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Agronomic and Functional Quality Traits in Various Underutilized Hot Pepper Landraces

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Abstract: Landraces are considered a crucial component of biodiversity conservation, serving as a reservoir of genetic diversity. Consequently, the collection, cultivation, and detailed characterization of such landraces constitute an inherent aspect of the world's natural resource heritage. This effort holds promise for the development of elite varieties capable of thriving amidst continuous global climate fluctuations. In this context, we conducted a comprehensive assessment of the main agronomic attributes, physico-chemical properties, and functional quality traits of the major hot pepper landraces adapted to diverse climatic conditions in Tunisia. These landraces include 'Dhirat', 'Semmane', 'Beldi', 'Nabeul', 'Jerid', 'Mahdia', 'Cayenne', 'Kairouan', and 'Baklouti'. Most of the pepper landraces exhibited satisfactory yields, ranging from 1163.25 to 1841.67 g plant^{-1} in 'Jerid' and 'Kairouan', respectively, indicating robust productivity, especially under prevailing climatic changes and high temperatures during both growing cycles. The levels of antioxidants comprising capsaicinoids, carotenoids, phenolics, and tocopherols, as well as radical scavenging activity, emerged as key discriminating factors among pungent pepper landraces. Irrespective of genotype, capsaicin and dihydrocapsaicin constituted the major capsaicinoids, accounting for 44–91% of the total capsaicinoids content. Total capsaicinoids ranged from 1.81 µg g^{-1} fw to 193.71 µg g^{-1} fw, with 'Baklouti' and 'Jerid' identified as the most pungent landraces. Total carotenoids ranged from 45.94 µg g^{-1} fw to 174.52 µg g^{-1} fw, with 'Semmane' and 'Jerid' exhibiting the highest levels. Considerable variation was observed in β-carotene content, spanning from 3% to 24% of the total carotenoids. α-Tocopherol content ranged from 19.03 µg g^{-1} fw in 'Kairouan' to 30.93 µg g^{-1} fw in 'Beldi', exerting a notable influence on the overall tocopherol content. Conversely, the β- and γ-tocopherol isomers were detected at very low concentrations. The total vitamin C content ranged from 132 mg 100g^{-1} fw in 'Mahdia' to 200 mg 100 g^{-1} fw in 'Nabeul', indicating relatively low genetic variability. However, large variability was detected in total phenolics content, ranging from 168.58 mg GAE kg^{-1} fw in 'Beldi' to 302.98 mg GAE kg^{-1} fw in 'Cayenne'. Landraces such as 'Dhirat', 'Nabeul', 'Semmane', 'Kairouan', 'Cayenne', and 'Mahdia' appear suitable for both fresh consumption and processing, owing to their favorable average fruit weight, soluble solids content, and bioactive content. Among the pepper landraces tested, 'Cayenne' achieved the highest value of radical scavenging activity in

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both hydrophilic and lipophilic fractions (RSAHF and RSALF), with variations ranging from 59% to 120% for RSAHF and from 4% to 63% for RSALF. This study aims to preserve and enhance the value of local genetic resources and contribute to identify desirable traits for incorporation into breeding programs to develop high-quality, high-yielding landraces and elite lines.

Keywords: carotenoids; *Capsicum annuum* L.; capsaicinoids; tocopherols; antioxidant activity; vitamin C; phenolics

1. Introduction

Pepper (*Capsicum annuum* L.) is not only economically important but also ranks among the world's primary agricultural crops due to its nutritional value and versatile applications. The fruits are renowned for their rich content of secondary metabolites, which exhibit impressive bioactivities. In 2022, global pepper production totaled approximately 31 million tons, according to FAOSAT. In Tunisia, pepper cultivation covered around 16,000 hectares in 2023, yielding 420,000 tons, including 77,000 tons designated for processing [1]. The processed pepper sector in Tunisia produced 33,000 units in 2023, primarily exported to Europe and the United States [2]. Notably, Tunisia's renowned pepper paste, 'Harissa', was inscribed as UNESCO world heritage in 2022.

Hot or spicy peppers are savory food additives widely utilized and highly valued for their combination of color, taste, and pungency, attributable to capsaicinoids. Pepper fruits are consumed in various forms: either fresh, as immature green or mature red fruits, or processed into a range of products, including pastes, jams, paprika powders, and oleoresins. The characteristic vivid red color of *Capsicum* fruits is principally due to the pigments capsanthin and capsorubin. Recently, pepper fruits have gained recognition as natural sources of various bioactive compounds associated with reducing the risk of developing several chronic diseases due to their radical scavenging and antioxidant properties [3–5].

In recent decades, genetic erosion in pepper has increased dramatically, exacerbated by the widespread adoption of commercial cultivars and hybrids vulnerable to biotic and abiotic stresses, which require intensive inputs [6,7]. As a result, climate change, global warming, and rising food demands underscore the urgent need to recover, characterize, and valorize local pepper landraces for the benefit of plant breeders [7,8]. Pepper landraces are local genotypes selected by farmers and adapted over time to the specific agro-climatic conditions under which they have been long cultivated and maintained [9]. Landraces represent a critical repository and safety valve of genetic diversity, owing to their confirmed distinctive traits, including tolerance/resistance to abiotic stress and superior flavor and fruit quality compared to widely grown genotypes in different parts of the world [10–12]. Therefore, desirable traits can be introgressed into suitable and resilient new cultivars to cope with a constantly and rapidly changing climate [11]. Traditional pepper landraces have also proven to be well suited for various emerging farming systems, including dry-farming, low-input practices, organic cultivation, and urban agriculture [10].

Previously, significant variability has been reported and documented among pepper landraces and ancient genotypes from different geographical locations in terms of yields, physico-chemical traits [13,14], disease resistance [15], and fruit functional quality. This includes carotenoids, capsaicinoids, phenols, vitamins, and antioxidant activity assessed using different analytical methods [16–26]. Besides, increasing reports comparing pepper landraces have noted that certain landraces exhibit favorable horticultural traits, high functional quality, and yields comparable to currently available commercial hybrids, particularly under the ongoing climatic changes [6–9,15–17]. Using such genotypes for fresh consumption or processing, especially in rural areas where they are cultivated, has the potential to create new markets. These markets would cater to consumers willing to pay higher prices for fresh produce that meets high nutritional standards and offers improved taste, thereby increasing income for small farmers [7,9,27].

In Tunisia, as well as in other parts of the world, the large variability in agro-climatic regions has traditionally promoted the cultivation and preservation of numerous pepper landraces. Each landrace is typically named after the locality where it has been preferred and cultivated for many years, such as 'Kairouan', 'Nabeul', 'Mahdia', 'Jerid', and 'Baklouti'. However, some landraces are more widely distributed, such as 'Baklouti' and 'Beldi'. We hypothesize that most of these landraces exhibit desirable traits in terms of horticultural performance and functional quality, particularly when grown during hot seasons. Despite this, there is currently a lack of information about the horticultural performances, physico-chemical characteristics, and functional quality traits of these pepper landraces. Therefore, this study aimed to assess the primary agronomic traits and functional quality attributes of the main pungent pepper landraces grown under open field conditions over two consecutive growing seasons in 2022 and 2023.

2. Materials and Methods

The field experiments were conducted over two consecutive growing seasons in 2022 and 2023 at the Research and Experimental Station of Teboulba, Monastir, Tunisia (35.637178, 10.957276). The study utilized nine hot pepper landraces: 'Dhirat', 'Semmane', 'Jerid', 'Mahdia', 'Cayenne', 'Baklouti', 'Nabeul', 'Kairouan', and 'Beldi', which were selected and maintained by the Laboratory of Horticultural Crops at the National Agricultural Research Institute of Tunisia (Figure 1).

Figure 1. External appearance of different hot pepper landraces cultivated within the experimental field of Teboulba, Monastir, Tunisia.

For each growing year, sowing took place in plug-seedling trays during May, and seedlings were treated against *Pythium* spp. using 150 mL hL^{-1} Previcur Energy 840 SL (Bayer, Leverkusen, Germany). Pepper seedlings were hand transplanted at the beginning of June into a clay-loamy open field suitable for pepper cultivation. The soil was characterized by 22% clay, 17% loam, 16% sand, 14% calcareous substance, and 4.77% organic matter, with a pH of 7.92 and an EC of 1.10 mmho cm^{-1}. The spacing was approximately 0.4 m within the row and 0.7 m between rows, resulting in a density of about 3.5 plants per m^2. Irrigation was implemented using drippers with a flow rate of 4 L h^{-1}, positioned at 0.4 m intervals along the irrigation line. Drip irrigation was applied for 1–3 h at various day intervals, adjusted according to local evapotranspiration potential, prevailing climatic conditions, and crop coefficient. The cultivation schedule adhered to the practices employed by the research station and neighboring high-yield farmers. This method included the application of synthetic chemical fertilizers (99 kg N ha^{-1}, 30 kg P_2O_5 ha^{-1}, 147 kg K_2O ha^{-1}, 22 kg Cao ha^{-1}, 15 kg MgO ha^{-1}) added to the irrigation water through pump injection twice a week. Additionally, production methods involved manual weeding and controlling plant pathogens such as powdery mildew using 40 mL hL^{-1} Score 250 EC (Syngenta, Basel, Switzerland) and aphids using 75 mL hL^{-1} Decis EC 25 (Bayer Group, Leverkusen, Germany), applied once per cycle.

The experimental design employed a randomized complete block with three replicates (blocks) each year. Throughout the growing seasons of 2022 and 2023, the average temperature ranged between 25–33 °C and 20–34 °C, respectively. Relative humidity varied between 55–88% in 2022 and 56–90% in 2023, while rainfall ranged from 0–5 mm and 0–15 mm during the respective years (Figure 2).

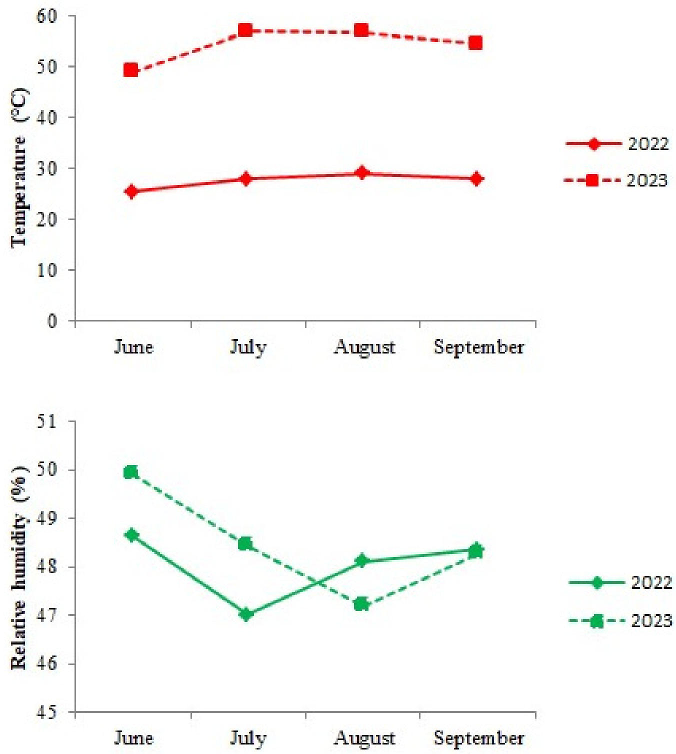

Figure 2. Temperature (°C) and relative humidity (%) data recorded by the Teboulba, Monastir weather station, the closest to the field trials. The reported values cover the entire pepper landrace growing seasons of 2022 and 2023.

2.1. Fruit Sampling

Pepper fruits were harvested from each plant in the rows and the middle of each plant at the red-ripe stage, which occurs approximately at the end of August and the beginning of September. Healthy, fresh pepper fruits were handpicked from each block and promptly transported to the laboratory. Triplicate sampling was conducted each year upon reaching the red-ripe stage. For each growing season, yield performance was estimated based on data from 20 plants per replicate. Quality trait analyses were conducted at least three times, starting from sub-samples of four independent harvests. The selected pepper fruits were thoroughly washed with deionized water and then cut into small pieces. These pieces were homogenized using a laboratory blender (Waring Laboratory Science, Torrington, CT, USA), and the resulting homogenates were stored at −20 °C. The homogenates were used within a few days to assess capsaicinoids, tocopherols, carotenoids, vitamin C, total phenolics content, and antioxidant activity, minimizing potential nutrient degradation.

2.2. Evaluation of the Main Agronomic Characteristics

Yield was assessed by determining the weight of fruits per plant, expressed as grams of fresh weight (fw)·plant^{-1}. The average fruit weight was calculated by dividing the weight of a random sample of pepper fruits by the number of fruits within the sample, and it was expressed in grams of fresh weight. Fruit length was measured using a Vernier caliper. Soluble solids concentration was determined by placing a small sample of blended pepper juice on the prism of an Atago PR-100 digital refractometer equipped with automatic temperature adjustment. Titratable acidity was measured as a percentage of citric acid after titrating the diluted pepper juice with a 0.1 M sodium hydroxide solution until a pH of 8.1 was reached. Redness (a*) and yellowness (b*) were estimated using a Minolta chroma mether CR-400 (Konica Minolta, Tokyo, Japan), and the ratio (a*/b*) was subsequently calculated [28].

2.3. Determination of Capsaicinoid Content

Capsaicinoid content was determined following the methods of Daood et al. [29] and Duah et al. [3]. Briefly, three grams of pepper homogenate were crushed with quartz sand in a crucible mortar. Then, 50 mL of analytical-grade methanol was added, and the mixture was transferred into a 100 mL Erlenmeyer flask fitted with a stopper. After three minutes of ultrasonication, the mixture was filtered through Whatman No. 1 paper. The filtrate was further filtered using a 0.20 µm PTFE syringe filter (Chromfil Xtra, Macherey-Nagel, Düren, Germany) into vials after being diluted ten times (9:1 by vol.). Using an Eppendorf pipette, 1 mL of methanol and 1 mL of the filtrate (from the syringe filter) were further diluted into vials. HPLC separation of capsaicinoids was performed on a Purospher C18, 2.7 µm, 150 × 4.6 mm column with isocratic elution using 48:52 water-acetonitrile at a flow rate of 0.8 mL min^{-1}. The compounds were detected fluorometrically, with exitation at 285 nm and emission at 320 nm. Capsaicinoids were identified and quantified based on the retention times (Figure 3) and calibration curves of external standards (capsaicin, dihydrocapsaicin, and nordihydocapsaicin), with their content expressed as mg kg^{-1} fw.

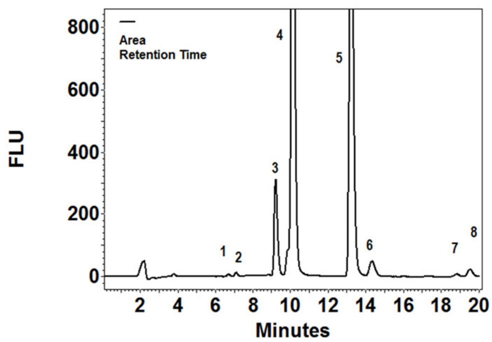

Figure 3. HPLC profile of capsaicinoids from pungent red pepper fruit. Peak identification: 1: nornorcapsaicin, 2: norcapsaicin, 3: nordihydocapsaicin, 4: capsaicin, 5: dihydrocapsaicin, 6: dihydrocapsaicin isomer, 7: homodihydrocapsaicin-1, 8: homodihydrocapsaicin-2.

2.4. Determination of Carotenoid and Tocopherol Content

Carotenoids and tocopherols were simultaneously extracted and quantified following the protocols of Nagy et al. [30] and Duah et al. [3]. Approximately 2.5 g of homogenized pepper samples from different landraces were ground in a crucible mortar with quartz sand and 20 mL of analytical-grade methanol. The homogenate was then transferred into a separation funnel and diluted with 60 mL of a methanol mixture (1:5 *v*/*v*), followed by vigorous shaking. After the addition of 1 mL of distilled water, the mixture was shaken again to ensure thorough mixing. The pigmented phase was collected and passed through filter paper containing anhydrous sodium sulfate. The filter was subsequently washed with 5 mL of dichloroethane. The solvent was then evaporated at 70 °C under vacuum. The resulting residue was diluted with 5 mL of methanol, sonicated, and filtered through a 0.22 µm PTFE membrane syringe. Finally, the prepared sample was injected into the HPLC column for analysis.

An HPLC system (Hitachi Chromaster, Tokyo, Japan) equipped with a 5110 Pump, a 5210 Auto Sampler, a 5430 Diode Array Detector, and a 5440 Fluorescence Detector was used for compound analysis. Carotenoids and tocopherols were separated using a Nucleodur C18, 3 µm, 240 × 4.6 mm column (Machery Nagel, Dürer, Germany) with a gradient elution starting from 7% water in methanol, transitioning to methanol/2-propanol-acetonitrile (10:90, *v*/*v*) at a constant flow rate of 0.6 mL min^{-1}. Carotenoids were detected within the wavelength range of 190 to 700 nm, and their concentrations were expressed as mg kg^{-1} fw. Individual carotenoid peaks were identified based on the retention times of external standards (Figure 4). All chemicals, including carotenoid standards, analytical and HPLC-grade solvents, were purchased from VWR (Budapest, Hungary, and Darmstadt, Germany). Tocopherols were detected using a fluorescent detector with excitation and emission wavelengths set at 295 nm and 325 nm, respectively. Isomers of α-, β-, and γ-tocopherols were identified using external standards (Sigma-Aldrich, Budapest, Hungary) (Figure 5), and their contents were expressed as mg kg^{-1} fw.

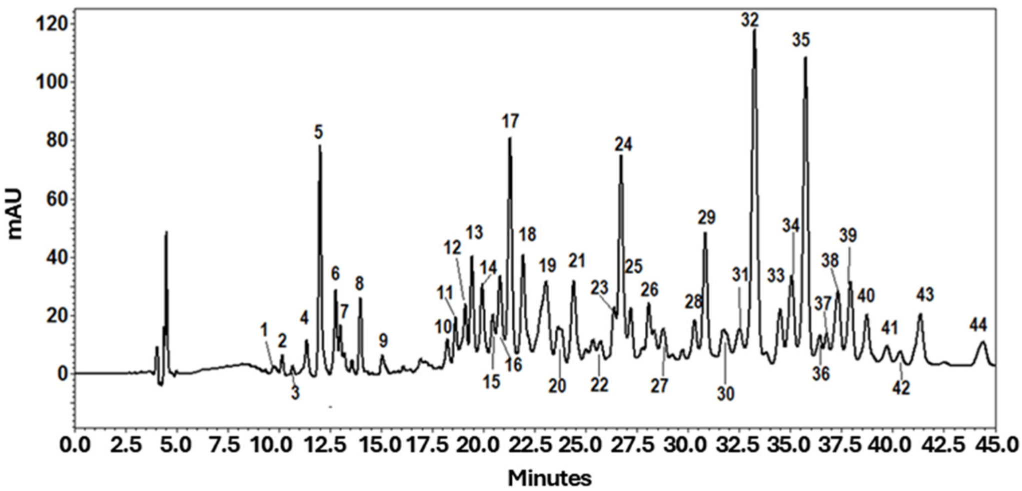

Figure 4. HPLC profile of carotenoids from pungent red pepper fruit. Peak identification: 1: capsorubin, 2: 2,5-dihydroxykarpaxanthin, 3: violaxanthin, 4: capsanthin epoxide, 5: capsanthin, 6: cucurbitaxanth, 7: *cis*-capsanthin, 8: zeaxanthin, 9: β-cryptocapsin, 10: β-cryptocapsin ME, 11: cucurbitaxanthin ME, 12: β-cryptoxanthin, 13: capsanthin epoxide ME, 14: capsanthin ME, 15: antheraxanthin ME, 16: *cis*-capsanthin ME, 17: capsanthin ME, 18: antheraxanthin ME, 19: *cis*-capsanthin ME, 20: capsanthin ME, 21: antheraxanthin ME, 22: β-cryptocapsin ME, 23: ζ-carotene, 24: β-carotene, 25: capsorubin DE, 26: *cis*-β-carotene, 27: capsorubin DE, 28: *cis*-capsorubin DE, 29: capsanthin DE, 30: β-cryptoxanthin ME, 31: *cis*-capsanthin DE, 32: capsanthin DE, 33: capsanthin DE, 34: *cis*-capsanthin DE, 35: capsanthin DE, 36: zeaxanthin DE, 37: *cis*-capsanthin DE, 38: *cis*-capsanthin DE, 39: capsanthin DE, 40: zeaxanthin DE, 41: *cis*-capsanthin DE, 42: *cis*-capsanthin DE, 43: zeaxanthin DE, 44: *cis*-zeaxanthin DE. (ME = monoester; DE = diester).

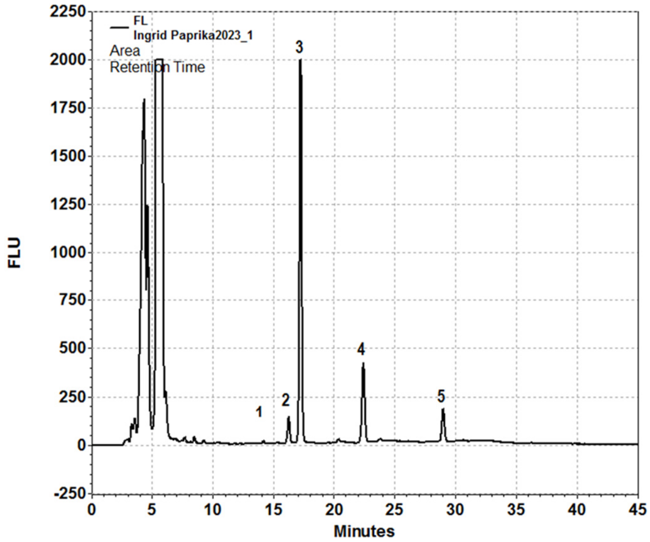

Figure 5. HPLC profile of tocopherols from pungent red pepper fruits Peak identification. 1: mixture of β- and γ-tocopherol, 2: α-tocopherol hydroquinone, 3: α-tocopherol, 4: α-tocopherol acetate, 5: α-tocopherol ester.

2.5. Determination of Total Vitamin C Content

Total vitamin C (AsA + DHA) was extracted and quantified from 0.2 g samples of homogeneous pepper juice, according to Kampfenkel et al. [31]. Absorbance was measured using a Cecil BioQuest CE 2501 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK) at 525 nm and expressed in mg 100 g^{-1} fw. The standard curve for AsA was linear between 0 and 750 µmol.

2.6. Determination of Total Phenolic Content

The extraction and measurement of total phenolic content followed the method outlined by Martínez-Valverde et al. [32]. Briefly, 0.1 g of pepper homogenate was combined with 5 mL of 80% methanol and 50 µL of 37% HCl, then extracted for 2 h at 4 °C and 300 rpm. After extraction, the mixture was centrifuged for 20 min at 10,000× g. A 125 µL sample of the supernatant was transferred to a test tube and mixed with 500 µL distilled water, followed by the addition of 125 µL Folin–Ciocalteu reagent. After 3 min, 1250 µL of 7% sodium carbonate solution was added, and the final volume was adjusted to 3 mL with distilled water. Each sample was allowed to stand for 90 min at ambient conditions, and the absorbance was measured at 760 nm against a blank using a Cecil BioQuest CE 2501 spectrophotometer (Ceil Instruments Ltd., Cambridge, UK). Total phenolic content was expressed as mg of gallic acid equivalent (GAE) kg^{-1} fw.

2.7. Measurement of the Radical Scavenging Activity

The radical scavenging activity of the hydrophilic and lipophilic fractions (RSAHF and RSALF, respectively) was assessed using the Trolox Equivalent Antioxidant Capacity (TEAC) method, following Miller and Rice-Evans [33]. To extract hydrophilic and lipophilic antioxidants, 0.1 g of pepper homogenate was mixed with methanol (50%) or acetone (50%), respectively, at 4 °C under continuous shaking at 300 rpm for 12 h. The samples were then centrifuged at 10,000× g for 7 min. The collected supernatants were used to determine antioxidant activity at 734 nm using a Cecil BioQuest CE 2501 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK). Antioxidant activity was calculated and expressed as µM of Trolox 100 g^{-1} of fw.

2.8. Statistical Analysis

Since no significant differences were observed between growing years, the results were presented as the mean value ± standard error of six independent replicates (n = 6).

Each landrace was represented by six different batches of fruits, with each batch consisting of at least 3 kg from the same harvesting lot, analyzed separately.

Variation affecting the agronomic and functional quality of different pepper landraces was assessed by analysis of variance (ANOVA). Significant differences between means were determined using the Least Significant Difference (LSD) test ($p < 0.05$). Statistical analyses were performed using IBM SPSS Statistics software for Windows, Version 21.0. (IBM Corp., Armonk, NY, USA). Correlations among variables were determined using Pearson's correlation coefficient (r) to assess the strength and direction of the relationships between different agronomic and functional quality traits. This helps in understanding how different variables influence each other, which can be crucial for breeding programs and agricultural practices. Additionally, Principal Component Analysis (PCA) for the main bioactive classes of molecules (total contents) identified in the various hot pepper landraces was performed using the Past software, Version 4.17 (University of Oslo, Oslo, Norway).

3. Results

3.1. Agronomic and Physico-Chemical Traits

The main agronomic attributes and physicochemical traits of different hot pepper landraces are reported in Tables 1 and 2, respectively. The studied agronomic attributes and physicochemical traits exhibited significant variations among the tested genotypes ($p < 0.05$). Under open field conditions, the pepper genotypes displayed determinate growth habits, showing vigorous growth with excellent foliage coverage. Earliness varied from early for 'Dhirat', 'Beldi', and 'Jerid' to very late for 'Cayenne'. The fruit shape was triangular for 'Jerid' and 'Baklouti' and elongate for the remaining landraces (Figure 1). All genotypes exhibited satisfying yields, ranging from 1163.25 g plant^{-1} in 'Jerid' to 1841.67 g plant^{-1} in 'Kairouan', indicating good productivity despite ongoing climatic changes and high temperatures recorded during the growth period. The average fruit weight ranged from 6.5 g in 'Jerid' to 37.75 g in 'Kairouan', with 'Semmane', 'Beldi', 'Nabeul', 'Mahdia', and 'Baklouti' showing similar weights appreciated by fresh market consumers.

Table 1. Agronomic attributes of pungent pepper landraces grown under open field conditions during two growing seasons and harvested at the red-ripe stage. Different superscript letters indicate significant differences among landraces at the 0.05 significance level, according to the LSD test. Data are expressed as the mean ± S.E. of six replicates (2022 and 2023 sampling data).

Landraces	Earliness	Fruit Shape	Intended Use	Yeald per Plant (g Plant^{-1})	Average Fruit Length (cm)	Average Fruit Weight (g)
Dhirat	Early	Elongate	Fresh market	1480.58 ± 36.11 ^{d}	11.67 ± 1.11 ^{de}	22.25 ± 0.83 ^{d}
Semmane	Late	Elongate	Fresh market	1687.25 ± 40.00 ^{c}	17.33 ± 1.78 ^{abc}	34.33 ± 1.11 ^{bc}
Beldi	Early	Elongate	Fresh market/Processing	1755.08 ± 56.22 ^{c}	18.00 ± 2.00 ^{abc}	34.33 ± 1.11 ^{bc}
Nabeul	Late	Elongate	Fresh market	1720.83 ± 56.22 ^{c}	18.67 ± 2.44 ^{ab}	35.58 ± 0.89 ^{bc}
Jerid	Early	Triangular	Pickling	1163.25 ± 47.17 ^{e}	5.33 ± 1.56 ^{f}	6.5 ± 0.5 ^{f}
Mahdia	Late	Elongate	Fresh market	1634.92 ± 22.55 ^{c}	13.67 ± 1.11 ^{cde}	33.83 ± 0.39 ^{c}
Cayenne	Very late	Elongate	Pickling	1997.33 ± 11.61 ^{a}	9.33 ± 1.56 ^{ef}	14.25 ± 0.5 ^{e}
Kairouan	Late	Elongate	Fresh market/processing	1841.67 ± 17.94 ^{b}	20.33 ± 2.22 ^{a}	37.75 ± 0.17 ^{a}
Baklouti	Late	Triangular	Fresh market/processing	1644.08 ± 29.61 ^{c}	14.67 ± 1.78 ^{bcd}	35.75 ± 0.17 ^{b}

Soluble solids content (Table 2) ranged from 9.1° Brix in 'Semmane' to 12.2° Brix in 'Beldi', with the high content in 'Beldi' making it suitable for processing. pH values spanned from 4.72 in 'Baklouti' to 5.6 in 'Cayenne', while titratable acidity ranged from 0.16% in 'Semmane' to 0.31% in 'Nabeul', with 'Mahdia' showing similar acidity to 'Semmane'. The color index (a*), indicating the intensity of red color, ranged from 33.94 in 'Nabeul' to 41.35 in' Kairouan', while the color index (b*), indicating yellowness, ranged from 39.32 in 'Nabeul' to 44.81 in 'Semmane'. Consequently, the (a*/b*) ratio, useful for characterizing the quality and maturity of pepper pods, spanned from 0.87 in 'Nabeul' to 0.97 in 'Cayenne'.

Table 2. Physicochemical properties of pungent pepper landraes grown under open field conditions during two growing seasons and harvested at red-ripe stage. Different superscript letters indicate significant differences among landraces at the 0.05 significance level, according to the LSD test. Data are expressed as the mean ± S.E. of six replicates (2022 and 2023 sampling data).

Landraces	Soluble Solids (°Brix)	pH	Titratable Acidity (%)	a*	b*	a*/b*
Dhirat	10.6 ± 0.3 [bc]	4.95 ± 0.01 [c]	0.21 ± 0.01 [bcd]	36.68 ± 0.76 [bc]	40.47 ± 0.04 [bc]	0.91 ± 0.02 [bcd]
Semmane	9.1 ± 0.1 [e]	4.93 ± 0.05 [cd]	0.16 ± 0.04 [f]	40.33 ± 1.27 [a]	44.81 ± 1.52 [a]	0.90 ± 0.01 [bcd]
Beldi	12.2 ± 0.36 [a]	4.97 ± 0.02 [c]	0.19 ± 0.01 [def]	40.9 ± 1.38 [a]	43.59 ± 0.76 [a]	0.94 ± 0.01 [abc]
Nabeul	10.9 ± 0.9 [bc]	4.85 ± 0.02 [cd]	0.31 ± 0.03 [a]	33.94 ± 1.23 [c]	39.32 ± 0.94 [c]	0.87 ± 0.03 [d]
Jerid	10.63 ± 0.06 [bc]	5.02 ± 0.01 [c]	0.22 ± 0.02 [bcd]	40.57 ± 0.98 [a]	42.9 ± 1.20 [ab]	0.95 ± 0.00 [ab]
Mahdia	10.6 ± 0.53 [bc]	5.35 ± 0.00 [b]	0.16 ± 0.00 [ef]	34.82 ± 1.43 [c]	38.36 ± 1.87 [c]	0.91 ± 0.01 [bcd]
Cayenne	9.6 ± 0.1 [de]	5.60 ± 0.35 [a]	0.20 ± 0.30 [cde]	38.81 ± 1.2 [ab]	39.82 ± 1.16 [c]	0.97 ± 0.00 [a]
Kairouan	10.1 ± 0.7 [cd]	5.02 ± 0.00 [c]	0.24 ± 0.03 [bc]	41.35 ± 0.59 [a]	44.48 ± 1.68 [a]	0.93 ± 0.02 [abc]
Baklouti	11.4 ± 0.2 [ab]	4.72 ± 0.01 [e]	0.25 ± 0.01 [b]	35.43 ± 0.77 [c]	39.58 ± 0.53 [c]	0.89 ± 0.03 [cd]

3.2. Functional Quality Traits

3.2.1. Capsaicinoid Content

The content of individual capsaicinoids and their total level in different hot pepper landraces harvested at the red-ripe stage is presented in Table 3. The content of individual capsaicinoids and their total content exhibited significant variation among pepper landraces ($p < 0.05$). Irrespective of genotype, capsaicin and dihydrocapsaicin were the main capsaicinoids detected in the hot pepper landraces under investigation, accounting for between 44% and 91% of the total capsaicinoids. Nordihydrocapsaicin comprised up to 15.4% of total capsaicinoids, ranging from 0.02 μg g^{-1} fw in 'Nabeul' to 23.12 μg g^{-1} fw in 'Baklouti', demonstrating substantial variability among the investigated landraces. Similarly, homodihydrocapsaicin ranged from 0.92 μg g^{-1} fw in 'Kairouan' to 3.9 μg g^{-1} fw in 'Jerid'. Homocapsaicin was detected in 'Dhirat', 'Semmane', 'Nabeul', and 'Cayenne' only, with values ranging from 0.007 μg g^{-1} fw in 'Nabeul' to 1.26 μg g^{-1} fw in 'Dhirat'. Capsaicin content ranged from 0.54 μg g^{-1} fw in 'Kairouan' to 108.6 μg g^{-1} fw in 'Jerid', while dihydrocapsaicin ranged from 0.49 μg g^{-1} fw in 'Mahdia' to 68.36 μg g^{-1} fw in 'Baklouti'. Overall, total capsaicinoids exhibited considerable variability among the tested pepper landraces, ranging from 1.81 μg g^{-1} fw in 'Kairouan' to 194.02 μg g^{-1} fw in 'Jerid'.

Table 3. Individual capsaicinoids comprising of nordihydrocapsaicin, homocapsaicin, homodihydrocapsaicin, capsaicin, dihydrocapsaicin, and total capsaicinoids (μg g^{-1} fw) from pungent red pepper fruits. Different superscript letters indicate significant differences among landraces at the 0.05 significance level, according to the LSD test. Values represent the mean ± standard error of six replicates (2022 and 2023 sampling data).

Landraces	Capsaicin (μg g^{-1} fw)	Dihydrocapsaicin (μg g^{-1} fw)	Nordihydrocapsaicin (μg g^{-1} fw)	Homocapsaicin (μg g^{-1} fw)	Homodihydrocapsaicin (μg g^{-1} fw)	Total Capsaicinoids (μg g^{-1} fw)
Dhirat	67.36 ± 6.46 [c]	67.60 ± 7.65 [a]	12.62 ± 1.28 [b]	1.26 ± 0.12 [a]	3.09 ± 0.15 [b]	151.94 ± 15.66 [b]
Semmane	19.84 ± 0.18 [d]	12.48 ± 0.16 [b]	1.60 ± 0.01 [d]	1.15 ± 0.01 [b]	1.58 ± 0.04 [e f]	36.65 ± 0.32 [c]
Beldi	0.72 ± 0.26 [e]	2.37 ± 1.06 [c]	0.07 ± 0.01 [e]	n.d.	2.52 ± 0.08 [c]	5.68 ± 1.41 [d]
Nabeul	0.78 ± 0.06 [e]	0.83 ± 0.09 [c]	0.02 ± 0.02 [e]	0.01 ± 0.00 [c]	2.12 ± 0.07 [c d]	3.76 ± 0.03 [d]
Jerid	108.6 ± 0.67 [a]	68.21 ± 0.47 [a]	12.99 ± 0.13 [b]	n.d.	3.90 ± 0.05 [a]	193.71 ± 1.32 [a]
Mahdia	1.07 ± 0.01 [e]	0.49 ± 0.01 [d]	0.12 ± 0.00 [e]	n.d.	1.32 ± 0.08 [fg]	3.01 ± 0.09 [d]
Cayenne	19.97 ± 0.46 [d]	12.40 ± 0.78 [c]	6.48 ± 0.81 [c]	0.16 ± 0.00 [c]	3.11 ± 0.47 [b]	42.12 ± 2.52 [c]
Kairouan	0.54 ± 0.14 [e]	0.29 ± 0.03 [d]	0.06 ± 0.01 [e]	n.d.	0.92 ± 0.01 [g]	1.81 ± 0.19 [d]
Baklouti	93.25 ± 1.79 [b]	68.36 ± 1.92 [d]	23.12 ± 0.46 [a]	n.d.	1.94 ± 0.03 [de]	186.67 ± 4.13 [a]

n.d.: not detected.

3.2.2. Carotenoid Content

Individual carotenoid content, including capsorubin, violaxanthin, lutein, zeaxanthin, β-cryptoxanthin, and β-carotene, as well as total carotenoid content in different hot pepper landraces harvested at the red-ripe stage are presented in Table 4. Significant variations ($p < 0.05$) were observed in the contents of capsorubin, violaxanthin, lutein, zeaxanthin, β-cryptoxanthin, and β-carotene among the investigated hot pepper landraces. Based on their content, zeaxanthin, β-cryptoxanthin, and β-carotene were the main carotenoids detected in red hot pepper landraces, accounting for between 74% and 93% of the total content of carotenoids. The content of β-cryptoxanthin ranged from 7.32 μg g^{-1} fw in 'Nabeul' to 27.11 μg g^{-1} fw in 'Semmane', while zeaxanthin content ranged from 7.24 μg g^{-1} fw in 'Beldi' to 27.8 μg g^{-1} fw in 'Semmane'. β-Carotene, the predominant carotenoid, ranged from 14.32 μg g^{-1} fw in 'Kairouan' to 113.68 μg g^{-1} fw in 'Semmane', reflecting significant variability. The contribution of β-carotene to the total carotenoid content ranged from 26.1% in 'Kairouan' to almost 70% in 'Semmane', suggesting a significant contribution to the recommended daily intake of vitamin A content.

Table 4. Individual carotenoids comprising capsorubin, violaxanthin and lutein, zeaxanthin, β-cryptoxanthin, β-carotene, and total carotenoids (μg g^{-1} fw) from pungent red pepper fruits. Different superscript letters indicate significant differences among landraces at the 0.05 significance level, according to the LSD test. Values represent the mean ± standard error of six replicates (2022 and 2023 sampling data).

Landraces	Capsorubin (μg g^{-1} fw)	Violaxanthin (μg g^{-1} fw)	Lutein (μg g^{-1} fw)	Zeaxanthin (μg g^{-1} fw)	β-Cryptoxanthin (μg g^{-1} fw)	β-Carotene (μg g^{-1} fw)	Total Carotenoids (μg g^{-1} fw)
Dhirat	2.27 ± 0.12 ^{c}	1.58 ± 0.07 ^{cd}	4.55 ± 2.90 ^{b}	10.41 ± 1.01 ^{bc}	8.86 ± 0.41 ^{cd}	25.44 ± 16.80 ^{de}	53.12 ± 18.10 ^{d}
Semmane	2.95 ± 0.32 ^{bc}	2.36 ± 0.14 ^{b}	0.62 ± 0.04 ^{c}	27.80 ± 3.16 ^{a}	27.11 ± 3.17 ^{a}	113.68 ± 14.27 ^{a}	174.52 ± 21.09 ^{a}
Beldi	3.91 ± 0.09 ^{ab}	2.10 ± 0.08 ^{bc}	1.54 ± 0.11 ^{c}	7.24 ± 3.69 ^{c}	12.72 ± 0.27 ^{bc}	43.44 ± 2.07 ^{cd}	70.94 ± 1.26 ^{cd}
Nabeul	2.60 ± 0.14 ^{c}	1.51 ± 0.03 ^{cd}	0.96 ± 0.04 ^{c}	9.39 ± 0.09 ^{bc}	7.32 ± 0.12 ^{d}	32.97 ± 1.15 ^{cde}	54.75 ± 1.28 ^{d}
Jerid	4.89 ± 0.30 ^{a}	5.14 ± 0.28 ^{a}	0.88 ± 0.06 ^{c}	16.04 ± 1.87 ^{b}	26.76 ± 2.50 ^{a}	93.69 ± 7.69 ^{a}	147.40 ± 12.70 ^{a}
Mahdia	4.43 ± 0.43 ^{a}	1.48 ± 0.16 ^{cd}	7.19 ± 4.23 ^{a}	11.83 ± 1.14 ^{bc}	9.10 ± 0.81 ^{cd}	30.30 ± 3.39 ^{de}	64.33 ± 10.16 ^{d}
Cayenne	2.10 ± 0.01 ^{c}	1.65 ± 0.16 ^{bcd}	7.63 ± 4.86 ^{a}	14.98 ± 0.04 ^{b}	14.55 ± 0.06 ^{b}	55.68 ± 0.29 ^{bc}	96.60 ± 4.64 ^{bc}
Kairouan	5.01 ± 0.08 ^{a}	1.38 ± 0.01 ^{cd}	5.63 ± 3.18 ^{b}	10.77 ± 0.24 ^{bc}	8.83 ± 0.02 ^{cd}	14.32 ± 8.98 ^{e}	45.94 ± 5.97 ^{d}
Baklouti	1.85 ± 1.23 ^{c}	1.06 ± 0.71 ^{cd}	8.25 ± 5.32 ^{a}	7.35 ± 0.35 ^{c}	12.77 ± 1.32 ^{bc}	70.97 ± 2.69 ^{b}	102.25 ± 5.53 ^{b}

Capsorubin, violaxanthin, and lutein were detected at lower levels compared to zeaxanthin, β-cryptoxanthin, and β-carotene. Capsorubin content ranged from 1.85 μg g^{-1} fw in 'Baklouti' to 5.01 μg g^{-1} fw in 'Kairouan', while violaxanthin ranged from 1.06 μg g^{-1} fw in 'Baklouti' to 5.14 μg g^{-1} fw in 'Jerid'. Lutein content varied from 0.62 μg g^{-1} fw in 'Semmane' to 8.25 μg g^{-1} fw in 'Baklouti'. Regarding total carotenoids, the landrace 'Semmane' exhibited the highest content with 113.68 μg g^{-1} fw, mainly influenced by β-carotene level.

3.2.3. Tocopherol Content

The content of α-, β-, and γ-tocopherol isomers and their total content in different hot pepper landraces harvested at the red-ripe stage are presented in Table 5. The content of different tocopherol isomers and their total varied significantly ($p < 0.05$) between the studied hot pepper landraces harvested at the red-ripe stage. α-Tocopherol content ranged from 19.03 μg g^{-1} fw in 'Kairouan' to 30.93 μg g^{-1} fw in 'Beldi'. The content of β-tocopherol showed lower variability, ranging from 0.11 μg g^{-1} fw in 'Mahdia' to 0.66 μg g^{-1} fw in 'Jerid'. The γ-tocopherol isomer was detected in 'Dhirat', 'Semmane', 'Beldi', 'Nabeul', 'Jerid', and 'Mahdia', albeit in very low amounts ranging from 0.021 μg g^{-1} fw in 'Dhirat' to 0.18 μg g^{-1} fw in 'Beldi'. The total tocopherol content was highest in 'Beldi' and 'Jerid', ranging from 19.38 μg g^{-1} fw in 'Kairouan' to 31.36 μg g^{-1} fw in 'Beldi'. Consequently, the total tocopherol content was predominantly composed of α-Tocopherol, with the other isomers present in trace amounts across all analyzed pepper landraces.

Table 5. Tocopherol isomers and their total content (µg g^{-1} fw) from pungent red pepper fruits. Different superscript letters indicate significant differences among landraces at the 0.05 significance level, according to the LSD test. Values represent the mean ± standard error of six replicates (2022 and 2023 sampling data).

Landraces	α-Tocopherol (µg g^{-1} fw)	β-Tocopherol (µg g^{-1} fw)	γ-Tocopherol (µg g^{-1} fw)	Total Tocopherols (µg g^{-1} fw)
Dhirat	19.96 ± 2.11 ^{c}	1.15 ± 0.08 ^{a}	0.02 ± 0.01 ^{d}	21.13 ± 2.20 ^{c}
Semmane	22.84 ± 1.87 ^{bc}	0.33 ± 0.03 ^{de}	0.15 ± 0.02 ^{b}	23.32 ± 1.92 ^{bc}
Beldi	30.93 ± 1.23 ^{a}	0.25 ± 0.02 ^{ef}	0.18 ± 0.02 ^{a}	31.36 ± 1.26 ^{a}
Nabeul	26.92 ± 1.03 ^{ab}	0.59 ± 0.03 ^{bc}	0.14 ± 0.01 ^{b}	27.65 ± 1.07 ^{ab}
Jerid	30.55 ± 5.09 ^{a}	0.66 ± 0.11 ^{b}	0.03 ± 0.02 ^{d}	31.24 ± 5.21 ^{a}
Mahdia	19.67 ± 0.84 ^{e}	0.11 ± 0.02 ^{f}	0.07 ± 0.01 ^{c}	19.85 ± 0.86 ^{c}
Cayenne	22.34 ± 0.62 ^{bc}	0.42 ± 0.03 ^{de}	n.d.	22.77 ± 0.62 ^{bc}
Kairouan	19.03 ± 0.29 ^{c}	0.36 ± 0.01 ^{de}	n.d.	19.38 ± 0.30 ^{c}
Baklouti	19.50 ± 2.04 ^{c}	0.46 ± 0.10 ^{cd}	n.d.	19.96 ± 2.14 ^{c}

n.d.: not detected.

3.2.4. Total Vitamin C Content

Total vitamin C content in different hot pepper landraces harvested at the red-ripe stage is presented in Figure 6. The total vitamin C content varied significantly ($p < 0.05$) among pepper landraces. Across the study, total vitamin C ranged from 132 mg 100 g^{-1} fw in 'Mahdia' to 200 mg 100 g^{-1} fw in 'Nabeul', with variations ranging from 4% to 51% compared to 'Mahdia', indicating relatively low variability among the analyzed pepper landraces. Landraces 'Jerid', 'Dhirat', and 'Cayenne' showed statistically similar intermediate total vitamin C levels.

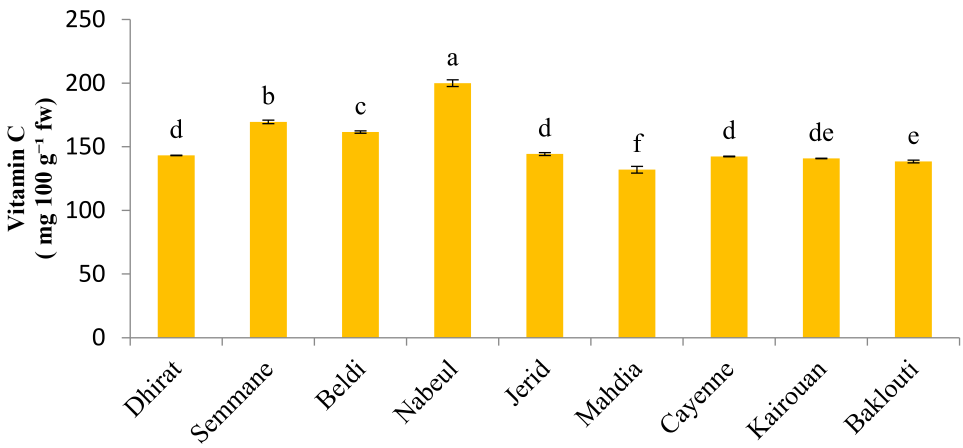

Figure 6. Total vitamin C content (mg 100 g^{-1} fw) from pungent red pepper fruits. Values represent the mean ± standard error of six replicates (2022 and 2023 sampling data). Bars marked with the same letters are not significantly different (LSD Test, $p < 0.05$).

3.2.5. Total Phenols

Total phenolics in different hot pepper landraces harvested at the red-ripe stage are presented in Figure 7. The content of total phenols varied significantly ($p < 0.05$) among the analyzed hot pepper landraces. Total phenolics content ranged from 168.58 mg GAE kg^{-1} fw in 'Beldi' to 302.98 mg GAE kg^{-1} fw in 'Cayenne', with variations ranging from 22% to 80% compared to 'Beldi', indicating substantial variability among the analyzed pepper landraces. 'Dhirat', 'Semmane', 'Nabeul', 'Jerid', and 'Mahdia' demonstrated statistically similar intermediate total phenolic values.

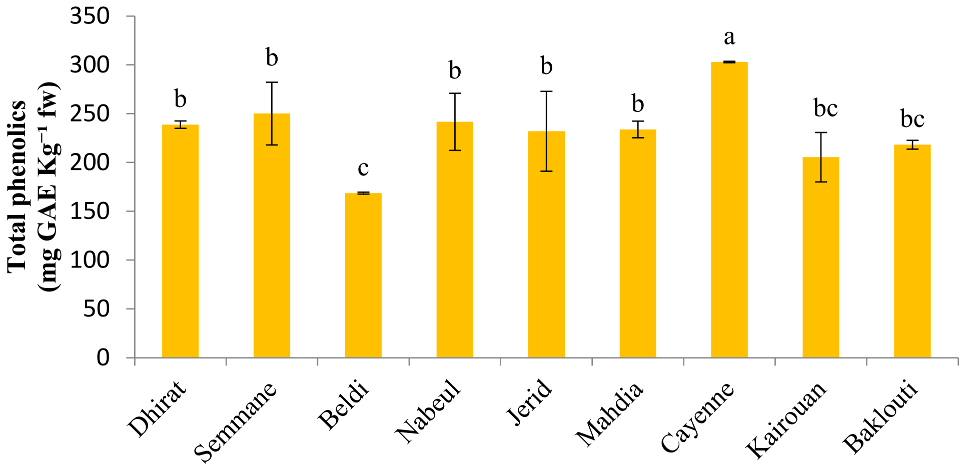

Figure 7. Total phenolics (mg GAE kg^{-1} fw) from pungent red pepper fruits. Values represent the mean ± standard error of six replicates (2022 and 2023 sampling data). Bars marked with the same letters are not significantly different (LSD Test, $p < 0.05$).

3.2.6. Radical Scavenging Activity

The RSAHF and RSALF measured in different hot pepper landraces are presented in Figure 8. The RSAHF and RSALF values varied significantly ($p < 0.05$) among the studied pepper landraces. RSAHF values ranged from 763.31 µM Trolox 100 g^{-1} fw in 'Baklouti' to 1680.27 µM Trolox 100 g^{-1} fw in 'Cayenne', with variations spanning from 59% to 120% compared to 'Baklouti'. Conversely, RSALF values ranged from 1043.85 µM Trolox 100 g^{-1} fw in 'Beldi' to 1707.28 µM Trolox 100 g^{-1} fw in 'Cayenne', exhibiting variations ranging from 4% to 63% compared to 'Beldi'. While the landrace 'Cayenne' displayed the highest values for both RSAHF and RSALF, 'Baklouti' statistically ranked the last for both traits.

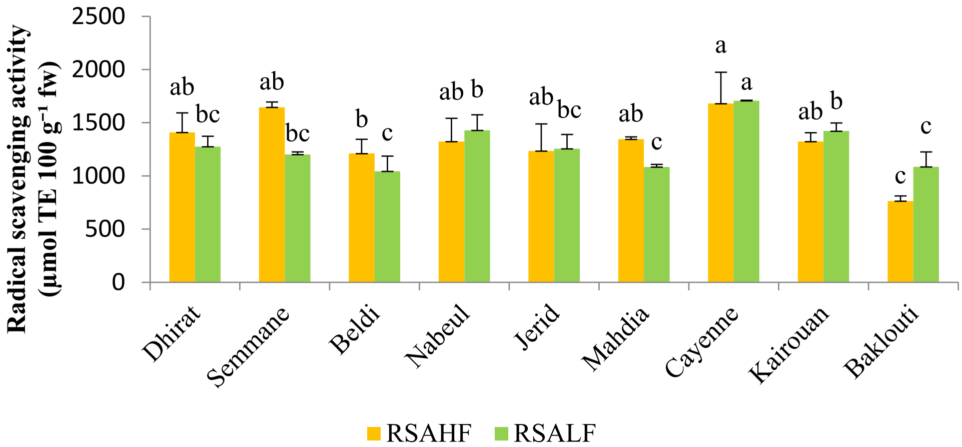

Figure 8. RSAHF and RSALF (µmol Trolox Equivalent 100 g^{-1} fw) from pungent red pepper fruits. Values represent the mean ± standard error of six replicates (2022 and 2023 sampling data). Bars with the same color marked with the same letters are not significantly different (LSD Test, $p < 0.05$).

3.3. Correlation Analysis

The Pearson correlation matrix between agronomic, physico-chemical, and functional quality attributes of the hot pepper landraces under investigation is presented in Figure 9. The relationships between the studied traits were examined using Pearson correlation analysis at $p < 0.01$. A significant positive correlation was observed between RSAHF and total phenolics (r = 0.603). Furthermore, the color indexes a* and the ratio (a*/b*) exhibited significant correlations with the content of various pungent pepper quality traits, including homodihydrocapsaicin (r = 0.476), total carotenoids (r = 0.320), β-cryptoxanthin (r = 0.286), violaxanthin (r = 0.415), α-tocopherol (r = 0.245), as well as RSAHF (r = 0.331) and RSALF (r = 0.360). These findings suggest the potential utility of color indexes in predicting key quality traits among the investigated parameters, particularly during breeding and selection, including large samples.

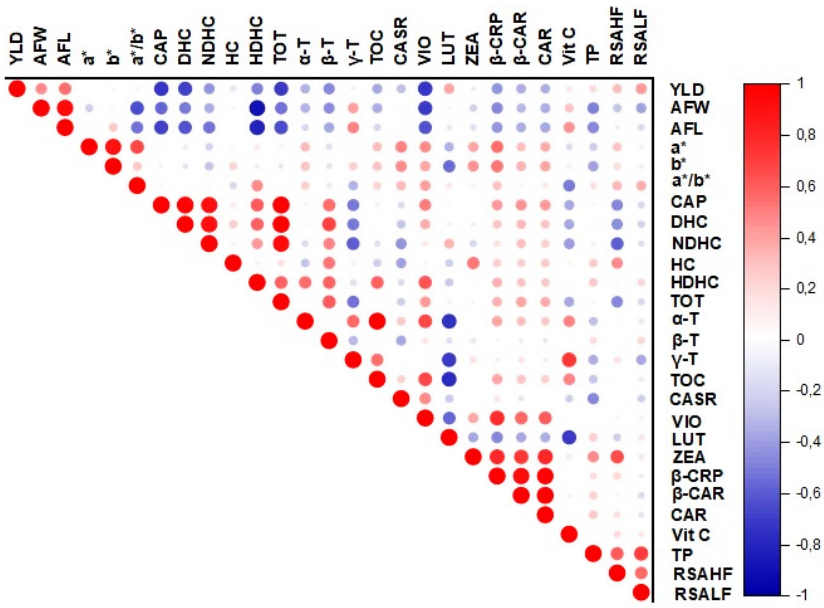

Figure 9. Pearson correlation matrix between agronomic, physico-chemical, and functional quality attributes of the hot pepper landraces under investigation. Reddish tones express higher positive correlations, whereas blueish tones indicate negative correlations. Larger circle diameters denote higher modules of the correlation coefficient (r). White intersections exhibit non-significant correlations (significance was set at p correlation). Abbreviations: YLD, yield per plant; AFW, average fruit weight; AFL, average fruit length; TA, titratable acidity; a*, redness; b*, yellowness; (a*/b*), a*/b* ratio; CAP, capsaicin; DHC, dihydrocapsaicin; NDHC, nor-dihydrocapsaicin; HC, homocapsaicin; HDHC, homodihydrocapsaicin; TOT, total capsaicinoids; α-T, α-tocopherol; β-T, β-tocopherol; γ-T, γ-tocopherol; TOC, total tocopherols; CASR, capsorubin; VIO, violaxanthin; LUT, lutein; ZEA, zeaxanthin; β-CRP, β-cryptoxanthin; β-CAR, β-carotene; CAR, total carotenoids; Vit C, Vitamin C; TP, total phenols; RSAHF, radical scavenging activity of the hydrophilic fraction antioxidant activity; RSALF, radical scavenging activity of the lipophilic fraction.

3.4. Principal Component Analysis (PCA)

The principal component analysis (PCA) biplot PC1 vs. PC2 and PC1 vs. PC3 of the main bioactive classes of molecules (total contents) identified in the different pepper genotypes under investigation is presented in Figure 10. To gain a deeper understanding of the relationships between hot pepper landraces, we conducted PCA using the biochemical data as input variables. The first three principal components explained 69.558% of the observed variation, with PC1 contributing 25.826%, PC2 contributing 23.482%, and PC3 contributing 20.076%. PC1 showed positive correlations with total phenols, vitamin C, total carotenoids, and total tocopherols, as well as RSAHF and RSALF, but it exhibited negative correlations with total capsaicinoids. Conversely, PC2 displayed positive correlations with total carotenoids, total phenols, total capsaicinoids, and RSAHF, but it showed negative correlations with total tocopherols, total vitamin C, and RSALF. PC3 positively correlated with total tocopherols, total carotenoids, total capsaicinoids, total phenols, and vitamin C, had no correlation with RSAHF, and showed a negative correlation with RSALF. Regardless of the growing year, 'Semmane' and 'Nabeul' consistently scored positively along PC1, while 'Mahdia' and 'Kairouan' consistently scored negatively along PC3. 'Baklouti' and 'Jerid' exhibited strong correlations with total capsaicinoid contents.

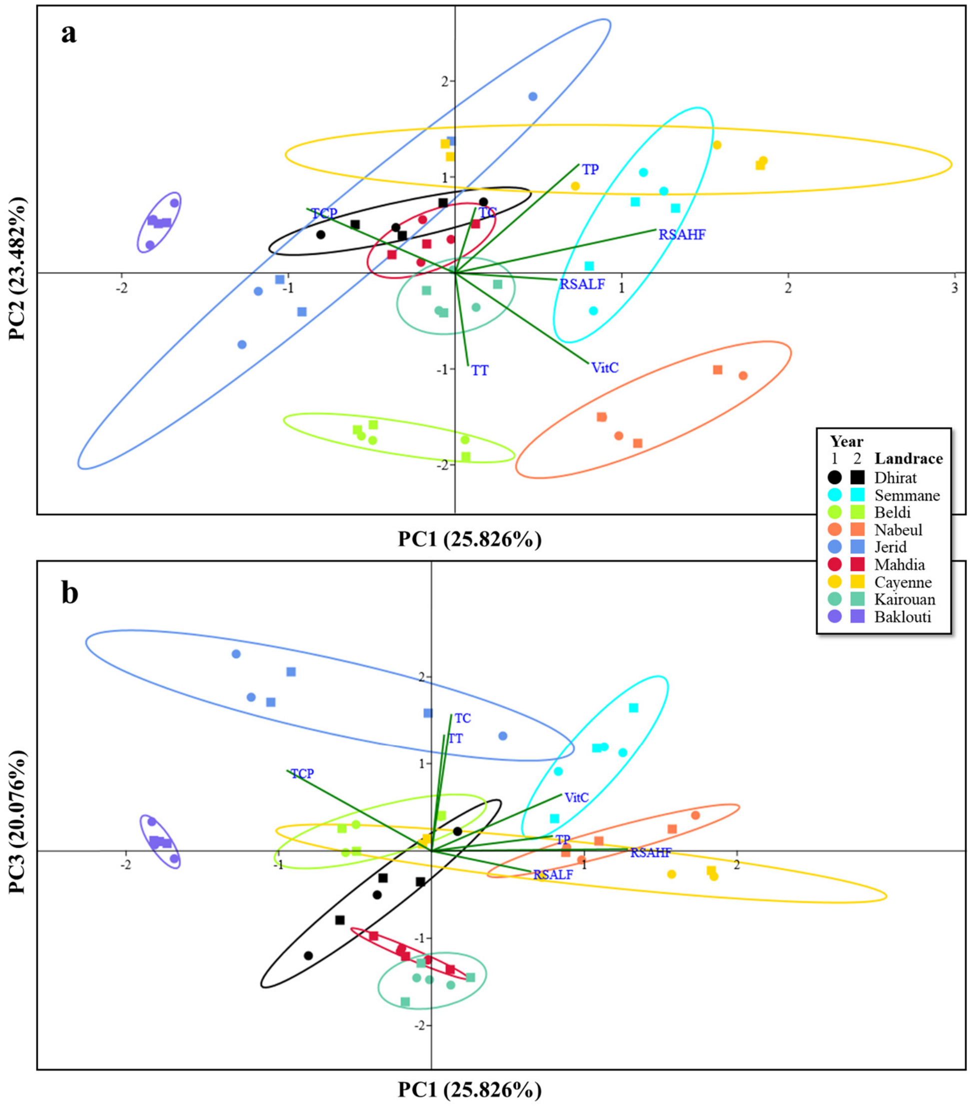

Figure 10. Principal component analysis (PCA) biplots PC1 vs. PC2 (**a**) and PC1 vs. PC2 (**b**) of the main bioactive classes of molecules (total contents) identified in the different pepper genotypes under investigation. The variance (%) explained by each PCA axis is given in brackets. The length of the vectors is correlated to their significance within each population. Between vectors and between a vector and an axis, there is a positive correlation if the angle is <90°, whereas the correlation is negative if the angle is >90°. There is no linear dependence if the angle is 90°. Ellipses enclose the 75% confidence interval. Abbreviations: TCP, total capsaicinoids; TP, total phenols; TT, total tocopherols; TC, total carotenoids; Vit C, total Vitamin C; RSALF, radical scavenging activity of the lipophilic fraction; RSAHF, radical scavenging activity of the hydrophilic fraction.

4. Discussion

The assessed pepper landraces demonstrated desirable agronomic traits, though significant variations in yield were observed due to genetic factors. For instance, Ilić et al. [13] noted significant variations in fruit weight among traditional Serbian pepper landraces (Nizača, Lokošnička, and Turšijara), highlighting the impact of both environmental and genetic factors on yield. Based on the qualitative chemical traits, we underscore the possible use of most of the investigated landraces for various purposes, such as fresh consumption, processing, and picking, as well as mixed uses, confirming their traditional multi-use and aligning with various studies. Brilhante et al. [14] reported similar ranges in soluble solids (5.0 to 24.3 °Brix), pH values (4.69 to 5.94), and total acidity (0.25 to 1.60 mEq% fw) while assessing a Brazilian *Capsicum* germplasm collection. Similarly, Ilić et al. [13] found soluble solids ranging from 8.81 to 14.42 in traditional Serbian pepper landraces.

Color is a critical attribute of vegetable food, and the a*, b*, and a*/b* ratio indices are invaluable in characterizing the quality and maturity of pepper berries. Our b* readings were consistent with those reported by Ilić et al. [13] for Serbian pepper landraces (13.78 to 22.72), although a* readings were slightly higher (30.41 to 37.05) than ours, possibly due to differences in maturity, climate, or agronomic practices enhancing the accumulation of carotenoids and other metabolites responsible for the red color of the ripe fruits.

Regarding functional quality, our study highlighted the prevalence of capsaicin and dihydrocapsaicin as the primary capsaicinoids, comprising 44% and 91%, respectively, of the total capsaicinoid content. This aligns closely with Barbero et al. [34], who reported an even higher dominance of the two compounds, accounting for 79% to 90% of total capsaicinoids during the ripening of Cayenne pepper grown in Spain. Nordihydrocapsaicin emerged as the third major capsaicinoid in the studied landraces, constituting 0.5% to 15.4% of the total content, while homodihydrocapsaicin and homocapsaicin collectively accounted for 1% to 56%, in agreement with Barbero et al. [34]. Indeed, the authors observed nordihydrocapsaicin levels ranging between 6% and 14% of total capsaicinoids in Cayenne pepper, with homodihydrocapsaicin and homocapsaicin present at lower levels (2% to 4%), depending on the fruit's ripening stage. Notably, 'Baklouti' and 'Jerid' exhibited the highest accumulation of total capsaicinoids, solidifying their reputation as among the spiciest and most pungent genotypes in Tunisia. Conversely, 'Mahdia' and 'Kairouan' accumulated the lowest levels, making them preferred choices for consumers seeking milder options. Genotype significantly influences the content of pepper capsaicinoids, as previously reported by Jeeatid et al. [16]. Substantial variation in capsaicin (0 to 9948 μg g^{-1} dw), dihydrocapsaicin (0 to 4114.3 μg g^{-1} dw), and total capsaicinoids (0 to 14,062.3 μg g^{-1} dw) has been reported by Castillo-Aguilar et al. [35] in nine chili pepper landraces from Yucatan peninsula, Mexico, with the extreme values registered in 'Dulce' and 'Rosita', respectively. Alam et al. [18] found higher capsaicin and dihydrocapsaicin content in hot pepper landraces from Malaysia compared to sweet ones. Moon et al. [22] reported wide-ranging capsaicinoid content (0.00 to 1219.90 mg 100 g^{-1} fw) in a collection of *Capsicum annuum* and *Capsicum frutescens* pepper accessions. Díaz-Sánchez et al. [19] found variability in capsaicinoid content in 31 piquin pepper landraces, ranging from 135 to 1379 μg mL^{-1} and 301 to 3719 μg mL^{-1} for total capsaicinoids grown under field and greenhouse conditions, respectively.

Our findings on carotenoid content revealed that fresh red peppers from all analyzed landraces accumulated high levels of provitamin A carotenoids (β-carotene and β-cryptoxanthin), as well as zeaxanthin, collectively accounting for between 74% and 93% of the total carotenoids. β-carotene was the most abundant, comprising 26% to 70% of all carotenoids, consistent with Rodríguez-Rodríguez et al. [20] and Maiani et al. [21]. The obtained results also align with those reported by Martínez-Ispizua et al. [36] in an 18-pepper landrace collection from Valencia, Spain, with total carotenoids levels ranging from 12.17 to 103.88 μg g^{-1} fw at the red maturity stage. The authors also observed variability ranging from 2.64 to 13.11 μg g^{-1} fw in the level of total carotenoids in the same landraces harvested at the green stage. Additionally, Moon et al. [22] revealed an even larger variation in total carotenoids and β-carotene contents, ranging from 52.5 to 3496 μg g^{-1} fw and 5.97 to 392.74 μg g^{-1} fw, respectively, in 380 pepper accessions of *Capsicum annuum* grown in Korea. This was confirmed by Da Silveira et al. [23], who reported significant variability among pepper landraces regarding total carotenoids content, emphasizing the high influence of genotype on this trait.

Regarding total tocopherols, a strong correlation with α-tocopherol content was observed, highlighting that, unlike β- and γ-tocopherols, α-isomer is the main component of the total content [26,37]. Karaman et al. [38] detected α-tocopherol content of 1078.4 μg g^{-1} dw in fruits of recombinant inbred pepper lines from interspecies crosses (*Capsicum annuum* × *Capsicum frutescens*). Duah et al. [7] reported a low extent of variability in α-tocopherol content (392 to 448 μg g^{-1} dw) while assessing bioactive compounds in new hybrid hot chili peppers from Hungary.

Ascorbic acid plays a pivotal role in maintaining plant redox homeostasis by acting as an antioxidant that scavenges reactive oxygen species [39] and is crucial in defending against oxidative stress [38]. The obtained values for total vitamin C were consistent with those of Moon et al. [22], who found vitamin C content ranging from 0.10 to 18.5 mg 100 g^{-1} fw, and Martínez et al. [40], who detected vitamin C levels from 0.62 to 2.5 mg g^{-1} fw at the green stage and from 1.05 to 3.94 mg g^{-1} fw at the red stage in 18 sweet pepper landraces harvested at different ripening stages. It has been reported that variety, maturity, and pre-harvest and post-harvest practices are key factors influencing ascorbic acid content [38].

Our results on total soluble phenolics were in the range of those found by Lee et al. [41] (178 to 384.9 mg chlorogenic acid equivalent 100 g^{-1} fw) in fresh peppers. The results are also consistent with those of Kumar et al. [42], who recorded total phenolics content reaching 266 mg GAE kg $^{-1}$ in red peppers. Numerous authors have argued that phenolic accumulation varies depending on the variety, maturity stages, agronomic practices, climate conditions, and determination methodologies [42,43] with total phenolic levels ranging between 33 and 250 mg GAE 100 g^{-1} fw for different *Capsicum annuum* genotypes [44,45]. Alam et al. [18] found higher total phenolic content in hot *versus* sweet pepper landraces, ranging from 0.5 to 1.0 mg GAE g^{-1} dw in the Malaysian 'cili ungu' and 'cili burung' landraces, respectively, suggesting a positive correlation between phenolic content and pungency. Martínez-Ispizua et al. [36] observed large variability in phenolic content from 1.83 to 7.24 mg g^{-1} fw at the green stage and from 5.66 to 15.87 mg g^{-1} fw at the red ripe stages. Based on their phenolic content, the studied pepper landraces can be considered a good source of phenolics.

Regarding RSALF and RSAHF, the results align with those of García-Vásquez et al. [24], who assessed the antioxidant activity of ten pepper populations from Mexico using two analytical methods, finding variations using DPPH (13.8 to 28.4 μmol TE g^{-1}) and FRAP (36.6 to 63.4 μmol TE g^{-1}). Similarly, Constantino et al. [46] assessed antioxidant activity in 22 pepper accessions from four Brazilian states, with DPPH values ranging from 0.13 to 1.12 TEAC g^{-1} and FRAP values from 0.21 to 2.27 μmol TEAC g^{-1}, suggesting different sensitivities of the techniques and a variability related to genotype and cultivation place. Ramírez-Aragón et al. [25] reported differences ranging from 65 to 348 μmol Trolox g^{-1} dw in 14 chili pepper cultivars grown in Mexico. Martínez-Ispizua et al. [36] found antioxidant activity ranging from 49.68 to 96.31 mg TE g^{-1} fw, noting variability from 6.12 to 77.84 mg TE g^{-1} fw at the green stage and from 49.68 to 96.31 mg TE g^{-1} fw at the red stage.

Finally, the highly significant correlations recorded between color readings and various attributes suggest the potential utility of color indexes in predicting key quality traits among the investigated parameters. The RSAHF can be predicted based on the content of total phenolics and total vitamin C. Similarly, RSALF might be predicted using the content of several lipophilic antioxidants, such as homodihydrocapsaicin, β-tocopherol, lutein, and zeaxanthin.

5. Conclusions

The study provides comprehensive insights into both the agricultural traits and functional quality of various pungent pepper landraces and traditional genotypes. It demonstrates that many traditional genotypes possess valuable agronomic, physicochemical, and functional quality traits, making them suitable candidates for breeding programs aimed at developing high-yielding genotypes with minimal input and water requirements, particularly under the challenging conditions posed by global climate change.

The findings suggest that several quality traits of pepper landraces can be effectively estimated based on color readings. However, further analysis of other bioactive compounds and the use of additional analytical methodologies would enhance the development of genotypes with specific bioactive profiles. This approach could lead to the creation of pepper varieties tailored to meet specific nutritional and functional demands, thereby supporting sustainable agriculture and improving food security.

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