50^{ab} \pm 112{,}42$	$19{,}80^{b} \pm 1{,}73$	$3{,}84^{e} \pm 0{,}5$	$29{,}83^{b} \pm 9{,}77$	$0{,}21^{e} \pm 0{,}1$
G9	$77{,}99^{bcd} \pm 20{,}53$	$2021{,}00^{a} \pm 467{,}24$	$518{,}18^{a} \pm 184{,}77$	$680^{a} \pm 134{,}68$	$21{,}45^{b} \pm 1{,}49$	$12{,}51^{c} \pm 3{,}04$	$21{,}99^{c} \pm 6{,}02$	$1{,}35^{ab} \pm 0{,}1$
G10	$70{,}79^{bcd} \pm 32{,}25$	$1403{,}50^{ab} \pm 214{,}47$	$197{,}88^{bc} \pm 100{,}86$	$465{,}50^{abc} \pm 121{,}46$	$20{,}50^{b} \pm 0{,}71$	$1{,}92^{e} \pm 0{,}12$	$7{,}85^{e} \pm 0{,}27$	$0{,}63^{cd} \pm 0{,}16$

Values are averages ± SD ($n = 3$). Letters (a–e) denote statistical differences between assessed genotypes using Duncan's multirange mean comparison test ($\alpha = 0.05$). One-way ANOVA results revealing significant differences between genotypes are presented according to p ($p < 0.05$)

kidney stones or cardiovascular issues. It is showed that high mineral content, particularly iron and magnesium, can cause digestive problems such as constipation, diarrhea, or nausea. This can affect the overall acceptability of pearl millet as a staple food (Malik 2015; Chicaiza 2021).

Soluble sugar compositions

Soluble sugars' analysis of various *Pennisetum glaucum* grains indicated the presence of maltose, fructose, glucose, and sucrose (Table 3). A significant difference ($p < 0.05$) in sugar concentrations (fructose, glucose, sucrose, maltose) was observed between grains from the two different origins. G8, G9, and G7 exhibited the highest concentrations of detected sugars, with total amounts of 2163.86 mg/kg, 2064.9 mg/kg, and 2052.29 mg/kg of dry weight, respectively. Although the overall highest total amount was recorded in genotypes collected from Medenine region, the influence of genetic factors and geographical location is evident. Among the soluble sugars, glucose showed the highest concentration, followed by fructose and sucrose, while maltose showed the lowest values. Genotype G9 from Medenine exhibited the highest glucose content with an average of 1477.38 mg/kg of dry matter, whereas Genotype G5 displayed the lowest value at an average of 171.5 mg/kg of dry matter. Genotype G8 expressed a fructose content of 480.55 mg/kg dry matter, while Genotype G4 showed a lower concentration of 74.01 mg/kg dry matter. Sucrose values varied between a minimum of 62.74 mg/kg dry matter in Genotype G3 and a maximum of 212.8 mg/kg dry matter in Genotype G7. Maltose was only detected in populations derived from Medenine.

The differences in glucose, fructose, sucrose, and maltose contents among the different millet populations may be attributed to genetic and environmental factors. These results align with those reported by other researches (Badau et al. 2005; Shankaramurthy and Somannavar 2019), who also identified the presence of glucose, fructose, sucrose, and maltose in the studied millet grains. These concentrations exceeded those found in many other plants. Haila et al. (1992) reported total sugar content in fresh weight of about 4.24 g/100 g in redcurrant (*Ribes rubrum* L.), 5.26 g/100 g in strawberry (*Fragaria* × *ananassa Duch.*), and 5.41 g/100 g in blackcurrant (*Ribes nigrum* L.). Hence, our plant revealed an interesting nutritional composition of reducing sugars that could constitute a beneficial factor for human and animal health by providing immediate sources of energizing calories. Millet grains help in dealing with diabetes since it has low glycemic index; in fact, pearl millet flour has high amounts of magnesium in it, due to which it helps in controlling the glucose receptors in the body. In the populations that have bajra or magnesium-rich foods in their diet, the occurrence of diabetes is reduced by at least 30 per cent (Gupta et al. 2022; Samtiya et al. 2023).

Fatty acids content

Chromatographic analyses of oil and fatty acid methyl esters in grains from ten genotypes of *Pennisetum glaucum* L. are detailed in Table 4. The obtained results revealed significant variation in total fatty acid content among the studied genotypes, reflecting both genetic influences and geographical factors. *Pennisetum glaucum* L. grains exhibit a diverse array of fatty acids, with eight identified, three saturated, and five unsaturated. The fatty acid composition of the investigated genotypes ranged from C16–C20, depending on the total carbon number. Major fatty acids identified were palmitic acid (15.09–20.76%), octadecanoic acid (6.21–9.50%), oleic acid (12.42–27.61%), linolelaidic acid (21.78–44.85%), linoleic acid (21.29–44.27%), and elaidic acid (12.06–27.61%), each accounting for 13.21%

Table 3 Sugar composition of different *Pennisetum glaucum* L. grains (mg/ Kg)

Populations	Fructose	Glucose	Sucrose	Maltose	Total sugar
G1	$161{,}76^{cd} \pm 1{,}14$	$320{,}22^{c} \pm 27{,}64$	$77{,}21^{b} \pm 9{,}45$	ND	559.19
G2	$93{,}47^{cd} \pm 10{,}12$	$268{,}03^{c} \pm 5{,}97$	$75{,}56^{b} \pm 11{,}15$	ND	437.06
G3	$174{,}80^{c} \pm 52{,}96$	$180{,}40^{c} \pm 16{,}53$	$62{,}74^{b} \pm 0{,}25$	ND	417.94
G4	$74{,}01^{d} \pm 27{,}07$	$184{,}42^{c} \pm 6{,}09$	$65{,}55^{b} \pm 3{,}34$	ND	323.98
G5	$126{,}12^{cd} \pm 6{,}84$	$171{,}50^{c} \pm 71{,}09$	$89{,}61^{b} \pm 4{,}26$	ND	387.23
G6	$334{,}98^{b} \pm 54{,}31$	$1189{,}43^{ab} \pm 58{,}15$	$207{,}18^{a} \pm 7{,}81$	$135{,}05^{a} \pm 18{,}91$	1866.64
G7	$458{,}59^{a} \pm 34{,}21$	$1237{,}64^{ab} \pm 288{,}04$	$212{,}80^{a} \pm 3{,}43$	$143{,}26^{a} \pm 2{,}69$	2052.29
G8	$480{,}55^{a} \pm 29{,}36$	$1361{,}70^{a} \pm 75{,}52$	$189{,}95^{a} \pm 49{,}77$	$131{,}66^{a} \pm 2{,}31$	2163.86
G9	$366{,}20^{b} \pm 94{,}09$	$1477{,}36^{a} \pm 607{,}37$	$176{,}68^{a} \pm 31{,}55$	$44{,}66^{b} \pm 77{,}35$	2064.9
G10	$344{,}61^{b} \pm 72{,}35$	$892{,}13^{b} \pm 234{,}55$	$87{,}93^{b} \pm 12{,}03$	ND	1324.67

Values are averages ± SD ($n = 3$). Letters (a–d) denote statistical differences between assessed genotypes using Duncan's multirange mean comparison test ($\alpha = 0.05$). One-way ANOVA results revealing significant differences between genotypes are presented according to p ($p < 0.05$)

Table 4 Fatty acids contents of different *Pennisetum glaucum* L. grains (%)

	Palmitic acid	Octadecanoic acid	Oleic acid	Linolelaidic acid	α-Linolenic acid	Elaidic acid	Linoleic Acid
G1	15,57^{b} ± 3,03	9,50^{a} ± 5,17	13,05^{a} ± 13,05	44,85^{a} ± 3,88	1,11^{b} ± 1,11	13,21^{a} ± 13,21	_
G2	20^{a} ± 1,07	6,21^{a} ± 0,65	13,01^{a} ± 13,01	22,19^{ab} ± 22,19	2,07^{ab} ± 0,17	13,21^{a} ± 13,21	22,11^{ab} ± 12,76
G3	19,80^{a} ± 1,23	6,97^{a} ± 1,58	27,61^{a} ± 2,79	43,72^{a} ± 1,25	1,04^{b} ± 1,04	_	_
G4	18,18^{a} ± 2,70	6,88^{a} ± 0,91	26,24^{a} ± 0,56	24,95^{ab} ± 24,95	1,01^{b} ± 1,01	_	22,17^{ab} ± 12,80
G5	15,09^{b} ± 0,31	6,75^{a} ± 0,11	14,26^{a} ± 14,26	23,97^{ab} ± 23,96	1,97^{ab} ± 0,48	13,62^{a} ± 13,62	24,32^{ab} ± 14,04
G6	20,76^{a} ± 0,12	8,62^{a} ± 0,29	12,42^{a} ± 12,42	42,94^{a} ± 0,47	1,10^{b} ± 1,10	13,51^{a} ± 13,51	_
G7	20,20^{a} ± 0,35	6,23^{a} ± 0,27	13,16^{a} ± 13,16	21,78^{ab} ± 21,78	2,04^{ab} ± 0,11	_	21,38^{ab} ± 12,3
G8	19,18^{a} ± 1,02	9,18^{a} ± 1,70	12,77^{a} ± 12,77	43,32^{a} ± 1,19	2,23^{ab} ± 0,00	13,29^{a} ± 13,29	_
G9	19,86^{a} ± 0,30	8,41^{a} ± 1,90	12,65^{a} ± 12,65	22,12^{ab} ± 22,12	2,14^{ab} ± 0,85	_	21,29^{ab} ± 12,2
G10	19,88^{a} ± 1,13	5,92^{a} ± 1,01	14,29^{a} ± 14,29	–	2,51^{a} ± 0,23	12,06^{a} ± 12,06	44,27^{a} ± 0,51

Values are averages ± SD ($n = 3$). Letters (a–b) denote statistical differences between assessed genotypes using Duncan's multirange mean comparison test ($\alpha = 0.05$). One-way ANOVA results revealing significant differences between genotypes are presented according to p ($p < 0.05$)

of the total. Arachidic acid (0.53–2.23%) and linolenic acid (1.01–2.23%) were present in relatively abundant amounts. Linoleic acid and αlinoleic acid were the most frequently occurring. Genotype G1 derived from Djerba showed a higher linoleic acid content (44.85%), and genotype G10 from Medenine contained up to 44.27% linoleic acid.

We detected the presence of linoleic and **α**-linolenic acids, commonly known as omega-6 and omega-3 fatty acids, respectively. These fatty acids were deemed the most significant in the millet under study. Various studies have compared the fatty acid content of millet with that of oats and quinoa, revealing similar fat content across the three plants (Slama et al. 2020; Samtiya et al. 2023). These findings align with the research conducted by Marmouzi et al. (2018), who also observed comparable fat content among millet, oats, and quinoa.

Identification of phenolic compounds by HPLC–MS

The analyzed phenolic extracts exhibited identical chromatographic profiles, while quantitatively, significant variations were observed (Table 5). Grains from different millet genotypes were found to contain varying and substantial quantities of phenolic compounds. The LC–MS analysis results enabled the identification of eight phenolic acids, with quinic acid being the predominant one. In addition, two flavonoids were detected: quercetin and apigenin.

The results have shown that millet is a plant rich in polyphenols, which is consistent with our previous finding (Triki et al. 2022a, b) and the findings of Xiang et al. (2019a, b). Also, previous research has reported that the millet grains can be a good source of phenolic compounds (Talhaoui et al., 2015). Millet, especially the Medenine ecotype, is a food of significant importance due to its high total phenol content, which imparts beneficial health properties and

Table 5 Phenolic acid contents (μg/g DM) identified in the ethanol extracts of different *Pennisetum glaucum* L. grains

Populations	Quinic acid	1,3-di-O-caffeoyquinic acid	Gallic acid	Protocatechuic acid	Caffeic acid	p-Coumaric acid	Trans-ferulic	Quercetin	Apigenin
G1	99,42^{bc} ± 2,17	1,89^{b} ± 0,02	0,12^{cd} ± 0,01	0,53^{bcd} ± 0,07	0,95^{ef} ± 0,11	2,26^{cd} ± 0,17	0,59^{c} ± 0,00	0,22^{a} ± 0,00	0,06^{c} ± 0,00
G2	70,72^{e} ± 11,23	1,90^{b} ± 0,01	0,11^{d} ± 0,00	0,52^{bcd} ± 0,1	0,94^{ef} ± 0,28	2,21^{cd} ± 0,17	0,47^{d} ± 0,00	0,21^{a} ± 0,00	0,05^{d} ± 0,00
G3	92,74^{cd} ± 2,53	1,48^{d} ± 0,05	0,13^{cd} ± 0,02	0,58^{bc} ± 0,01	1,29^{cd} ± 0,08	4,99^{a} ± 1,69	0,50^{cd} ± 0,03	0,17^{bc} ± 0,00	0,08^{b} ± 0,01
G4	86,95^{cd} ± 4,47	1,72^{c} ± 0,03	0,14^{bcd} ± 0,01	1,06^{a} ± 0,29	0,78^{f} ± 0,11	1,49^{cd} ± 0,04	0,75^{b} ± 0,072	0,16^{cd} ± 0,00	–
G5	79,46^{de} ± 3,22	1,79^{bc} ± 0,09	0,13^{cd} ± 0,02	–	1,13^{de} ± 0,06	2,36^{c} ± 0,40	0,56^{cd} ± 0,10	0,17^{bc} ± 0,03	–
G6	87,56^{cd} ± 7,29	2,03^{a} ± 0,07	0,17^{bc} ± 0,00	0,00^{e} ± 0	0,91^{ef} ± 0,08	4,00^{ab} ± 0,20	0,52^{cd} ± 0,11	0,20^{ab} ± 0,01	0,05^{d} ± 0,00
G7	132,03^{a} ± 6,18	1,83^{bc} ± 0,10	0,28^{a} ± 0,04	0,63^{bc} ± 0,04	1,03^{def} ± 0,03	4,25^{ab} ± 0,46	1,19^{a} ± 0,02	0,17^{c} ± 0,02	0,06^{c} ± 0,01
G8	111,26^{b} ± 17,85	1,78^{bc} ± 0,08	0,16^{bcd} ± 0,01	0,71^{b} ± 0,07	1,81^{a} ± 0,17	3,60^{b} ± 0,20	0,77^{b} ± 0,04	0,13^{d} ± 0,01	0,16^{a} ± 0,00
G9	109,93^{b} ± 0,23	1,79^{bc} ± 0,01	0,11^{d} ± 0,00	0,46^{cd} ± 0,04	1,65^{ab} ± 0,15	3,72^{b} ± 0,06	0,70^{b} ± 0,04	0,13^{d} ± 0,01	0,08^{b} ± 0,00
G10	79,30^{de} ± 0,38	1,91^{b} ± 0,063	0,19^{b} ± 0,06	0,35^{d} ± 0,06	1,47^{bc} ± 0,20	1,23^{d} ± 0,12	0,59^{c} ± 0,02	0,22^{a} ± 0,00	–

Values are averages ± SD ($n = 3$). Letters (a–e) denote statistical differences between assessed genotypes using Duncan's multirange mean comparison test ($\alpha = 0.05$). One-way ANOVA results revealing significant differences between genotypes are presented according to p values as follows: ns: $p \geq 0.05$; $^{*}0.01 \leq p < 0.05$; $^{**}0.001 \leq p < 0.01$; and $^{***}p < 0.001$

medicinal advantages. It can be considered a nutraceutical and functional food. Previous studies have also reported variations in these compounds, demonstrating high levels of phenolic and flavonoid compounds (Klepacka et al. 2011; Bhoyar et al. 2018; Slama et al. 2020). Grains from different millet genotypes exhibited varying and high levels of phenolic compounds. Among these compounds, quinic acid, identified in our study, is widely used in the pharmaceutical industry for drug synthesis. It is recognized for its beneficial effects against colds, flu, migraines, and fever (Chethan et al. 2008; Triki et al. 2022a, b). Other phenolic compounds such as p-coumaric acid, protocatechuic acid, syringic acid, trans-ferulic acid, and trans-cinnamic acid have been studied for their effectiveness in inhibiting eye lens cataracts (Chethan et al. 2008; Triki et al. 2022a, b). Furthermore, caffeic, p-coumaric, ferulic, and protocatechuic acids are known for their antifungal properties (Dragland et al. 2003; Shahidi and Chandrasekara 2013; Sandhu et al. 2020; Triki et al. 2022a, b). In summary, we observe that millet genotypes from Djerba have lower flavonoid content compared to those from Medenine. Previous studies suggest that millet extracts containing flavones such as kaempferol, apigenin, luteolin, and quercetin may have anti-proliferative effects against human colon HT-29 cells. In addition, quercetin, one of the most active flavonoids, has demonstrated high anti-tumor activity by blocking epidermal receptors responsible for tyrosine kinase activity. It is also known for its strong antioxidant power (Sandhu et al. 2020; Riahi et al. 2024).

Multi-criteria analysis and clustering of millet genotypes

To highlight the relationships among the overall assessed traits and identify the most discriminant ones, and to demonstrate their correlation with the studied genotypes, principal component analysis (PCA) coupled with a heatmap analysis (Fig. 2) was conducted based on Pearson's correlations. The results revealed that PC1, primarily associated with minerals, accounted for 30.20% of the total variability, while PC2, related to phenolics and antioxidant activities, explained 22.33%. The heatmap analysis (Fig. 2) enabled us to distinguish four distinct groups. Among these groups, two groups stand out individually. The first individual group is represented by population G7 from Medenine region. This population shows a strong correlation with sugar content and phenolic acids, suggesting marked biochemical similarity in

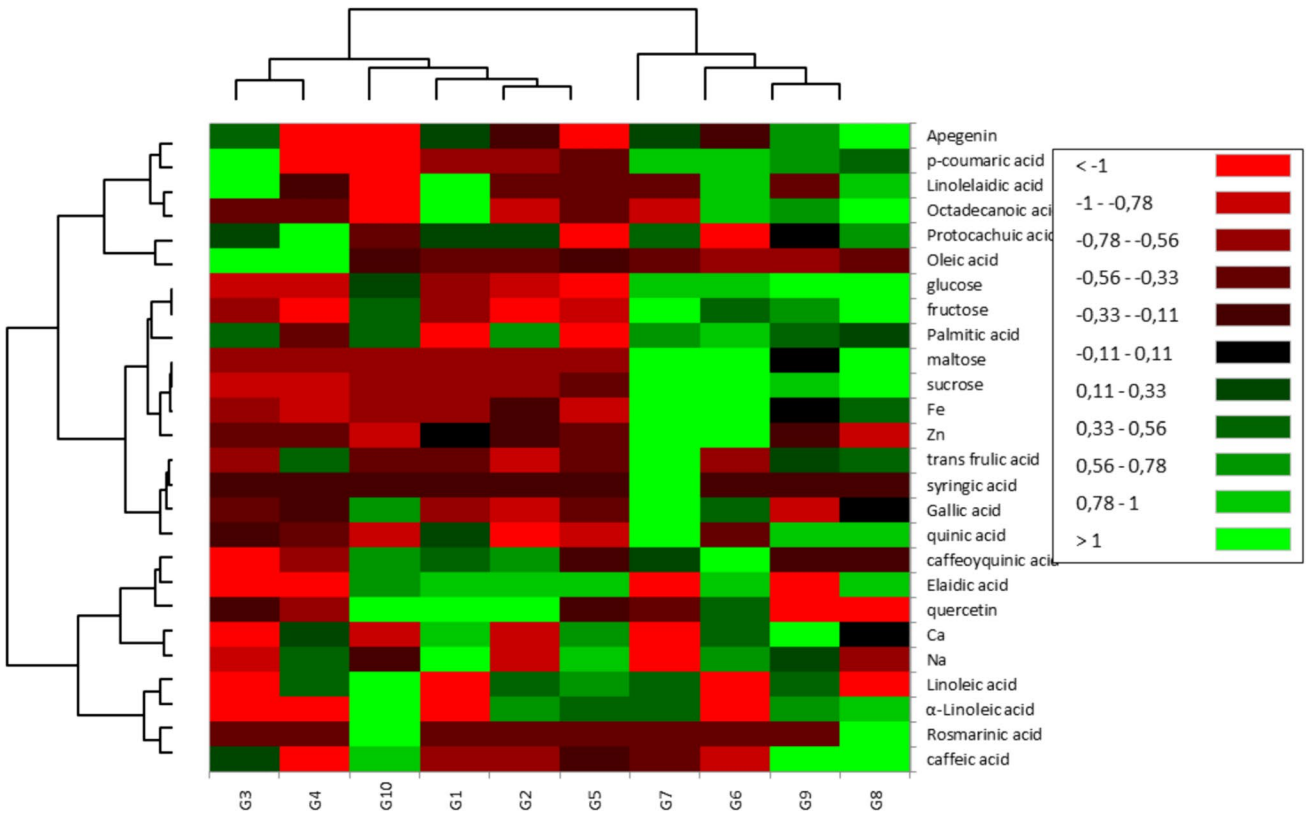

Fig. 2 Heatmap and hierarchical clustering for phenolic contents, sugars, fatty acids, and mineral composition of pearl millet genotypes from southern Tunisia. Genotypes names are presented on the horizontal axis; green color presents the highest contents of minerals (Mn, Cu, K, Mg, Ca, Na, Zn, and Fe), total polyphenols (PT), total phenolic acids, total flavonoids, sugars, and fatty acids

this genotype regarding these two parameters. The second individual group is represented by population G5 originating from Djerba. This genotype is distinguished by its richness in minerals. By combining the PCA and the two-dimensional heatmap analysis, it is possible to visualize and distinguish the different groups of millet populations based on their biochemical characteristics. The third group primarily consists of genotypes from Djerba, namely G1, G2, G3, and G4, as well as population G10 from Medenine. This group is characterized by a richness in some phenolic acids. The last group is exclusively composed of genotypes from Medenine region, namely G6, G8, and G9. These genotypes are distinguished by high levels of phenolic acids, and sugars.

The methods of the multivariate hierarchical clustering and the PCA were employed following the establishment of correlations between morphological, phenological, and chemical composition of forage parts. These approaches helped identify the most pertinent traits for comprehending the sources of variability in local pearl millet genotypes. Our results obtained through the heatmap clustering could serve as a valuable tool for selecting high-performing genotypes for subsequent pearl millet breeding programs.

Conclusion

The results obtained in our research have highlighted the significant presence of bioactive compounds in the analyzed grains, thereby demonstrating a remarkable antioxidant capacity manifested through phenolic composition. In addition, the results show that millet grains are rich in total sugars, lipids, and nutrients, including mineral elements. Consequently, these grains can be considered as functional foods due to their nutritional and therapeutic value. Furthermore, we have observed genetic variability within this germplasm; this diversity is reflected in the differences in the levels of the studied biochemical markers. Indeed, certain genotypes, particularly those of continental origin (Medenine), exhibit an interesting qualitative potential in terms of phenols and minerals. The findings from this study provide a solid foundation for future research and development in the identification of millet genotypes with superior nutritional profiles that can guide breeding programs aimed at enhancing the nutritional value of millet crops. Also, the genetic diversity observed in the studied germplasm offers opportunities for developing millet varieties that are more resilient to environmental stresses and adopted to southern Tunisia, thereby promoting sustainable agricultural practices.

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Data availability The data used to support the findings of this study are included within the article, and a supplementary information file was also provided.

Declarations

Conflicts of interest The authors declare no conflicts of interest.

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