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Agronomic and Phytochemical Characterization of Chickpea Local Genetic Resources for the Agroecological Transition and Sustainable Food Systems

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Abstract: Legume crops play a key role in hastening both the agroecological and protein transition and improving the sustainability of cropping systems. Among legumes, chickpea (*Cicer arietinum* L.) is a valuable source of protein, fibers, and nutraceutical compounds, providing important agri-environmental effects. Nevertheless, few studies have explored the effect of genetic characteristics on production and quality traits in chickpea. Chickpea landraces seem particularly interesting for their positive agronomic and quality characteristics, opening the door for innovation in sustainable food systems. Thus, the present study aimed to characterize two chickpea Tuscan landraces (Rugoso della Maremma and Cappuccio della Valtiberina) in comparison with widely distributed commercial chickpea varieties (Ares, Maragià, Pascià, Principe, Reale, Sultano, and Vittoria). Our findings highlighted positive agronomic traits of landraces in terms of seed yield and yield components, demonstrating performance that is either superior or comparable to commercial varieties. Notably, Cappuccio della Valtiberina showed the highest 1000-seed weight (425.50 g), followed by Maragià (432.92 g), Principe (392.32 g), and Reale (382.79 g), and the highest harvest index (0.55), similar to Reale (0.55). Overall, landraces achieved 18.75% higher yields than commercial varieties. Regarding chickpea quality, landraces exhibited profiles comparable to those of commercial genotypes in terms of protein and oil content, as well as nutraceuticals. Interestingly, the two landraces had the most favorable ω-6/ω-3 ratios (Cappuccio della Valtiberina, 12.45; Rugoso della Maremma, 13.71) among the genotypes except for Maragià (11.78), indicating better nutritional quality compared to commercial varieties (>14.00). These results demonstrated that landraces could offer promising prospects for future chickpea breeding programs, aiding in the selection of genotypes capable of adapting to changing growing conditions and supporting the development of sustainable food systems.

Keywords: *Cicer arietinum* L.; plant-based proteins; legumes; landraces; agrobiodiversity; phenolic compounds; antioxidant activity; sustainable cropping systems

Citation: Abou Chehade, L.; Tavarini, S.; Bozzini, M.F.; Koskey, G.; Caturegli, L.; Antichi, D.; Angelini, L.G. Agronomic and Phytochemical Characterization of Chickpea Local Genetic Resources for the Agroecological Transition and Sustainable Food Systems. *Agronomy* **2024**, *14*, 2229. https://doi.org/10.3390/agronomy14102229

Academic Editors: Massimiliano Renna, Genuario Belmonte, Luigi De Bellis, Elena Ciani, Andrea Pieroni and Monica Marilena Miazzi

Received: 31 July 2024
Revised: 23 September 2024
Accepted: 24 September 2024
Published: 27 September 2024

1. Introduction

In recent years, growing attention has been directed towards the environmental impact of animal-based protein production in response to their substantial contribution to greenhouse gas (GHG) emissions, land use and degradation, water use, and nutrient pollution (e.g., acidification and eutrophication) [1]. Within this context, pulses emerge

as a promising source of high-quality dietary plant-based proteins, providing a viable alternative to meat yet relatively underutilized [2,3]. Another notable attribute is the lower fat content of pulses. This is related to the growing awareness of consumers towards healthy food, obtained with environmentally friendly production methods, for which they are willing to pay more [4–6].

Over time, agronomic research and breeding have paid little attention to legumes, resulting in their current limited distribution. The situation for local legume landraces is even worse, with many varieties facing the threat of extinction. This neglect has posed significant challenges to their cultivation, such as reduced genetic diversity, lower yields, and decreased resilience to pests and diseases. In European countries, for example, these crops occupy a niche of crop rotations, although the situation is slightly better under organic farming [7]. Recently, attempts have been made to define prospects for greater development of legume crops with a view to sustainability. Incorporating legumes into diversified farming systems and crop rotations represents, in fact, a good opportunity for addressing many problems associated with short rotations or monocultures, contributing to improving soil fertility, and pest management while providing market opportunities for farmers and related sectors [8–10]. Furthermore, the valorisation of these essential minor crops is foreseen, as pulses play a key role in the farm-to-fork (F2F) supply chain and in the EU protein strategy, fully aligning with the sustainable goals of the European Green Deal (EGD) [11], by enhancing biodiversity, agricultural resilience, and climate adaptation in response to environmental challenges [12,13].

Chickpea (*Cicer arietinum* L.) is currently the world's third most important pulse, after bean and pea. The global land area devoted to chickpea cultivation covers approximately 14 million hectares, with the majority of production concentrated in India [14]. Chickpea acreage in Italy has declined to less than 3500 hectares, with almost all located in southern regions [15].

From a nutritional point of view, chickpea represents a rich and affordable source of protein, with a high bioavailability, good digestibility, and a suitable balance of amino acids. Chickpea oil content varies from 4% to 10%, with a notable concentration of unsaturated fatty acids, mainly linoleic and oleic. The dry seeds are also a good source of carbohydrates (60–70%), dietary fibers, microelements, and group B vitamins [16].

Moreover, chickpea is characterized by numerous bioactive phytochemicals with beneficial health effects. Among them, phenolic compounds are notably represented by biochanin A and formononetin isoflavones, mainly concentrated in the seed coat, with a proven oxygen radical adsorption capacity and an antiproliferation potential. Chickpea seeds are also rich in carotenoids, including β-carotene, lutein, zeaxanthin, β-cryptoxanthin, lycopene, and α-carotene [17]. In addition, protein hydrolysis has shown a wide range of chickpea polypeptides, exhibiting relevant functional biological effects, such as antioxidant, immune-enhancing, hypoglycaemic, hypotensive, and hypolipidemic properties [18].

However, only a few studies have explored the effect of genetic characteristics on production and quality traits in chickpea. The local chickpea varieties seem particularly interesting for their positive agronomic and quality characteristics, opening the door for innovation in sustainable food systems. These varieties have generally evolved under conditions of low agronomic inputs, and their genetic diversity is extremely useful for a quicker and more adequate response, both to extreme environmental events and changes in selection criteria. This is why they can be effectively employed in organic farming systems [10]. Characterization of these genetic resources and the evaluation of their interaction with agroecological practices are also needed to hasten the agroecological transition. Moreover, identifying and highlighting the quality peculiarities, i.e., biotechnological, nutritional, and organoleptic, of these resources may represent an added value that could respond to market demands and target changing dietary habits.

Therefore, the present study aimed to characterize, from an agronomic (phenological, morphological, and productive traits) and quality (crude protein and oil content, fatty acid composition, and phytochemical characteristics, such as total phenols and antioxidant

activity) point of view, two local Tuscan varieties of chickpea (Rugoso della Maremma and Cappuccio della Valtiberina, namely Rugoso and Cappuccio, respectively). The local varieties were compared to common commercial varieties (Ares, Maragià, Pascià, Principe, Reale, Sultano, and Vittoria) in a field experiment carried out under the Mediterranean environment of central Italy.

2. Materials and Methods

2.1. Plant Material, Experimental Setup and Site Characteristics

A field experiment was set up in the 2022 growing season at the Centre for Agri-environmental Research Enrico Avanzi (CiRAA) of the University of Pisa (Pisa, Italy, 43°40′ N; 10°19′ E; 1 m elevation). The area's climate is typical of the North Mediterranean region, featuring a long-term average annual rainfall of 907 mm, primarily in spring and fall. The yearly average temperature is 15.5 °C.

In the present trial, nine cream-colored Kabuli chickpea genotypes (Table 1) were involved, including seven commercial varieties and two local Tuscan (Italy) genotypes. All genotypes except Sultano, Ares, and Vittoria have rough seed surface texture. The experiment followed a randomized complete block design with three replications. In total, 27 plots were established, each measuring 3 m × 2.5 m (six rows of 2.5 m in length, spaced 50 cm apart).

Table 1. List of the compared commercial and local chickpea genotypes.

Type	Genotype	Provider	Origin
Commercial	Ares	SAIS [1]	Italy
	Maragià	ISEA [2] S.r.l.	Italy
	Pascià	ISEA S.r.l.	Italy
	Principe	SAIS	Italy
	Reale	ISEA S.r.l.	Italy
	Sultano	ISEA S.r.l.	Italy
	Vittoria	SAIS	Italy
Landrace	Cappuccio della Valtiberina	Germplasm bank of the DAFE [3]	Italy
	Rugoso della Maremma	Germplasm bank of the DAFE	Italy

[1] SAIS = Società Agricola Italiana Sementi (Via Ravennate 214, 47521, Cesena (FC), Italy); [2] Società Produttori Sementi S.P.A. (via Macero, 1, 40050 Argelato (BO), Italy); [3] DAFE = Department of Agriculture, Food and Environment, University of Pisa (via del Borghetto 80, 56124, Pisa, Italy).

The chickpea genotypes were sown on 10 March 2022, following mouldboard plowing at 30 cm in February and subsequent seedbed preparation. A target plant density of 33.3 plants m^{-2} was adopted. The physical and chemical characteristics of the soil, determined on samples collected at a depth of 0–30 cm at the beginning of the experiment, are reported in Table 2. The soil was sandy loam, according to the United States Department of Agriculture (USDA) soil texture classification, with a sub-alkaline reaction, good soil organic matter (SOM) content, and a low level of total nitrogen. Soil pH was determined using a 1:2.5 soil water suspension following the McLean procedure [19]. Electrical conductivity was measured at 20 °C using a GLP-31 Crison conductometer (52.93 electrode) (Montepaone s.r.l., San Mauro Torinese, Torino, Italy). Total nitrogen was assessed using the macro-Kjeldahl digestion procedure [20], nitrate (NO_3-N) concentration was determined by ion-exchange chromatography (Dionex ICS 45001; AS4A column), the available phosphorus by colorimetric analysis using the Olsen method [21], and the exchangeable K using the Thomas method [22]. SOM was calculated by multiplying the soil organic carbon concentration, measured using the modified Walkley–Black wet combustion method [23], by 1.724. For the evaluation of active $CaCO_3$, the ammonium oxalate-titration method was used. The cation exchange capacity followed the Mehlich method [24]. The trial was managed following organic farming practices (Regulation (EU) 2018/848). Chickpea genotypes were cultivated in rainfed conditions, and no fertilizers were applied before or during the growing season. Weed control was conducted by two inter-row cultiva-

tion interventions during the early growth stages of the plants. Seeds were harvested between 7 and 18 July 2022, when genotypes reached physiological maturity (90% of pods turned yellow).

Table 2. Soil physical and chemical characteristics (0–30 cm depth) at the start of the experiment.

Sand (%)	58.70
Silt (%)	27.50
Clay (%)	13.80
pH	7.85
Electric conductivity (mS cm^{-1})	0.14
Total N (g kg^{-1})	0.89
NO_3-N (mg kg^{-1})	50.70
Available P Olsen (mg kg^{-1})	4.5
Exchangeable K (mg kg^{-1})	141.5
Organic matter (%)	2.15
Active $CaCO_3$ (%)	2.83
Cation exchange capacity (meq 100 g^{-1})	7.24

Temperature and rainfall data for the study period were collected from a meteorological station near the experimental site. Following sowing, the monthly maximum and minimum air temperatures gradually increased (Figure 1), reaching a monthly maximum temperature of 37.7 °C in July. The total rainfall recorded between February and August 2022 was 243.5 mm, with the majority occurring in March and April. July was the driest month, with no rainfall at all.

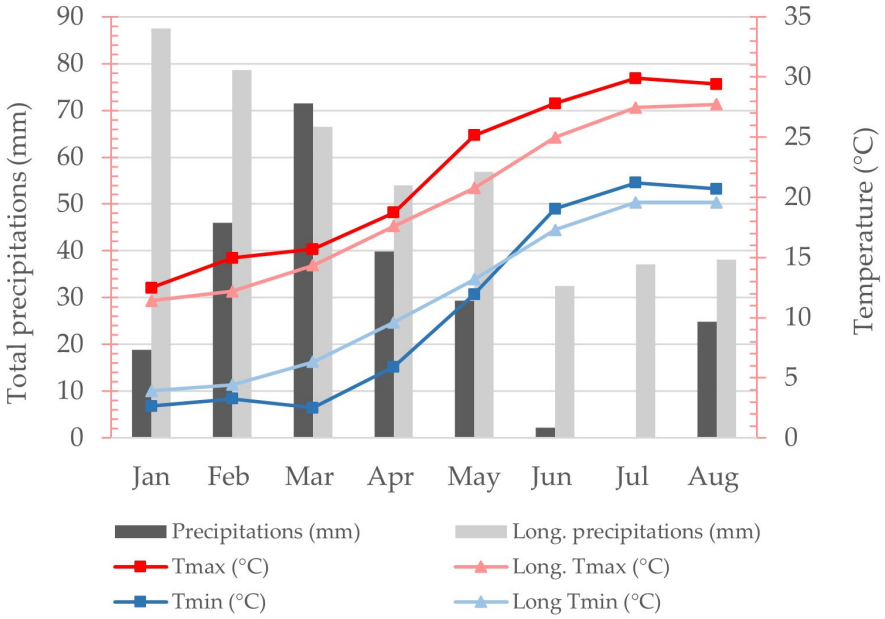

Figure 1. Meteorological data with mean maximum and minimum air temperatures (°C) and monthly precipitations during the trial period (March 2022–July 2022) in Central Italy (Pisa, Italy, 43°40′ N; 10°19′ E; 1 m elevation), compared to long-term (1992–2021) mean values.

2.2. Sampling and Measurements

At full maturity (90% of pods have turned golden yellow), ten plants were selected from the two central rows of each plot for morphological and yield component evaluation. For each selected plant, the height of the plant and first pod, the number of primary and secondary branches per plant, the number of pods per plant, and the seed number per pod were determined. Seed production per plant and plant density were assessed within a central area of 1 m^2 in each plot.

Subsequently, all plants were separated into different organs (seeds, pods, straw) using a fixed-point thresher to determine the fresh and dry weight (DW). DWs were measured after oven-drying at 60 °C until constant weight.

The thousand-seed weight (TSW) was measured on representative seed samples from each plot following the international rules for seed testing [25]. The Harvest Index (HI) was calculated as the ratio of seed dry weight to the total dry aboveground biomass. The total nitrogen content in chickpea seeds was determined by combustion using an elemental analyzer (Eurovector) and gas chromatography. During the chickpea growing season, key phenological stages were recorded, including the number of days from sowing to emergence, full flowering (when 50% of plants had at least one open flower), and maturity (when 90% of pods have turned golden yellow). Growing degree days (GDD) were afterwards calculated using the following formula:

$$GDD = \sum(T_m - T_b) \quad (1)$$

where T_m is the daily average temperature (°C), and T_b is the base temperature for chickpea considered 0 °C, as specified in Rocchetti et al. [26].

2.3. Phytochemical Analyses

2.3.1. Chemicals

Folin–Ciocalteu reagent, sodium carbonate, DPPH (2,2-diphenyl-1-picrylhydrazyl), TPTZ (2,4,6-tri(2-pyridyl)-triazine), ferric chloride, sodium acetate trihydrate, gallic acid monohydrate (3,4,5-trihydroxybenzoic acid), and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were obtained from Sigma-Aldrich Chemical Co. (Milan, Italy). All chemicals and solvents used in the present study were of analytical grade.

2.3.2. Protein, Oil Content, and Fatty Acids Composition

Seed oil and protein contents were evaluated on representative samples from each plot.

Seed oil content was determined following the procedure described by Clemente et al. [27] using a Soxhlet extractor apparatus (mod. R 306) from Behr Labor-Technik (Düsseldorf, Germany). Fatty acid (FA) profiles were determined through direct esterification following the method previously reported by Christie [28]. In summary, internal standard and 3 mL of methanolic HCl (10%) were added to 200 mg of finely ground seeds. After vigorous shaking, the samples were left for incubation at 50 °C overnight. Subsequently, 1 mL of *n*-hexane and 10 mL of 6% K_2CO_3 were added, and the mixture was vortexed and centrifuged at 5000 rpm at 4 °C for 10 min. The organic phase was separated, washed, and dried under nitrogen flux before gas chromatography analysis, performed with a GC2010 Shimadzu gas chromatograph (Shimadzu, Columbia, MD, USA) equipped with a flame-ionization detector and a high polar fused-silica capillary column (Chrompack CP-Sil88 Varian, 152 Middelburg, The Netherlands; 100 m, 0.25 mm i.d.; film thickness 0.20 µm). The analytical parameters were set as follows: The initial oven temperature was 40 °C, gradually increasing to 163 °C at a rate of 2 °C min^{-1}, where it was maintained for 10 min. Afterwards, the temperature was raised to 180 °C at 1.5 °C min^{-1}, held for 7 min, followed by an increase to 187 °C at a rate of 2 °C min^{-1}. Finally, the temperature reached 220 °C at a rate of 3 °C min^{-1}, held for 25 min. The injector and detector temperatures were set at 270 °C and 300 °C, respectively, with hydrogen as the carrier gas at 1 mL min^{-1}. The injection volume was 1 µL, with a split ratio of 1:40. Individual FA methyl esters were identified by comparison with a standard mixture of 52 Component FAME Mix (Nu-Chek Prep Inc., Elysian, MN, USA). The FA composition was reported as a relative percentage of the total peak area.

Seed crude protein content was calculated by multiplying the total nitrogen percentage by 6.25.

2.3.3. Sample Extraction and Total Phenolic and Antioxidant Activity Analyses

Chickpea seeds were ground into fine powder, and then a portion of 0.2 g was extracted with 2 mL of 80% (*v/v*) methanol. The mixture was sonicated for 30 min and centrifuged (135,00 rpm for 10 min). The supernatant was filtered with a syringe filter (Ø 0.45 µm), and the obtained extracts were stored at −20 °C until subsequent analyses.

Total phenols were determined using the Folin–Ciocalteu colorimetric method according to Dewanto et al. [29] and expressed as gallic acid equivalents (mg GAE g^{-1} DW).

For the investigation of the antioxidant activity, the ferric-reducing antioxidant power of methanolic extracts was determined following Benzie and Strain [30], and the free radical-scavenging activity was evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazil radical) scavenging method, according to Brand-Williams et al. [31]. Trolox was used as a standard for constructing calibration curves, and the antioxidant capacity was expressed as µmol Trolox equivalents (TE) per gram sample DW (µmol TE g^{-1} DW).

The spectrophotometric assays were carried out by UV–Vis spectrophotometer (Varian Cary 1E, Palo Alto, CA, USA).

2.4. Color Characterization

Chickpea flour color determination was performed by means of a tristimulus colorimeter (Eoptis, Mod. CLM-196 Benchtop, Trento, Italy) according to the CIE *L*a*b** color system. A glass cell filled with chickpea flour samples and covered with a white plate was placed above the light source. Chromatic coordinates *L**, *a**, and *b** were determined, indicating lightness, green-red, and blue-yellow components, respectively. The color differences among samples (ΔE^*_{ab}) were calculated by applying the following equation:

$$\Delta E^*_{ab} = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

2.5. Statistical Analysis

Data on chickpea morphological and agronomic traits were checked for normality by the Shapiro-Wilk test and heteroskedasticity by Levene's test before analysis. If the assumptions of linear mixed models were violated, data were fit to generalized linear mixed models with the best-fit family and link function (verified visually and with Akaike information criterion). Genotype was considered a fixed factor and block as a random one in the models fitted. Tukey's test at $p < 0.05$ was used to determine significant differences among predicted trait means of the genotypes. *T*-tests at $p < 0.05$ compared the marginal means between commercial and local genotypes. Pearson's correlation coefficient and statistical significance were calculated to trace the relationship between the yield and yield component variables.

Concerning the phytochemical analyses and the color determination, a one-way ANOVA was performed using the JMP® Pro 17.0.0 software package (SAS Institute, Cary, NC, USA). Averages were separated by Tukey's post hoc test. $p < 0.05$ was used to assess significant differences between means. The fatty acid composition data were analyzed using multivariate statistical techniques, namely principal component analysis (PCA) and hierarchical cluster analysis (HCA). The PCA was conducted on the covariance matrix (dimension 9 × 23), selecting the two highest PCs derived from the linear regressions, accounting for a total explained variance of 84.1%. HCA was carried out using Ward's method, with squared Euclidean distances as the measure of similarity.

3. Results and Discussion

3.1. Weather and Plant Phenology

Hotter weather characterized the crop-growing season compared to the long-term trend, especially from May to July 2022 (from chickpea flowering to harvest) (Figure 1). In addition, drier conditions occurred before the chickpea cycle (from the end of March till May 2022) compared to the long-term trend. No considerable variability was observed among the chickpea genotypes regarding crop developmental stages (Figure 2). Plant

emergence was characterized by a dry period and required 19–21 days from sowing time. The genotypes required between 74 and 77 days to reach full flowering and 122 and 125 days to complete their growing cycle (Figure 2A). Accordingly, the thermal time needed for the genotypes ranged between 975.1 and 1031.5 °C and between 2081.7 and 2167.4 °C for full flowering and seed maturity, respectively (Figure 2B). These results are generally in line with those reported by other studies conducted on spring-sown chickpea in Mediterranean conditions, despite some differences related to genotypes, pedoclimatic conditions, and biotic or abiotic stresses like heat stress and moisture availability [32,33].

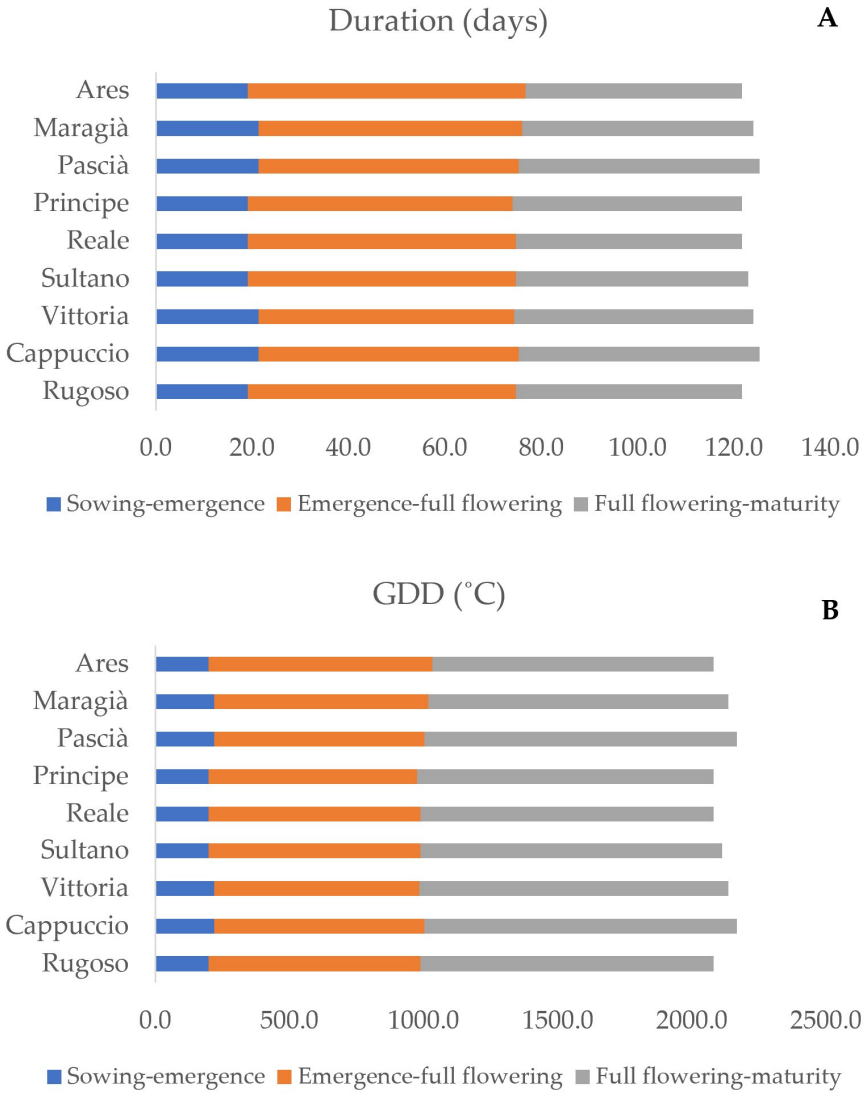

Figure 2. Duration in days (**A**) and growing degree days (GDD) (**B**) of the main phenological stages of chickpea genotypes.

3.2. Yield and Yield Components

Morphological and agronomic characteristics of the nine chickpea genotypes investigated are reported in Table 3. Plant height ranged from 45.85 cm in Pascià to 54.40 cm in Ares. However, pairwise comparisons did not return any significant differences among the heights of the genotypes. All the chickpea genotypes in our study grew well despite being exposed to dry and hot conditions during the season. The landraces were among the shortest genotypes, reaching almost 47 cm in height. Similar trends were observed with the height of the first pod, a key indicator for mechanical harvesting. An average bottom pod height above 27 cm was observed in landraces, suggesting that these genotypes might be suitable for mechanized combine harvest.

Table 3. Morpho-agronomic characteristics of the commercial and local chickpea genotypes compared in the study.

Genotype	Plant Height ‡	Height to the First Pod	Branches		Pods per Plant ‡	No. Seeds per Pod	1000-Seed Weight	Seed Production	Plant Density	Seed Yield	Harvest Index
			Primary	Secondary							
	cm	cm	No. Plant^{-1}	No. Plant^{-1}	No. Plant^{-1}	No. Pod^{-1}	g DW Plant^{-1}	g DW Plant^{-1}	No. Plant m^{-2}	g m^{-2}	
Commercial											
Ares	54.40 ± 1.82	33.20 ± 2.47	2.30 ± 0.28	10.37 ± 0.87 ^{a}	38.36 ± 5.64	0.83 ± 0.09	280.67 ± 13.49 ^{c}	8.75 ± 1.85	27.55 ± 3.36	241.68 ± 26.19	0.51 ± 0.01 ^{ab}
Maragià	45.97 ± 1.82	27.43 ± 2.47	2.40 ± 0.28	7.29 ± 0.66 ^{b}	21.77 ± 3.25	1.09 ± 0.13	432.92 ± 13.49 ^{a}	9.90 ± 1.85	27.22 ± 3.33	261.24 ± 26.19	0.53 ± 0.01 ^{ab}
Pascià	45.85 ± 1.81	25.59 ± 2.46	2.12 ± 0.26	7.29 ± 0.66 ^{b}	27.01 ± 4.02	0.93 ± 0.11	377.48 ± 13.49 ^{ab}	10.72 ± 1.85	26.22 ± 3.26	219.40 ± 26.19	0.53 ± 0.01 ^{ab}
Principe	50.27 ± 1.82	32.00 ± 2.47	2.53 ± 0.29	6.33 ± 0.60 ^{bc}	22.84 ± 3.40	0.85 ± 0.09	392.32 ± 13.49 ^{ab}	7.51 ± 1.85	32.53 ± 3.71	240.74 ± 26.19	0.52 ± 0.01 ^{ab}
Reale	46.70 ± 1.82	28.67 ± 2.47	2.70 ± 0.30	4.74 ± 0.49 ^{c}	21.55 ± 3.22	0.90 ± 0.10	382.79 ± 13.49 ^{ab}	7.15 ± 1.85	30.87 ± 3.59	220.63 ± 26.19	0.55 ± 0.01 ^{a}
Sultano	53.83 ± 1.82	33.80 ± 2.47	2.20 ± 0.27	8.48 ± 0.74 ^{ab}	28.19 ± 4.18	0.81 ± 0.09	283.44 ± 13.49 ^{c}	6.48 ± 1.85	35.85 ± 3.94	237.23 ± 26.19	0.50 ± 0.01 ^{ab}
Vittoria	49.73 ± 1.82	30.47 ± 2.47	2.87 ± 0.31	6.76 ± 0.63 ^{b}	30.41 ± 4.50	0.88 ± 0.10	331.57 ± 13.49 ^{bc}	8.79 ± 1.85	29.87 ± 3.52	245.15 ± 26.19	0.53 ± 0.01 ^{ab}
Local											
Cappuccio	47.07 ± 1.82	27.13 ± 2.47	2.50 ± 0.29	6.53 ± 0.61 ^{bc}	30.50 ± 4.51	0.78 ± 0.08	425.50 ± 13.49 ^{a}	10.49 ± 1.85	33.86 ± 3.80	292.83 ± 26.19	0.55 ± 0.01 ^{a}
Rugoso	47.23 ± 1.82	28.83 ± 2.47	2.83 ± 0.31	8.15 ± 0.72 ^{ab}	29.06 ± 4.30	0.96 ± 0.11	271.27 ± 13.49 ^{c}	7.40 ± 1.85	36.51 ± 3.98	272.45 ± 26.19	0.50 ± 0.01 ^{b}
Significance	*	n.s.	n.s.	***	*	n.s.	***	n.s.	n.s.	n.s.	*
Commercial	49.49 ± 1.53	30.11 ± 1.84	2.44 ± 0.11	7.07 ± 0.50	26.65 ± 2.29	0.90 ± 0.03	354.46 ± 14.16	8.47 ± 1.17	30.01 ± 1.98	238.01 ± 8.88 ^{b}	0.52 ± 0.01
Local	47.15 ± 1.64	27.98 ± 1.94	2.67 ± 0.21	7.21 ± 0.89	29.73 ± 3.94	0.87 ± 0.07	348.39 ± 26.49	8.95 ± 1.50	35.19 ± 3.05	282.64 ± 16.62 ^{a}	0.52 ± 0.01
Significance	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	n.s.

*** $p \leq 0.001$; * $p \leq 0.05$; n.s. $p > 0.05$, means followed by different superscript letters are significantly different ($p \leq 0.05$, Tukey's test), ‡ Tukey's test returns no differences.

The findings revealed the impact of genetic variability on the plant components. Ares was observed to be the highest branching genotype (10.37) and with the highest number of pods per plant (38.36), but with one of the lowest plant densities (27.55) in the field. Conversely, the genotype Reale had the fewest secondary branches (4.74) and the least number of pods per plant (21.55). Local genotypes, Cappuccio and Rugoso, had fair to high secondary branching (6.53 and 8.15, respectively) and numbers of pods per plant (30.50 and 29.06, respectively), along with potentially greater plant densities (33.86 and 36.51, respectively) at harvest compared to the other genotypes. Genotypic differences were marked for the thousand-seed weight. Maragià, Cappuccio della Valtiberina, Pascià, Principe, and Reale are large to medium-seeded genotypes (432.92 to 382.79 g TSW). In contrast, Vittoria, Sultano, Areas, and Rugoso della Maremma are relatively small-seeded genotypes (331.57 to 271.27 g TSW).

The production per plant was statistically invariable between genotypes. However, the results indicated a tendency for greater production per plant in the landrace Cappuccio della Valtiberina (10.49 g plant^{-1}) and the commercial genotype Pascià (10.72 g plant^{-1}) compared to the other genotypes. The least productive genotype was observed to be Sultano (6.48 g plant^{-1}). Likewise, there was no significant difference in seed yields among the individual genotypes, which ranged from 292.83 to 219.40 g m^{-2}. Nevertheless, when the genotypes were grouped into local and commercial varieties, an overall yield advantage of approximately 18.75% for landraces was noticed. Our results are higher than those reported on the yield of spring-sown chickpea in Central and South Italy [32,34] and sometimes that of winter-sown [32]. This makes most of the genotypes suitable and well-performing for spring sowing in the pedo-climatic conditions of coastal Tuscany if frost damage is to be avoided.

The HI, a proxy of the plant capacity of allocating biomass from source organs into sink organs, was relatively high and ranged between a minimum of 0.50 for Rugoso and Sultano and a maximum of 0.55 for Reale and Cappuccio. The harvest indices of the genotypes were generally in the range reported in the literature [32,33]. Differential responses in the HI can be related to environmental and genetic factors. Delayed sowing, for instance, can increase the remobilization and partitioning of photoassimilates into the developing grain [35]. Unfavorable conditions during the reproductive phase of the crop, like heat or moisture stress, might stimulate dry matter translocation to the seed [33,36]. Dry matter translocation is an adaptation mechanism and can be superior in local genotypes (although not verified in our case), which are selected under the local pedo-climatic conditions [33].

Pearson's correlation analysis between the plant production and yield components of the nine chickpea genotypes was conducted to point out the main drivers of chickpea production. No particular relationships were noticed when the grouped genotypes (local vs. commercial) were analyzed separately. The correlation analysis performed on the nine genotypes is presented in Table 4. As the results show, plant seed production seems highly correlated to the number of pods (r = 0.71, $p \leq 0.001$) and the plant density (r = -0.79, $p \leq 0.001$). As the density decreased, both the number of pods and the production per plant increased. No clear contribution of the thousand-seed weight to the chickpea production was found. The strong relationship between chickpea production and pod number per plant rather than seed size, corroborated by Richards et al. [35], is connected to their inherent greater plasticity and adaptability to agronomic and environmental conditions. A moderate positive correlation (r = 0.43, $p \leq 0.05$), was also found between the HI and the grain production per plant. Efficient source-sink relationships can positively contribute to grain yield and might be able to reduce yield gaps from contrasting stress conditions [35].

Table 4. Pearson's product moment correlation coefficients (r) and their statistical significance for the chickpea genotypes' yield components.

	Plant Height	First Pod Height	No. Primary Branches ‡	No. Secondary Branches ‡	No. Pods per Plant	No. Seeds per Pod	1000-Seed Weight	Seed Production ‡	Plant Density	Yield	Harvest Index
Plant height	1	-	-	-	-	-	-	-	-	-	-
First pod height	0.84 ***	1	-	-	-	-	-	-	-	-	-
No. primary branches‡	n.s.	n.s.	1	-	-	-	-	-	-	-	-
No. secondary branches‡	n.s.	n.s.	n.s.	1	-	-	-	-	-	-	-
No. pods per plant	n.s.	−0.39 *	n.s.	0.76 ***	1	-	-	-	-	-	-
No. seeds per pod	n.s.	n.s.	n.s.	n.s.	n.s.	1	-	-	-	-	-
1000-seed weight	n.s.	n.s.	n.s.	−0.44 *	n.s.	n.s.	1	-	-	-	-
Seed production ‡	−0.46 *	−0.77 ***	n.s.	0.42 *	0.71 ***	n.s.	n.s.	1	-	-	-
Plant density	0.48 *	0.69 ***	n.s.	n.s.	−0.47 *	−0.48 *	n.s.	−0.79 ***	1	-	-
Yield	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.51 *	1	-
Harvest index	−0.39 *	−0.42 *	n.s.	n.s.	n.s.	n.s.	0.51 **	0.43 *	−0.41 *	n.s.	1

*** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$; n.s. $p > 0.05$, ‡ per plant.

3.3. Phytochemical Evaluation and Antioxidant Activity

3.3.1. Seed Quality Analyses

The crude protein and oil content, along with the fatty acid composition, of both commercial and local chickpea genotypes are presented in Table 5.

Compared to other pulse crops, chickpea exhibits a higher lipid content, reaching up to 10%, featured by nutritionally important sterols, tocopherols, and tocotrienols [37]. Among the nine analyzed genotypes, the oil content ranged from 6.1% to 7.2%, with the lowest percentage observed in the Vittoria genotype (6.1%) and the highest in Pascià (7.2%) and the Cappuccio landrace (7.1%). Our results in terms of oil content align with the findings reported by Zia-Ul-Haq et al. [38] for four desi chickpea cultivars grown in Pakistan and by Zhao et al. (2021) [39] for six cultivars (five Kabuli and one desi) from China. However, other studies indicate a slightly lower oil content, approximately 4–5% [40,41].

The total fat content of chickpea mainly consists of polyunsaturated (PUFAs), monounsaturated (MUFAs), and saturated (SFAs) fatty acids [42]. In the selected samples, the fatty acid classes were detected in the following range of percentages: 11–12% of SFAs, 29–37% of MUFAs, and 51–57% of PUFAs, in accordance with prior research outcomes [43,44]. A chickpea-based diet prioritizes increased consumption of unsaturated fats over saturated fats, contributing to the maintenance of healthy cholesterol levels and mitigating the risk of obesity and diabetes [45].

The fatty acid profiles were dominated by linoleic (C18:2 n-6), oleic (C18:1 n-9), palmitic (C16:0), and α-linolenic (C18:3 n-3) acids, listed in descending order. More in detail, linoleic acid (LA), ranging from 46.89% to 52.96%, showed a significantly ($p \leq 0.05$) higher value in the Sultano (52.96%) and Vittoria (51.98%) commercial genotypes and in Rugoso (52.91%) among the local ones. The percentages of oleic acid varied between 27.13% (Rugoso, landrace) and 35.92% (Principe, commercial). The saturated lipid fraction was represented by palmitic acid, significantly higher in Vittoria (11.74%) compared to the other genotypes. Finally, polyunsaturated α-linolenic acid (ALA) ranged from 2.97% in Pascià to 3.98% in Maragià, with comparable values among the two landraces (Cappuccio, 3.80%; Rugoso, 3.86%). All the remaining analyzed genotypes exhibited intermediate values. The complete fatty acid compositions were also subjected to multivariate statistical analysis using HCA and PCA (Figure 3). The dendrogram generated by HCA revealed a red macro cluster comprising six genotypes, a more homogeneous blue cluster including Cappuccio and Maragià, and a green cluster formed by Principe itself. In the PCA score plot and loading plot, Principe was positioned in the upper left quadrant (PC1 < 0; PC2 > 0), primarily influenced by the oleic acid vector. The two genotypes within the blue cluster were in the lower left quadrant (PC1 < 0; PC2 < 0) due to their modest linoleic acid percentages. The six genotypes from the red cluster were plotted in the central upper right part of the score plot, characterized by a linoleic acid percentage greater than 50%, with only one exception (Reale).

The obtained percentages of the nutritionally essential fatty acids, LA and ALA, as well as oleic and palmitic acids, fell within the ranges previously reported in the literature [37,44,46]. However, a notable difference was observed concerning ALA, which was detected at significantly lower levels (<1%) in various desi chickpea genotypes [38,43]. Therefore, the obtained ω-6/ω-3 ratios were notably lower than the values reported in other studies [47,48], with the most favorable outcomes observed in the Maragià commercial genotype (11.78), followed by the two local ones (Cappuccio, 12.45; Rugoso, 13.71).

Table 5. Protein and oil content in chickpea seeds with main fatty acids profile as affected by genotype.

Genotype	Protein Content	Oil Content	C16:0	C18:1	C18:2	C18:3	ω-6/ω-3
	%						
Commercial							
Ares	18.87 ± 0.99	6.6 ± 0.09 ^{abc}	10.99 ± 0.09 ^{d}	31.04 ± 0.25 ^{bc}	50.50 ± 0.41 ^{b}	3.39 ± 0.03 ^{c}	14.90 ± 0.01 ^{f}
Maragià	18.24 ± 1.65	6.9 ± 0.18 ^{ab}	10.66 ± 0.08 ^{e}	30.71 ± 0.22 ^{cd}	46.89 ± 0.41 ^{c}	3.98 ± 0.03 ^{a}	11.78 ± 0.01 ^{i}
Pascià	19.66 ± 1.49	7.2 ± 0.14 ^{a}	9.98 ± 0.07 ^{f}	29.97 ± 0.21 ^{d}	50.38 ± 0.41 ^{b}	2.97 ± 0.01 ^{f}	16.96 ± 0.08 ^{b}
Principe	20.48 ± 1.26	6.9 ± 0.23 ^{ab}	11.25 ± 0.10 ^{cd}	35.92 ± 0.30 ^{a}	47.78 ± 0.41 ^{c}	3.08 ± 0.02 ^{e}	15.51 ± 0.03 ^{e}
Reale	19.10 ± 0.14	6.2 ± 0.32 ^{bc}	11.52 ± 0.07 ^{b}	31.37 ± 0.27 ^{bc}	49.92 ± 0.46 ^{b}	3.06 ± 0.02 ^{e}	16.31 ± 0.04 ^{c}
Sultano	17.45 ± 1.58	6.5 ± 0.24 ^{abc}	11.07 ± 0.09 ^{d}	31.50 ± 0.28 ^{b}	52.96 ± 0.50 ^{a}	2.98 ± 0.02 ^{f}	17.77 ± 0.05 ^{a}
Vittoria	19.08 ± 2.14	6.1 ± 0.26 ^{c}	11.74 ± 0.11 ^{a}	28.55 ± 0.20 ^{e}	51.98 ± 0.47 ^{a}	3.26 ± 0.01 ^{d}	15.94 ± 0.10 ^{d}
Local							
Cappuccio	18.55 ± 1.55	7.1 ± 0.09 ^{a}	10.57 ± 0.06 ^{e}	28.72 ± 0.25 ^{e}	47.31 ± 0.40 ^{c}	3.80 ± 0.03 ^{b}	12.45 ± 0.01 ^{h}
Rugoso	19.40 ± 0.47	6.5 ± 0.10 ^{abc}	11.42 ± 0.10 ^{bc}	27.13 ± 0.19 ^{f}	52.91 ± 0.49 ^{a}	3.86 ± 0.03 ^{b}	13.71 ± 0.02 ^{g}
Significance	n.s.	***	***	***	***	***	***

The results are expressed as mean (n = 3) on a DW basis ± standard deviation. Different superscript letters indicate statistically significant differences at $p < 0.05$ level according to Tukey's post hoc test. Asterisks (*) point out a significant influence of the genotype factor as follows: n.s., not significant at $p > 0.05$; ***, significant at $p \leq 0.001$ level.

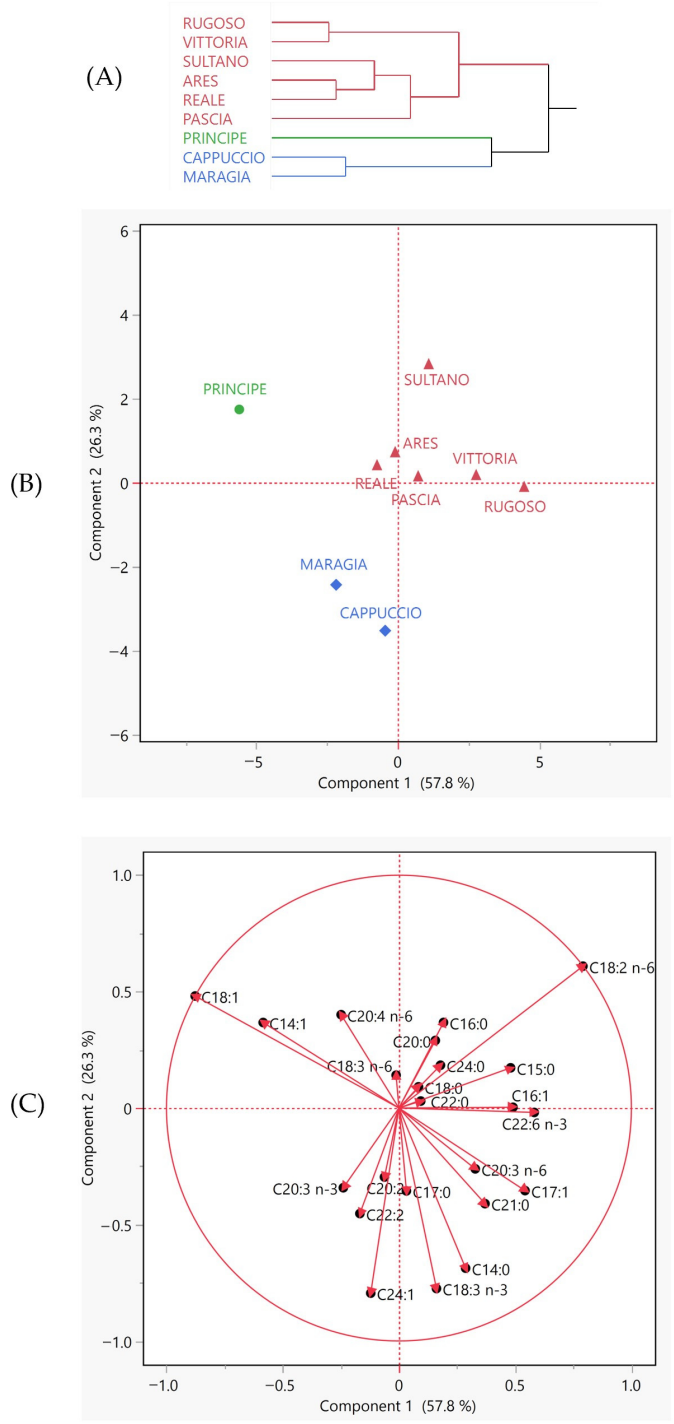

Figure 3. Multivariate statistical analysis performed on the fatty acid complete compositions. HCA dendrogram (**A**), PCA score plot (**B**), and loading plot (**C**) based on PC1 and PC2.

Chickpea seeds are considered an inexpensive source of high-quality proteins, mainly represented by globulin, glutelin, and albumin [49]. The amino acidic profile of chickpea is well-balanced and rich in lysin and arginine, but deficient in sulfur-containing amino acids [50]. In this study, the crude protein content of both the commercial and local genotypes was investigated. The statistical analysis revealed no significant effect of the genotype on the seed crude protein content, with mean values ranging from 17.45% to 20.48%. These findings align with the data previously reported in the literature [47,51].

From a nutritional point of view, our findings demonstrated that the landraces "Cappuccio della Valtiberina" and "Rugoso della Maremma" exhibited comparable profiles to

the analyzed well-known commercial genotypes in terms of protein and oil content, as well as fatty acid composition.

3.3.2. Bioactive Compounds and Antioxidant Activity

Chickpea seeds can provide health-protective bioactive components, such as anthocyanins, flavonoids, and phenolics, with antioxidant activity due to their capability to act as reducing agents, hydrogen-bond donors, and to neutralize free radicals [41].

The concentration of total phenols and the reported antioxidant activity of the hydroalcoholic extracts obtained from the nine analyzed chickpea genotypes are detailed in Table 6.

Table 6. Total phenolic content (TPC), DPPH scavenging capacity, and FRAP antioxidant activity of the analyzed commercial and local chickpea genotypes.

Genotype	TPC (mg GAE g^{-1} DW)	DPPH (μmol TE g^{-1} DW)	FRAP (μmol TE g^{-1} DW)
Commercial			
Ares	0.51 ± 0.01^{b}	0.51 ± 0.02^{cde}	0.94 ± 0.06^{d}
Maragià	0.44 ± 0.01^{d}	0.53 ± 0.04^{bcd}	0.81 ± 0.03^{e}
Pascià	0.46 ± 0.02^{bcd}	0.39 ± 0.06^{e}	0.88 ± 0.04^{de}
Principe	0.50 ± 0.03^{bc}	0.62 ± 0.03^{ab}	1.23 ± 0.02^{a}
Reale	0.50 ± 0.01^{bc}	0.61 ± 0.03^{abc}	0.98 ± 0.06^{cd}
Sultano	0.50 ± 0.00^{bc}	0.60 ± 0.03^{abc}	1.12 ± 0.01^{ab}
Vittoria	0.56 ± 0.01^{a}	0.67 ± 0.05^{a}	1.06 ± 0.00^{bc}
Local			
Cappuccio	0.45 ± 0.03^{cd}	0.43 ± 0.03^{de}	1.07 ± 0.02^{bc}
Rugoso	0.48 ± 0.03^{bcd}	0.54 ± 0.05^{bc}	1.09 ± 0.06^{bc}
Significance	***	***	***

The results are expressed as mean ($n = 3$) ± standard deviation. Different superscript letters indicate statistically significant differences at $p < 0.05$ level according to the Tukey's post hoc test. Asterisks point out a significant influence of the genotype factor as follows: ns, not significant at $p > 0.05$; ***, significant at $p \leq 0.001$ level. DW, dry weight; GAE, gallic acid equivalents; and TE, Trolox equivalents.

The Vittoria commercial genotype displayed the highest total phenolic content (0.56 mg GAE g^{-1}). Furthermore, it exhibited a good radical-scavenging activity (0.67 μmol TE g^{-1}), together with Principe (0.62 μmol TE g^{-1}), another commercial genotype, which also showed the highest antioxidant activity (1.23 μmol TE g^{-1}). Conversely, the DPPH assay evidenced the lowest radical-scavenging capacity for the Pascià and Cappuccio genotypes (0.39 and 0.43 μmol TE g^{-1}, respectively); likewise, the FRAP assay indicated the lowest total antioxidant activity for the Pascià and Maragià genotypes (0.88 and 0.81 μmol TE g^{-1}, respectively), the latter showing a small content of phenolic compounds (0.44 mg GAE g^{-1}) as well. The total phenolic content values of the analyzed chickpea genotypes are consistent with the range reported in the study of Kaur et al. (2019) [52] for Kabuli chickpea genotypes. Higher total phenols are described by Zhao et al. (2021) [39] for six beige and black-colored varieties of chickpea. The distribution of these bioactive secondary metabolites in the chickpea seeds shows the highest concentration of phenolic compounds in the seed coat, which acts as a protective barrier for the cotyledon [53]. The scavenging activity determined by DPPH of the chickpea extracts showed lower values than the average DPPH values reported in the literature (>1 μmol TE g^{-1} DW) [54]. The FRAP values of the analyzed genotypes were included in the range previously described by Quintero-Soto et al. (2018) [54] for nine Kabuli genotypes from Mexico (0.46–1.72 μmol TE g^{-1} DW) and were consistent with other data reported in the literature [55]. However, they were higher than the antioxidant activity values observed for six chickpea varieties from China (0.4–0.6 μmol TE g^{-1} DW) [39].

Concerning the two analyzed local genotypes, Rugoso della Maremma and Cappuccio della Valtiberina, the spectrophotometric assays revealed a medium content of secondary

metabolites and antioxidant activity in comparison to the commercial varieties. Between the two landraces, Rugoso showed a higher radical-scavenging capacity (0.54 µmol TE g^{-1}) than Cappuccio (0.43 µmol TE g^{-1}). However, no statistically significant differences between the two landraces were observed in terms of total phenolic content (Cappuccio, 0.45 mg GAE g^{-1}; Rugoso, 0.48 mg GAE g^{-1}) and total antioxidant activity (Cappuccio, 1.07 µmol TE g^{-1}; Rugoso, 1.09 µmol TE g^{-1}) as measured by the FRAP assay (Table 6).

3.4. Colorimetric Analysis

The color analysis outcomes are reported in Table 7. For color evaluation, *L**, *a**, and *b** values were observed for the flour samples of both commercial and local chickpea genotypes. These parameters were significantly affected by the genotype. Specifically, the lightness values (*L**) ranged from 84.87 for Rugoso, the darkest flour, to 87.41 for the Reale genotype. Our findings were in accordance with data previously reported in the literature for light-colored chickpea varieties [56]. The color parameters *a** (green–red) and *b** (blue–yellow) pointed out the local genotype Rugoso as the most intensively red (1.85) and yellow (27.32) colored one. All flour samples showed positive *a** values, which indicated a slight red tint rather than a green one. The main significant differences concerned the *b** parameter, whereby the commercial genotype, Principe, showed the lowest level of yellowness (22.80). The specific color characterizing legume seeds is the result of the presence of various pigments in their seed coats and cotyledons [57]. In another chickpea study, a positive correlation between higher yellow pigment intensity and carotenoid concentration has been observed [58]. The extraction and quantification of carotenoids represent expensive and time-consuming analyses. Hence, the evaluation of flour color may represent a possible strategy for a preliminary assessment of carotenoid concentration in chickpea seeds [59]. Moreover, a fast color analysis of the chickpea flour could be useful for the quality assessment of the product in relation to storage conditions, as reported in recent research [60].

Table 7. Color parameters for chickpea flours from commercial and local genotypes. Data are presented as a mean of three replicates.

Genotype	*L**	*a**	*b**
Commercial			
Ares	85.61 ± 0.08 ^{cd}	1.77 ± 0.03 ^{ab}	26.52 ± 0.06 ^{ab}
Maragià	86.40 ± 0.13 ^{abc}	1.40 ± 0.08 ^{abc}	23.73 ± 0.54 ^{cd}
Pascià	86.33 ± 0.01 ^{abc}	1.37 ± 0.03 ^{abc}	24.08 ± 0.57 ^{cd}
Principe	87.18 ± 0.07 ^{ab}	1.27 ± 0.00 ^{bc}	22.80 ± 0.10 ^{d}
Reale	87.41 ± 0.03 ^{a}	1.21 ± 0.02 ^{c}	24.88 ± 0.13 ^{bcd}
Sultano	86.10 ± 0.47 ^{bc}	1.63 ± 0.23 ^{abc}	25.72 ± 0.08 ^{abc}
Vittoria	87.08 ± 0.01 ^{ab}	1.17 ± 0.01 ^{c}	24.46 ± 0.05 ^{bcd}
Local			
Cappuccio	86.37 ± 0.47 ^{abc}	1.38 ± 0.09 ^{abc}	23.73 ± 0.45 ^{cd}
Rugoso	84.87 ± 0.5 ^{d}	1.85 ± 0.28 ^{a}	27.32 ± 1.35 ^{a}
Significance	**	*	**

** $p \leq 0.01$; * $p \leq 0.05$; in the same column, different superscript letters indicate significant differences among samples.

The CIE *L***a***b** color difference values (ΔE^*_{ab}) among the flour samples are described in Table 8.

ΔE^*_{ab} represents the expression of the metric distances among the chromaticity coordinates. As shown in Table 8, noticeable color differences can be observed among most samples, especially for the chickpea flours obtained from the commercial Principe and Reale genotypes and the local Rugoso, with $\Delta E^*_{ab} > 3$ values compared to many other genotypes.

Table 8. CIE *L*a*b** color differences among the analyzed chickpea flour genotypes.

ΔE^*_{ab}	Maragià	Pascià	Principe	Reale	Sultano	Vittoria	Cappuccio	Rugoso
Ares	2.92	2.58	4.07	2.5	0.95	2.6	2.92	1.09
Maragià	-	0.35	1.22	1.54	2.03	1.02	0.03	3.93
Pascià		-	1.54	1.18	1.68	0.86	0.35	6.08
Principe			-	10.47	2.09	3.13	1.67	1.24
Reale				-	1.61	0.54	1.56	3.58
Sultano					-	1.66	2.02	2.03
Vittoria						-	1.04	3.68
Cappuccio							-	3.92
Rugoso								-

4. Conclusions

All nine chickpea genotypes evaluated in this study grew well despite the dry and hot weather conditions experienced during the season. This indicates a high level of adaptability of the evaluated genotypes to the Mediterranean pedo-climatic conditions of Central Italy. The results of this study demonstrate the significant potential of Tuscan chickpea landraces, Rugoso della Maremma and Cappuccio della Valtiberina, as valuable genetic resources for future breeding programs targeting low-input management systems such as organic farming. Both landraces exhibited favorable agronomic traits, such as seed yield, 1000-seed weight, and harvest index, comparable to widely distributed commercial varieties. Additionally, their superior nutritional quality, evidenced by favorable ω-6/ω-3 ratios, highlights their importance in promoting healthier and more sustainable diets. These findings underscore the relevance of chickpea landraces in supporting the agroecological transition and enhancing crop diversity in the face of changing environmental conditions. Additional trials across different seasons remain necessary to unravel their potential under different abiotic and biotic stress conditions and to further define the best agronomic practices to optimize their agronomic and quality traits.

Author Contributions: Conceptualization, L.G.A., D.A. and S.T.; methodology, L.G.A., D.A., L.A.C. and S.T.; formal analysis, L.A.C., G.K. and M.F.B.; investigation, L.G.A., D.A., L.A.C. and S.T.; resources, L.G.A. and D.A.; data curation, L.A.C., M.F.B. and L.C.; writing—original draft preparation, L.A.C., M.F.B., L.C., S.T. and L.G.A.; writing—review and editing, S.T., L.A.C., M.F.B., L.C., D.A., G.K. and L.G.A.; visualization, L.A.C., L.C. and M.F.B.; supervision, L.G.A.; project administration, L.G.A.; funding acquisition, L.G.A. and D.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Ente Terre Regionali Toscane (Tuscany Region) within Rural Development Program 2014/2022, sub-measure 10.2. Support for the conservation and sustainable use of genetic resources in agriculture; under the project "Ex situ conservation of genetic resources of endangered herbaceous species in the Region of Tuscany".

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Acknowledgments: The authors would like to thank the staff of the Center for Agri-Environmental Research Enrico Avanzi (CiRAA) of the University of Pisa for their contribution to the field experiment set-up and sample collection.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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