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Research Article

Variation in Phenolic, Mineral, Dietary Fiber, and Antioxidant Activity across Southern Tunisian Pearl Millet Germplasm

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Pearl millet crop, reputed as one of the most important food sources cultivated in arid and semiarid parts of Africa and Asia, is known to be a source of many bioactive molecules with potential health-promoting properties. In Tunisia, this crop presented historically rich and diversified germplasm, which is being threatened by genetic erosion. The preservation programs of these species have been held for more than 20 years *via* participatory breeding schemes. A prospection was undertaken to collect pearl millet cultivars preserved in the last two decades from south-eastern Tunisian farmers to estimate their variability and performances. The aim of this study was to assess the profiles of phenolic compounds, antioxidant capacities, mineral composition, and dietary fiber contents of ten pearl millet cultivars in south-eastern Tunisia. The total phenolics and flavonoids in the free fraction ranged from 506.33 to 1287.71 $\mu g.g^{-1}$ DM ferulic acid equivalent (FAE) and 4.17 to 12.53 $\mu g.g^{-1}$ DM catechin equivalent (CE), respectively. The highest polyphenolic content from all genotypes was 1134.96 $\mu g{\cdot}g^{-1}$ DM (genotype Med.AG1.3). LC-MS analysis of individual phenol compounds allowed the identification of eight phenolic acids in millet grains. The quinic acid, *p*-coumaric acid, and caffeic acid were predominant phenolic acids, and six flavonoid compounds with cirsiliol and silymarin were the predominant flavonoids. The ranges of mineral contents variation were 693.10 to 1075.40 and 80.75 to 175.40 $\mu g{\cdot}g^{-1}$ for Ca and Mg, respectively, and 9.55 to 32.80, 0.75 to 8.60, 1.84 to 12.21, and 3.63 to 11.40 $\mu g{\cdot}g^{-1}$ for Na, Zn, Cu, and Fe, respectively. The content of NDF, ADF, and ADL per dry weight varied from 20 to 31%, 1 to 4.2%, and 0.4 to 2.3%, respectively. Overall, considering the variability among the assessed attributes, heatmap analysis showed the association between each of the traits as related to the clustered genotypes.

1. Introduction

Millet is the main food source of nutrients and dietary energy for people in arid and semiarid parts of Africa and Asia [1]. In Tunisia, pearl millet (*Pennisetum glaucum*) is the most grown millet species and has generated high interest as a substitute for sorghum. This annual cereal is cultivated in the south of the country as a fully irrigated summer crop in about 3000 ha [2], and it shows a significant genetic diversity, which needs further preservation and valorization [3–5]. The grain of pearl millet generally has a higher fat and hence higher energy, higher protein content, and high-quality

protein than most other cereal grains [6]. Many traditional foods and beverages are produced from pearl millet, including couscous, flatbreads, doughs, porridges, gruels, nonalcoholic beverages, and beers. Also, millet is superior to other cereals as a source of antioxidants [7]. In fact, antioxidants refer to a group of compounds that can delay or inhibit the oxidation of lipids [8]. Phenolic compounds and carotenoids are the most important fraction of whole grain phytochemicals [9]. Several health benefits are associated with the polyphenol contents of millet [10]. Phenolic acids play a key role as antioxidants by giving hydrogen or electrons, while carotenoids act as antioxidants by reducing single oxygen and free radicals [11]. Several studies have reported millet as a cheap source of protein and energy. It is considered exclusive among the cereals due to its high polyphenol, mineral, dietary fiber, and carbohydrate contents [12]. Millet is also rich in vitamins B and A, calcium, iron, and zinc. It contains potassium, phosphorus, magnesium, zinc, copper, and manganese [13]. Iron- and zinc-biofortified pearl millet has been developed for improved nutrition [14]. Also, it is a good source of phenolic acids with high antioxidant and antiproliferative potential. Millet phenolics are present in either free or conjugated form. These phenolics can be classified into two types: hydroxycinnamic and hydroxybenzoic acids. The hydroxybenzoic acids are derivatives of benzoic acid, that is, vanillic, *p*-hydroxybenzoic, gallic, and syringic acid, whereas hydroxycinnamic acids include caffeic, coumaric, and ferulic acid with a structure of C6-C3 [15]. Today, there is a growing interest in these compounds due to their benefits to human health [16].

However, little information is currently available on lipid composition, phenolic acids, flavonoids, carotenoids, and antioxidant activity in pearl millet. The objective of this study is to evaluate the profiles of phenolic compounds, antioxidant capacities, and the mineral composition of pearl millet cultivars grown in southern Tunisia to assess its potential uses in food and health purposes.

2. Material and Methods

2.1. Plant Material. Ten autochthonous pearl millet (*Pennisetum glaucum* (L.) R. Br.) cultivars characterized by different panicle forms (Table S1) were collected from different Tunisian farmers of Medenine (continental) and Djerba (coastal) (Table 1) to conduct this phytochemical characterization study. Panicles of ten randomly sampled plants from each cultivar were harvested separately, and then air-dried seeds were ground. Three homogenous samples of 100 g each were prepared. Each sample was obtained after mixing 10 g from seed powders of each plant per cultivar. The ground samples were stored at −20°C prior to extraction. Reagents and solvents used in this study were purchased from Sigma–Aldrich (Steinheim, Germany).

2.2. Mineral Contents. The mineral analysis was performed referring to the method described by Al-Showiman [17]. 1 *g* of milled plant material was burned in the muffle furnace at 530°C for 5 h. Then, the obtained ash was dissolved using 5 mL hydrochloric acid (20%), and the final volume of the dissolved solution was adjusted with distilled water into a volumetric flask of 50 mL. Finally, separate analysis was performed for each mineral element by atomic absorption photometer (Shimadzu A 6800, Kyoto, Japan).

Table 1: The 10 *P. glaucum* genotypes and their location of collection with geographic coordinates.

Genotype	Form	Origin	Longitude	Latitude
Med.AG1.3	Cylindrical	Medenine	33°33'.28"N	10°40'.00"E
Med.AG2.4	Oblanceolate	Medenine	33°35'.31"N	10°42'.01"E
Med.AG3.1	Conical	Medenine	33°36'.14"N	10°41'.16"E
Med.AG4.5	Cylindrical	Medenine	33°34'.23"N	10°41'.29"E
Med.AG4.6	Cylindrical	Medenine	33°35'.25"N	10°41'.20"E
Jer.AG5.2	Spindle	Djerba	33°49'.13"N	10°47'.38"E
Jer.AG6.1	Spindle	Djerba	33°49'.14"N	10°47'.52"E
Jer.AG6.2	Candle	Djerba	33°49'.14"N	10°47'.52"E
Jer.AG7.1	Cylindrical	Djerba	33°49'.09"N	10°48'.25"E
Jer.AG7.2	Spindle	Djerba	33°49'.09"N	10°48'.25"E

2.3. Preparations of Extracts from the Seed Parts. Free phenolic compounds extraction was performed by maceration at room temperature as described by Xiang et al. [18] with some modifications. 1 *g* of each ecotype grain was suspended in 10 mL of each sample. The mixtures were homogenized by an ULTRA-TURRAX® (IKA T 25 digital ULTRA-TURRAX®) for 5 min under agitation for 24 h and then centrifuged (Gyrozen, Korea) at 3000 rpm for 30 min, and the obtained supernatants were evaporated under vacuum in a rotatory evaporator (Cole-Parmer Rotary Evaporator System, US). The methanol extracts were freeze-dried using a freeze-dryer (Bioblock Scientific Christ ALPHA 1-2, Illkirch Cedex, France) and stored at 4°C in tightly closed dark vials until analysis.

2.4. Determination of Phenolic Compounds. 20 mg of each plant extract was dissolved in 1 mL of methanol, and the mixture was 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 elution: 0–45 min, 10–100% B; 45–55 min, 100% B. Equilibration time is controlled at 5 minutes 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 from 50 to 2000 m/z (Bedford, MA, USA).) Phenolic compounds present in different samples were identified by comparing retention times and spectra with 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.

2.5. DPPH, ABTS, and FRAP Activities. The DPPH radical scavenging activity of grains was estimated according to the method explained by Cheung et al. [19] with some modifications. Aliquots of 3.9 mL of 0.1 mM DPPH methanol were mixed with 0.1 mL of the extracts. The mixtures were vigorously shaken and left to stand for 10 min under subdued light. The absorbance at 515 nm was measured against water as a blank. The ability to scavenge the DPPH radical was expressed as percentage inhibition and calculated using the following equation:

$$\text{DPPH scavenging activity}\,(\%) = \frac{(A0 - A1)}{A0} \times 100, \quad (1)$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

The scavenging activity of the millet grains for ABTS radical cations was determined according to the method of Re et al. [20] with some modifications. 7 mM ABTS was added to a 2.45 mM potassium persulfate solution, and the mixture was stored overnight at room temperature in the dark. 1 mL of diluted ABTS radical cation solution (having an absorbance of 1.4–1.5 at 414 nm) was added to 0.5 mL of extract or ascorbic acid standard solution, and the absorbance was read at 414 nm after 1 h. The radical cation scavenging activity of ABTS was expressed as milligrams of ascorbic acid equivalent antioxidant activity (AEAC) per gram DM of the sample (mg.g-1 DM) [21]. The reducing power (FRAP) of grain extracts was determined using the method of Oyaizu [22]. The volumes of 0.25 mL of methanolic grain extract, 100 mM sodium phosphate buffer (0.25 mL, pH 6.6), and 2% potassium ferricyanide (0.20 mL) were mixed and incubated in a water bath at 50°C. After 20 min, 0.25 mL of 10% trichloroacetic acid (w/v) was added to the mixture and centrifuged at 1200 rpm (240 g) for 15 min. The supernatant (0.5 mL) was then mixed with an equal volume of distilled water and ferric chloride solution (0.1%, w/v). The intensity of the blue-green color was measured at 700 nm using a spectrophotometer (Shimadzu UV-1600). Results were expressed as the percentage of reducing power determined by the following equation:

$$\text{FRAP}\,(\%) = \frac{(A0 - A1)}{A0} \times 100, \quad (2)$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

2.6. Determination of Fiber Contents. The dried grains (48 h at 60°C) of the collected millet genotypes were sieved to 1 mm for fiber analysis. NDF, ADF, and ADL were determined using the method of Van Soest and Robertson [23]. Fiber Analyzer (ANKOM220: Ankom Technology Co., Fairport, NY, USA) was used to determine NDF and ADF in the absence of thermostable amylase. After 1 hour incubation of samples at 75°C, ADL was measured using 72% sulfuric acid for 3 hours. Ash content was determined after calcining 1 g of each dry sample ($n = 3$) at 550°C for 6 h.

2.7. Statistical Analyses. The differences in mean values among different millet phenolic fractions were evaluated using a one-way analysis of variance (ANOVA) followed by Duncan's multirange mean comparison test at a $p < 0.05$ significance level. The results were presented as mean ± standard deviation (SD). Graphics were generated using GraphPad Prism 5.0. Multivariate analyses (Heatmap and PCA) were performed 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.

3. Results and Discussion

3.1. Grain Mineral Composition. In literature, few emerging studies have reported the bioaccessibility potential of pearl millet bioactive compounds especially for the mineral's compounds [24, 25]. Herein, we found that, from the seven elements determined in millet grain genotypes, calcium was the predominant mineral followed by potassium (K), magnesium (Mg), sodium (Na), iron (Fe), and zinc (Zn) (Table 2). The mineral analysis has shown that grain of millet plant offers a good source of mineral elements that depend on genotype. Among the 10 genotypes, the variation was significant ($p < 0.05$) only for K, Ca, and Mg contents. Except the Ca, the genotype Med.AG1.3 (from Medenine) exhibited the highest values of all mineral compositions (Na (32 mg/100 g), K (482.65 mg/100 g), Mg (175.40 mg/100 g), Cu (12.21 mg/100 g), Zn (8.60 mg/100 g), and Fe (11.40 mg/100 g)). Also, the genotypes from Medenine (continental origin) showed high mineral contents compared to the other grain's genotypes collected from Djerba (littoral origin). In fact, this quantitative evaluation of the phytochemicals as well as mineral elements is an important insight into the pharmacological actions of plants. The mineral contents in millet grains, such as Na, K, Ca, Mg, Fe, Cu, and Zn, were reported to be higher than those of corn [26]. As compared with previous studies on pearl millet, our results were higher than those reported by Malik [27], which confirmed that millets have at least twice up to thirty times higher calcium amounts than rice. The same work reported the health-promoting abilities of pearl millet due to its richness in minerals, such as the effectiveness in reducing migraine attacks and severe respiratory problems for asthmatic

Table 2: Mineral compounds (mg.100 g^{-1} DM) determined by atomic absorption spectrometry in millet genotypes grains.

Genotype	Na	K	Ca	Mg	Cu	Zn	Fe
Med.AG1.3	32.80 ± 13.90 ab	482.65 ± 50.75 a	834.30 ± 7.10 bc	175.40 ± 24.90 a	12.21 ± 5.71 a	8.60 ± 0.71 a	11.40 ± 2.14 a
Med.AG2.4	31.70 ± 8.50 a	352.20 ± 37.30 b	1075.40 ± 273.00 a	118.30 ± 8.40 bc	2.44 ± 0.14 b	4.45 ± 0.58 b	10.11 ± 3.04 a
Med.AG3.1	9.55 ± 3.35 d	271.30 ± 27.60 b	912.90 ± 32.50 abc	80.75 ± 5.95 c	1.89 ± 0.09 b	0.78 ± 0.18 c	4.53 ± 0.02 b
Med.AG4.5	24.10 ± 4.50 bc	358.80 ± 3.00 ab	1009.20 ± 0.00 ab	130.30 ± 8.70 b	2.25 ± 0.02 b	1.01 ± 0.37 c	5.39 ± 1.28 b
Med.AG4.6	14.20 ± 0.80 cd	384.15 ± 42.85 ab	714.40 ± 141.20 c	127.30 ± 16.20 b	1.84 ± 0.10 b	0.76 ± 0.03 c	3.63 ± 0.55 b
Jer.AG5.2	9.90 ± 0.20 d	354.40 ± 46.30 b	813.05 ± 92.15 bc	107.00 ± 6.20 bc	3.78 ± 0.06 b	1.02 ± 0.04 c	4.77 ± 1.18 b
Jer.AG6.1	14.95 ± 2.75 cd	374.60 ± 48.70 ab	814.60 ± 53.60 bc	120.45 ± 7.25 bc	3.42 ± 0.13 b	1.29 ± 0.36 c	5.67 ± 0.42 b
Jer.AG6.2	12.50 ± 5.00 cd	288.65 ± 74.85 b	730.90 ± 71.50 c	95.70 ± 34.50 bc	2.19 ± 0.96 b	0.75 ± 0.26 c	3.75 ± 0.34 b
Jer.AG7.1	13.70 ± 2.70 cd	337.15 ± 103.95 b	698.45 ± 41.35 c	113.70 ± 24.50 bc	3.65 ± 0.81 b	0.87 ± 0.28 c	3.78 ± 0.48 b
Jer.AG7.2	15.25 ± 1.65 cd	331.15 ± 119.45 b	693.10 ± 154.20 c	115.55 ± 46.06 bc	2.75 ± 0.99 b	0.82 ± 0.35 c	5.72 ± 0.62 b
R^2	0.744	0.517	0.617	0.626	0.785	0.985	0.854
CV%	47.96	16.28	16.02	21.00	84.95	126.21	46.04
ANOVA	***	*	*	*	***	***	***

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: $^{*}0.01 \leq P < 0.05$; $^{**}0.001 \leq P < 0.01$; and $^{***}P < 0.001$.

patients thanks to higher amounts of magnesium; and they help to increase Hb to avoid anemia thanks to the bioavailability of iron [27]. Though, iron and zinc contents in the studied genotypes showed remarkable differences. This variability depends on the genotype and the grain fraction effects [28]. Krishnan and Meera [28] discussed the bioaccessibility of pearl millet's minerals, among iron and zinc, and reported that, at the household level, there are several traditional food processing and preparation methods that can improve the bioavailability of micronutrients in a grain-based diet. These processes include soaking, blanching, peeling, hydrothermal treatment, germination, acid treatment or fermentation, or a combination of treatments and a combination of enhancers.

3.2. Antioxidant Compounds of the Methanolic Extracts from Grains. Herein, methanol was selected as an extraction solvent. Because methanol is a relatively polar organic solvent compared to other extracting solvents, including ethanol, ethyl acetate, acetone, and hexane, most polyphenolics evaluated in this study are likely polar compounds.

It has been found that polyphenolic compounds are one of the most effective antioxidative constituents in plant foods, including fruit, vegetables, and grains [29]; hence, it is important to quantify polyphenolic contents and to assess their contribution to antioxidant activity. The polyphenolic contents in the grain methanolic extracts of the ten genotypes are shown in Table 3. The genotype Med.AG3.1 (from Medenine) was remarkably high in polyphenolic content compared to the other grains genotypes (1134.96 $\mu g \cdot g^{-1}$ DM). The genotype Jer.AG5.2 from Djerba contained about 2 times lower polyphenolics as compared with genotype Med.AG1.3 in the methanolic extracts (518.86 $\mu g \cdot g^{-1}$ DM). The total flavonoid content recorded in this genotype Med.AG1.3 was 12.53 $\mu g \cdot g^{-1}$ DM (Table 3). Hence, pearl millet (Medenine ecotype), with a high total flavonoid content, could be considered an important food as it contains health additives and medicinal benefits. It could be used as both a nutraceutical and functional food.

3.3. Antioxidant Activities. The stable DPPH radical, which has a maximum absorption at 515 nm, is widely used to evaluate the free radical scavenging activity of hydrogen donating antioxidants in many plant extracts [30,31]. The DPPH radical scavenging activity of the methanolic extracts is presented in Figure 1(b). The genotypes Med.AG4.5 (from Medenine), Jer.AG5.2, and Jer.AG7.1 (from Djerba) showed relatively the highest radical scavenging activity. Also, the ABTS method is widely employed for measuring the relative radical scavenging activity of hydrogen donating and chain-breaking antioxidants in many plant extracts [31, 32]. The ABTS radical cation scavenging activity of methanolic extracts, expressed as mg ascorbic acid equivalents per 1 g of dry matter, is presented in Figure 1(a). High pigmented red genotype Med.AG4.5 from Medenine (57 mg) showed much higher radical scavenging activity than the other samples (40–48 mg). However, many previous studies have reported a significant correlation between polyphenolics and antioxidant activities in fruits such as barley and mushrooms [21, 33].

The reducing power of the methanolic extracts (4 mg·mL^{-1}) is presented in Figure 1(c). In this method, the ferric-ferricyanide complex is reduced to the ferrous form depending on the presence of antioxidants [34]. Highly pigmented red genotype Med.AG4.5 collected from Medenine (11%) has relatively higher reducing power than other samples. This indicates that polyphenolics in methanolic extracts of grains may play a role as electron and hydrogen donors. As a result, genotype Med.AG4.5 collected from Medenine showed significantly higher antioxidant activities and contained higher polyphenolic contents than other samples in the methanolic extracts.

As a whole, ABTS, DPPH, and FRAP activities assessed in this study ranged from 36.71 to 54.86 mg·g^{-1} DM, from 66.67 to 95.08%, and from 7.39 to 12.01%, respectively. These findings slightly agree with those reported by Li et al. [31].

3.4. Profile of Individual Phenolic Compounds. The contents of free main phenolic acids and flavonoids in grains of pearl

Table 3: Total phenolic composition contents of the methanolic extracts obtained from the grain millet genotypes.

Genotype	Total phenolic acids ($\mu g \cdot g^{-1}$ DM)	Total flavonoids ($\mu g \cdot g^{-1}$ DM)	Total polyphenols ($\mu g \cdot g^{-1}$ DM)
Med.AG1.3	603.24 ± 103.96 d	8.44 ± 1.07 abc	611.68 ± 103.24 d
Med.AG2.4	956.76 ± 97.56 bc	8.81 ± 0.54 abc	965.57 ± 97.76 bc
Med.AG3.1	1127.52 ± 159.71 ab	12.53 ± 1.98 abc	1134.96 ± 161.65 ab
Med.AG4.5	717.98 ± 138.12 cd	9.90 ± 5.89 ab	727.88 ± 143.80 cd
Med.AG4.6	566.68 ± 165.19 d	9.16 ± 3.90 abc	575.84 ± 169.04 d
Jer.AG5.2	506.33 ± 199.17 d	7.45 ± 4.38 a	518.86 ± 203.54 b
Jer.AG6.1	980.23 ± 63.68 b	5.87 ± 1.87 bc	986.10 ± 65.40 bc
Jer.AG6.2	912.71 ± 166.90 bc	8.02 ± 1.78 abc	920.74 ± 165.18 a
Jer.AG7.1	1287.60 ± 100.50 a	7.87 ± 1.12 abc	1295.47 ± 99.37 a
Jer.AG7.2	973.75 ± 92.47 b	4.17 ± 0.87c	977.92 ± 93.30 bc
R^2	0.829	0.541	0.824
CV (%)	31.45	39.27	31.09
ANOVA	***	*	***

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 ***$P < 0.001$.

millet genotypes are shown in Tables 4 and 5. Phenolic acids and flavonoids made up the largest group among total phenolic compounds (Table 3). In total, 13 phenolic compounds were identified.

3.4.1. Free Phenolic Acids and Derivatives. Qualitatively, all analyzed phenolic extracts showed the same chromatographic profiles, whereas, quantitatively, significant differences appeared across genotypes (Table 4). The grains from different genotypes of millet were found to contain high and varying amounts of phenolic compounds. The analysis of the LC-MS results allowed the detection of eight phenolic acids from millet grains (Figure S1), which are quinic acid ([M-H]$^-$, $m/z = 191$, at RT = 1.973 min), protocatechuic acid (([M-H]$^-$, $m/z = 153$, at RT = 6.883 min), caffeic acid ([M-H]$^-$, $m/z = 179$, at RT = 14.457 min), syringic acid ([M-H]$^-$, m/$z = 197$, at RT = 16.056 min), 1,3-di-O- caffeoylquinic acid ([M-H]$^-$, $m/z = 515$, at RT = 16.988 min), *p*-coumaric acid ([M-H]$^-$, $m/z = 163$, at RT = 20.902 min), *trans*-ferulic acid ([M-H]$^-$, $m/z = 193$, at RT = 23.111 min), and salvianolic acid ([M-H]$^-$, $m/z = 717$, at RT = 28.114 min) (Table 4; Figure S1). Quinic acid, *p*-coumaric acid, and caffeic acid were the predominant identified phenolic acids. The highest amount was found in the genotype G3 from Medenine (1134.96 $\mu g \cdot g^{-1}$ DM), while the lowest was registered in genotype Med.AG3.1 from Djerba (518.86 $\mu g \cdot g^{-1}$ DM). These findings agree with those described in previous research, which reported that millet grains can represent a good source of phenolic compounds [18, 35].

Chethan et al. [36] reported that the constituents of millet grain coat phenolics like *p*-coumaric, protocatechuic, syringic, *trans*-ferulic, *trans*-cinnamic, and so on were known for their effectiveness in inhibiting cataract of the eye lens. Moreover, caffeic, *p*-coumaric, ferulic, and protocatechuic acids have been cited as antifungal agents [37, 38].

3.4.2. Flavone and Flavanone Derivatives. Five flavonoids were identified in pearl millet genotypes (Figure S1): quercetin ([M-H]$^-$, $m/z = 301$, at RT = 31.999 min), kaempferol ([M-H]$^-$, $m/z = 285$, at RT = 32.010 min), silymarin ([M-H]$^-$, $m/z = 481$, at RT = 33.848 min), apigenin ([M-H]$^-$, $m/z = 269$, at RT = 34.624 min), and cirsiliol ([M-H]$^-$, $m/z = 329$, at RT = 35.353 min) (Table 5). To the best of our knowledge, this is the first study that reported the presence of hyperoside ([M-H]$^-$, $m/z = 463$, at RT = 24.308 min) flavones in millet. These compounds have not been identified prior to this study in millet, although they are commonly recorded in amaranth, buckwheat, and quinoa for quercetin and in sorghum for catechin, as reported in a recent review [39]. However, Hithamani and Srinivasan [25] revealed 22 compounds and identified only 11 polyphenols as phenolic acids in the acidified methanol extract of finger millet without confirming the presence of any flavonoids. It is possible that flavonoids are included among the remaining 11 unidentified peaks. Ofosu et al. [40] reported in their study on Italian millet genotypes that all identified phenolics were flavonoids; among flavonols were the predominant subclass. Depending on the variety of millet, the quantity of flavonoids changes [41]. We conclude that the genotypes collected from the littoral origin Djerba have significantly ($p < 0.05$) lower flavonoid content than the genotypes from Medenine.

Shahidi and Chandrasekara [37] cited that flavones such as kaempferol, apigenin, luteolin, and quercetin in millets extracts could be involved in the antiproliferative activities *in vitro* against HT-29 human colon adenocarcinoma cells.

3.5. Fiber Contents. As in many arid and semiarid regions, farmers in the south of Tunisia pay attention to both grain and vegetative parts of pearl millet as human food and fodder yielding potential, respectively. Most of the fiber analysis reported in the literature focused on the feed organs. Very little information is available about the fiber contents in grains of pearl millet. In this study, the fiber components represented by neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were analyzed in grains of the pearl millet genotypes. The obtained results

Table 4: Phenolic acid contents ($\mu g \cdot g^{-1}$ DM) identified in the methanol extracts of grains of millet genotypes.

	Quinic acid	Protocatechuic acid	Caffeic acid	Syringic acid	1,3-di-O-Caffeoyquinic acid	*p*-Coumaric acid	*trans*-Ferulic acid	Salviolinic acid
Med.AG1.3	582.18 ± 105.40 d	—	3.87 ± 0.96 c	1.20 ± 1.32 a	0.96 ± 0.74 a	5.28 ± 1.91 e	7.76 ± 1.97 cd	1.72 ± 0.27 a
Med.AG2.4	920.89 ± 98.05 b	2.53 ± 1.53 a	5.96 ± 2.00 bc	1.20 ± 0.38 a	—	14.69 ± 2.00 d	9.61 ± 1.00 bcd	1.41 ± 0.62 a
Med.AG3.1	1072.36 ± 152.76 ab	3.33 ± 2.08 a	13.76 ± 4.04 a	1.52 ± 1.16 a	0.626 ± 0.48 a	24.35 ± 1.00 c	9.72 ± 1.00 bcd	1.87 ± 0.26 a
Med.AG4.5	649.32 ± 141.60 cd	—	14.30 ± 2.52 a	1.51 ± 1.00 a	—	35.80 ± 3.09 b	7.12± 2.08 ab	1.84 ± 0.28 a
Med.AG4.6	531.11 ± 166.23 d	—	5.43 ± 2.00 bc	1.74 ± 1.00 a	—	17.19 ± 1.53 d	9.60 ± 1.00 bcd	1.48 ± 0.50 a
Jer.AG5.2	461.65 ± 205.53 d	2.37 ± 1.00 a	12.46 ± 4.17 a	1.48 ± 1.00 a	—	14.49 ± 0.99 d	12.01 ± 2.00 d	1.53 ± 0.52 a
Jer.AG6.1	933.08 ± 64.29 b	—	9.76 ± 2.08 ab	2.15 ± 1.41 a	—	22.74 ± 2.00 c	10.93 ± 1.53 abc	1.08 ± 0.25 a
Jer.AG6.2	834.92 ± 175.30 bc	2.66 ± 1.41 a	11.20 ± 2.50 ab	1.74 ± 1.09 a	—	48.34 ± 2.86 a	12.19 ± 1.49 ab	1.57 ± 0.73 a
Jer.AG7.1	1202.88 ± 99.14 a	2.75 ± 1.29 a	12.37 ± 2.08 a	2.66 ± 1.53 a	—	51.44 ± 1.00 a	13.91 ± 2.52 a	1.67 ± 0.78 a
Jer.AG7.2	923.44 ± 89.80 b	2.52 ± 0.98 a	9.64 ± 1.50 ab	1.66 ± 1.12 a	—	23.12 ± 2.62 c	11.96 ± 1.65 ab	1.58 ± 0.71 a
R^2	0.813	0.687	0.732	0.167	0.673	0.987	0.677	0.199
CV%	30.22	87.49	37.05	26.115	216.55	58.35	20.18	14.51
ANOVA	***	**	***	ns	**	***	**	ns
Chromatographic analysis parameters								
Mw	192	154	180	198	516	164	194	718
Ionization form	[M-H]-	[M-H]-	[M-H]-	[M-H]-	[M-H]-	[M-H]-	[M-H]-	[M-H]-
m/z	191	153	179	197	515	163	193	717
Retention time	1.973	6.883	14.457	16.056	16.988	20.902	23.111	28.114
LOD^{++} (ppm)	0.616	0.122	0.031	0.297	0.624	0.384	0.373	0.092
LOQ^{++} (ppm)	1.867	0.369	0.093	0.901	1.890	1.165	1.131	0.280

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 $^{***}P < 0.001$. $^{++}$ The limit of detection LOD = 3.3σ / S, and the limit of quantification LOQ = 10σ / S; σ is the standard deviation of the response, and S is the slope of the calibration curve.

are shown in Figure 2. NDF, ADF, and ADL contents varied between 20 and 31%, 1 and 4.2%, and 0.4 and 2.3%, respectively, on a dry weight basis. The genotypes from Djerba were remarkably high in fiber contents compared to the other grain's genotypes collected from Medenine. Previous reports have shown that the NDF content of finger millet was 12% [42] and 11.5 [43, 44] on a fresh weight basis. Furthermore, the study of dietary fiber contents in Indian finger millet showed that the NDF content can reach 13.44% on a dry weight basis [45]. Based on NDF contents (20–31% DW), the grains of Tunisian genotypes studied here could be considered highly rich in cellulose, hemicelluloses, and lignin if compared to values obtained in previous reports (11.3% on a fresh weight basis) [44]. According to Malik [27], dietary fibers are effective in dealing with constipation, and they help in the process of weight loss. In addition, the richness of pearl millet in fibers, especially in their insoluble fraction, is known to lead as an agent preventing gall stone and reducing risks of its occurrence by the inhibition of excessive bile secretion. Moreover, the lignin and phytonutrients in millet act as strong antioxidants, thus preventing heart-related diseases [27]. On the other hand, Krishnan and Meera [28] mentioned that fibers and phytate chelate minerals form a fiber-phytate-mineral complex and that processing by germination is useful to raise soluble fiber fraction and reduce insoluble one in pearls.

3.6. Multicriteria Analysis and Clustering of Millet Genotypes. To underling the relations between overall assessed traits to determine those that were the most discriminant and to show their correlation with the studied genotypes, principal compounds (PCA) (Figure S2) coupled with heatmap (Figure 3) were analyzed based on Pearson's correlations. Obtained results showed that PC1 presented by minerals was the source of 39.01% of the total variability, while 22.66% were presented by PC2 explicated by phenolics and antioxidant activities (Figure S2). Heatmap (Figure 3) allowed us

Table 5: Flavonoid compounds ($\mu g \cdot g^{-1}$ DM) identified in the methanol extracts of grains of millet genotypes.

	Quercetin	Kaempferol	Silymarin	Apigenin	Cirsiliol	Hyperoside
Med.AG1.3	1.12 ± 0.53 a	0.22 ± 0.03 b	1.78 ± 0.31 cd	0.24 ± 0.07 b	3.68 ± 2.02 c	0.31 ± 0.26 b
Med.AG2.4	0.58 ± 0.10 b	0.26 ± 0.02 b	1.83 ± 1.22 cd	0.19 ± 0.03 bc	5.51 ± 1.04 abc	—
Med.AG3.1	0.60 ± 0.10 b	0.52 ± 0.10 a	_d	0.24 ± 0.07 b	4.85 ± 1.00 abc	—
Med.AG4.5	0.58 ± 0.30 b	0.18 ± 0.02 b	4.44 ± 2.00 a	0.14 ± 0.03 c	3.36 ± 2.00 c	0.18 ± 0.07 b
Med.AG4.6	0.46 ± 0.20 b	0.18 ± 0.02 b	3.71 ± 1.82 ab	0.19 ± 0.02 bc	3.90 ± 2.00 bc	0.05 ± 0.02 b
Jer.AG5.2	0.49 ± 0.30 b	0.24 ± 0.10 b	2.04 ± 1.00 bc	0.38 ± 0.02 a	3.34 ± 1.00 c	4.28 ± 2.00 a
Jer.AG6.1	0.49 ± 0.08 b	0.21 ± 0.02 b	_d	0.24 ± 0.04 b	4.94 ± 2.00 abc	—
Jer.AG6.2	0.46 ± 0.06 b	0.16 ± 0.04 b	0.55 ± 0.20 cd	0.15 ± 0.02 bc	6.70 ± 2.00 ab	—
Jer.AG7.1	0.46 ± 0.20 b	0.15 ± 0.01 b	_d	0.18 ± 0.08 bc	7.09 ± 1.00 a	—
Jer.AG7.2	0.53 ± 0.20 b	0.16 ± 0.05 b	0.65 ± 0.21 cd	0.16 ± 0.05 bc	2.66 ± 0.57 c	—
R^2	0.459	0.855	0.771	0.733	0.548	0.86
CV	34.28998476	51.02691864	105.0947844	32.83744084	32.27193126	32.27193126
ANOVA	ns	***	***	***	*	***
Chromatographic analysis parameters						
Mw	302	286	482	270	330	464
Ionization form	[M-H]-, [2M-H]-	[M-H]-, [2M-H]-	[M-H]-, [2M-H]-	[M-H]-, [2M-H]-	[M-H]-	[M-H]-, [2M-H]-
m/z	301	285	481	269	329	463
Retention time	31.999	32.010	33.848	34.624	35.353	24.308
LOD++ (ppm)	0.085	0.181	0.030	0.821	0.143	0.887
LOQ++ (ppm)	0.258	0.548	0.090	2.489	0.432	2.688

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 $^{***}P < 0.001$. $^{++}$ The limit of detection LOD = 3.3σ / S, and the limit of quantification LOQ = 10σ / S; σ is the standard deviation of the response, and S is the slope of the calibration curve.

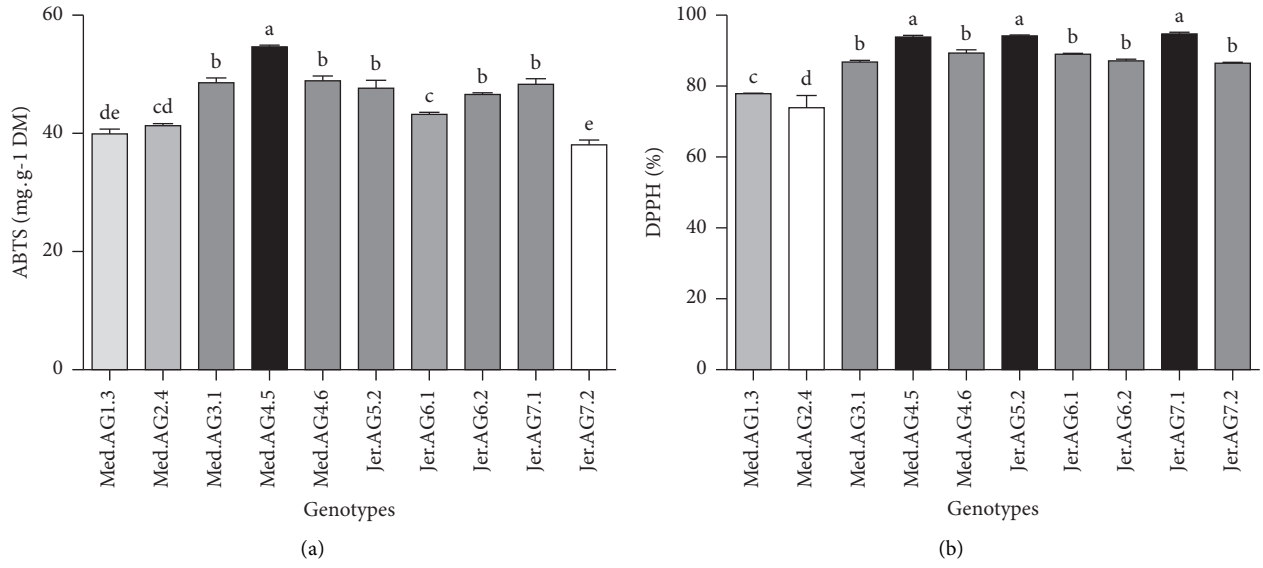
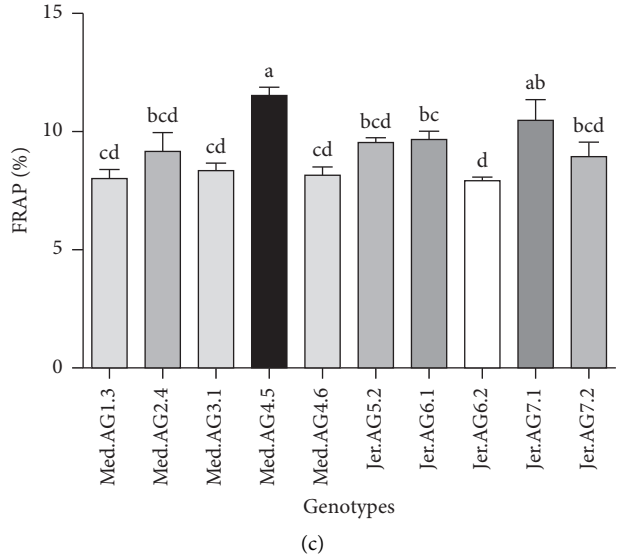
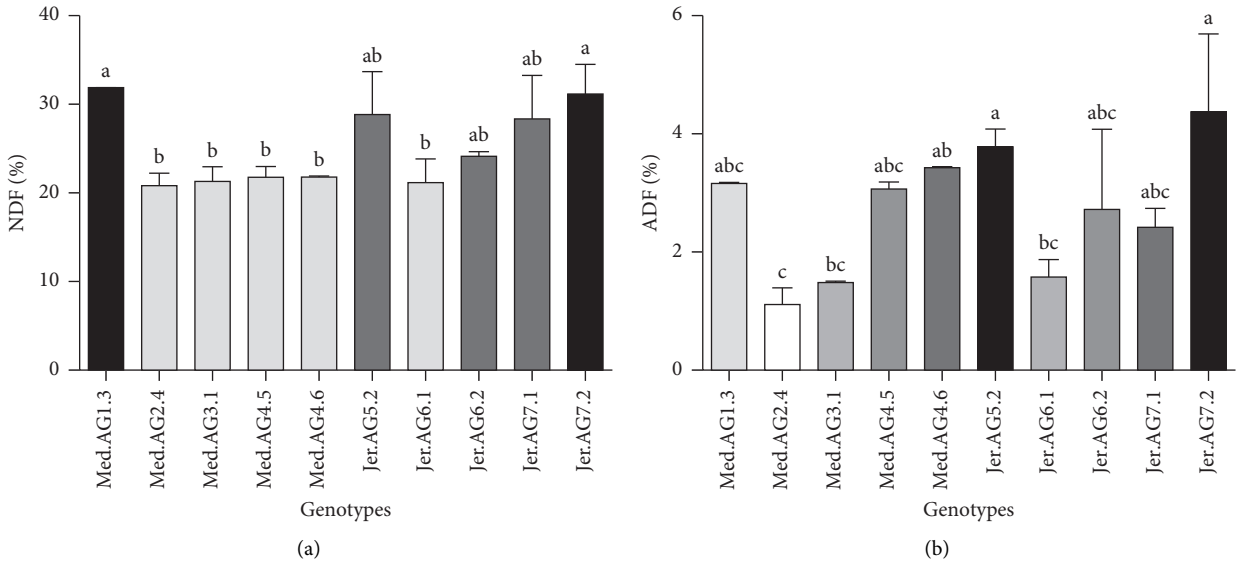


Figure 1: Continued.



(c)

Figure 1: Antioxidant activities of grain extracts from the assessed millet genotypes; (a) ABTS, (b) DPPH, and (c) FRAP. Values are averages ± SD ($n = 3$). Letters a–e denote statistical differences between assessed genotypes using Duncan's multirange mean comparison test ($\alpha = 0.05$).



(a) (b)

Figure 2: Continued.

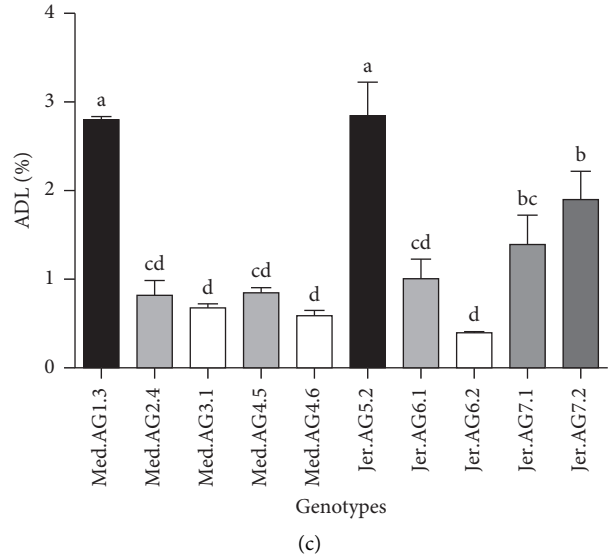


Figure 2: Fiber contents in the grains of the assessed millet genotypes; (a) neutral dietary fiber (NDF), (b) acid detergent fiber (ADF), and (c) acid detergent lignin (ADL). *Values are averages* ± *SD* (n = 3). Letters a–e denote statistical differences between assessed genotypes using Duncan's multirange mean comparison test ($\alpha = 0.05$).

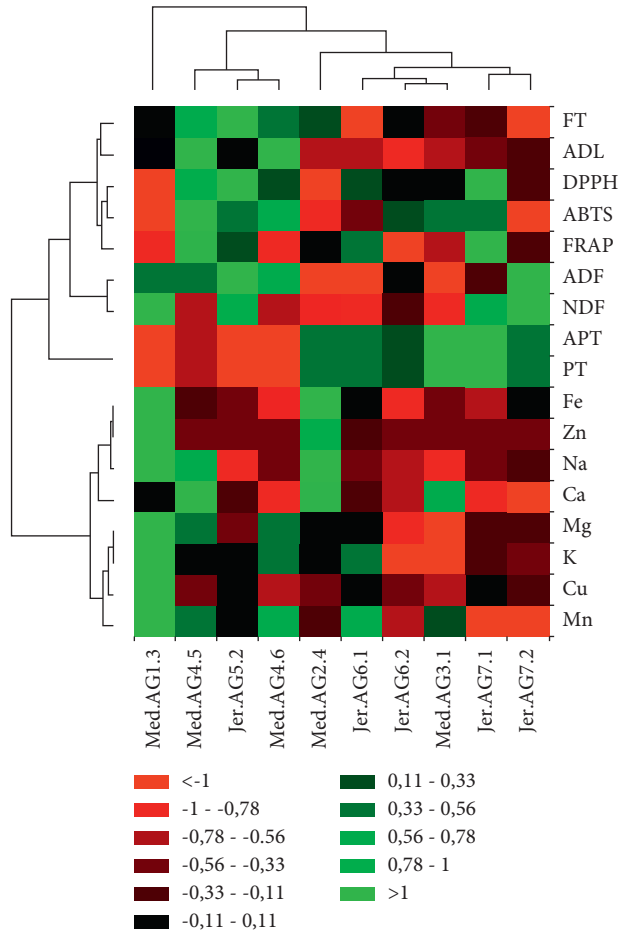


Figure 3: Heatmap and hierarchical clustering for phenolic contents, antioxidant activities, and chemical composition of pearl millet cultivars from southern Tunisia. Genotypes names are presented on the horizontal axis; green color presents the highest contents of minerals (Mn, Cu, K, Mg, Ca, Na, Zn, and Fe), total polyphenols (PT), total phenolic acids (APT), total flavonoids (FT), fibers (NDF, ADF, and ADL), and antioxidant activities (DPPH, ABTS, and FRAP).

to distinguish between four clusters: the first and third clusters were represented individually by Med.AG1.3 and Med.AG2.4 that were correlated with high levels of minerals; genotypes Med.AG4.5, Med.AG4.6, and Jer.AG5.2 that were correlated with antioxidant activities composed the second cluster, while the fourth cluster gathered the genotypes Jer.AG6.2, Jer.AG7.1, Jer.AG7.2, and Med.AG3.1 that presented high fiber and phenolic contents. Loumerem [4] studied millet breeding lines that originated from the same accessions as part of the germplasm assessed for his breeding work which was undertaken over 20 years ago. The approaches of multivariate hierarchical clustering and PCA after defining the correlations between morphological, phenological, and chemical composition of forage parts were used to define the most relevant traits to understand the sources of the variability in the local pearl millet genotypes. Our findings *via* heatmap clustering may be useful as a tool in selecting performant genotypes for further pearl millet breeding programs.

4. Conclusion

Genotype-dependent variation in antioxidant activities, chemical composition (phenolic compounds and mineral elements), and fiber contents quality traits have been observed among the currently considered cultivars. Some cultivars, especially from continental origin (Medenine), have an interesting quality potential in terms of antioxidant and fiber contents. Considering the level of pearl millet cultivation in the region and the smallholder farming context, a breeding program in nutritional quality traits based on the selected genotypes could have an important role in enhancing grain production and improving the livelihood of farmers in Tunisia.

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.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Tebra Triki, Samir Tlahig, and Ferdaous Guasmi designed the experiment and wrote the manuscript; Samir Tlahig, Hédi Yahia, and Mohamed Loumerem collected the plant material; Tebra Triki, Mohamed Bagues, and Khaled Belhouchette prepared plant extracts and performed lab spectrometry analysis; Tebra Triki and Samir Tlahig performed fiber analysis; Mahmoud Mabrouk performed LC-MS analysis; Samir Tlahig performed and computed statistical analysis; Tebra Triki, Samir Tlahig, Mohamed Ali Benabderrahim, Walid Elfalleh, and Ferdaous Guasmi discussed the results and revised the manuscript; Ferdaous Guasmi and Mohamed Loumerem supervised the work and provided chemicals. All authors validated the current version of the manuscript. Tebra Triki and Samir Tlahig contributed equally.

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Supplementary Materials

The data related to the morphological description of the studied millet genotypes (Table S1) and the PCA of the assessed parameters (Figure S1) are presented in the supplementary file (Supplementary Materials). (*Supplementary Materials*)

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