

Article

Bulgarian Cowpea Landraces—Agrobiological and Morphological Characteristics and Seed Biochemical Composition

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Abstract: To face climate change, searching for alternative crops resistant to drought and heat stress becomes necessary, along with efficient germplasm management. Old landraces well-adapted to local climatic conditions, pests, and pathogens could be used as a source of desired traits. Cowpea (*Vigna unguiculata* L. Walp.), grown mainly in the tropical and subtropical areas, presents superior drought and heat stress adaptation compared to other legumes. Limited information is available on field performance and nutrient qualities of cowpea landraces originating from southern Bulgaria. The aim of the present study was to compare in field conditions and their impacts on plant performance, yield, and the seed biochemical composition of Bulgarian cowpea accessions, including fourteen landraces and one variety. Higher-yielding, earlier-maturing accessions were discerned. Among the landraces studied, B1E0103 was the most productive under the agro-climatic conditions in Sadovo, central Bulgaria; BOE0035 had the earliest maturity. The seed moisture content was $11.5 \pm 0.3\%$, and the energy value was 347.9 ± 1.2 kcal/100 g. The crude protein content varied from 22.5 to 27%, the lipids were 1.6–2.55%, the carbohydrates were 56.5–61.4%, ash was 3.8–4.3%, dietary fibers were 3.1–4.5%, tannins were 16–22%, phenols were 1.3–4.4 mg/g, flavonoids were 1.85–3.7 mg/g, and the trypsin-inhibiting activity was 0.7–2.5 units/mg FW, with the lowest in BOE0010, the variety “Hrisi”, and B1E0103 and the highest in BOE0035, A9E1230, and A8E0562. Landraces are promising genetic material for future research and breeding purposes.

Keywords: cowpea; landraces; yield; proximates; phenols; tannins; antitrypsins



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1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important grain legume crop covering tropic and subtropics areas in Africa, Asia, and Central and South America [1–3]. Cowpea is a *Dicotyledonea* belonging to the *Fabaceae* family tribe *Phaseoleae*, the subtribe *Phaseolinae*, and the genus *Vigna*, with several species, which according to different authors, vary from 184 [4] to 170 [5] and between 170 and 150 [6]. About 84 of them have an indigenous origin in Africa [7]. All cultivated cowpeas are grouped under *V. unguiculata* subsp. *unguiculata* [7,8]. Cowpea originated from Africa, although the locality is still uncertain [9,10]. Vavilov [1] postulated that an area with intensive variation was one where the crop must have been cultivated for a long time, as in that area there would have been time for a large numbers of mutations and gene recombination as a result of interbreeding among different varieties and populations. It is generally considered that a very large number of varieties or the high variation of the species was found in the area of the distribution of the crop [11]. Some reports give information that cowpea has been cultivated in Europe since the eighteenth century, while other reports suggest that cowpea was introduced in Europe around 300 years BC [1,12]. Nowadays, cowpea is cultivated in sub-Saharan Africa

(about 95% of the global world production), in tropical regions of Asia and America, and in the Mediterranean part of Europe, including Spain, Portugal, Italy, Cyprus, Greece, North Macedonia, Serbia, and Bulgaria [13–20]. In Bulgaria, cowpea is grown predominantly in the southern regions, where it is known under the vernacular names “papuda”, “bebridja”, “roglio”.

In Europe, cowpea is a minor crop compared with the three most widely grown grain legumes, which are faba bean (*Vicia faba* L.), pea (*Pisum sativum* L.), and soybean (*Glycine max* (L.) Merr.). It is usually cultivated by smallholder farmers and not in an intensive manner. The quantity of cowpea production for 2022 was reported to be 9.8 million metric tons, which constitutes almost three times less than the world production of common bean at 28.3 million metric tons [21]. Compared to other legume crops, cowpea tolerates quite well drought and heat stress and grows in a range of soil types, including infertile sandy soils [22–24], which makes this crop essential for semiarid regions but also with a potential for meeting climate change challenges in temperate regions with an increasing frequency of drought and heat periods. As a legume crop, cowpea has an important agricultural role because of its ability to enrich the soil with nitrogen due to the symbiotic nitrogen fixation between legume crops and tuber bacteria (*Rhizobium*). This specific ability of legume crops makes them excellent in crop rotation by ameliorating the soil’s quality for subsequent crops, and, from another point of view, it is expected to reduce the need for nitrogen fertilization. About half of the greenhouse gas (GHG) emissions from agriculture come from nitrogen fertilizers; it is established that legumes emit five to seven times less GHG per unit area compared with other crops [25,26]. In this way, global warming could be mitigated indirectly through cultivation of grain legumes. Grain legumes in general, with their relatively low carbon footprint, and, in particular, cowpea, could have a substantial impact on sustainable agriculture.

Cowpea is a multipurpose crop grown mainly for grain and fodder, but its leaves and green pods are also consumed; it plays an important role in feeding populations in developing countries given the high nutritional value of the seeds, and it has excellent fodder value [27]. The whole plant of cowpea and, in particular, its seeds represent a valuable source of protein, fibers, vitamins, minerals, polyphenols, antioxidants, and other nutritional compounds [28,29]. The nutritional qualities of cowpea are comparable with those of common bean (*Phaseolus vulgaris* L.) [29,30], a legume species that since 19th century has been widely cultivated in Bulgaria and become traditional in Bulgarian cuisine; however, as a species, common bean is much more susceptible to heat and drought stresses than cowpea [30,31]. Comparative data on seed composition in common bean and cowpea are reported as follows. Common bean has crude protein at 22.9%, lipids at 1.3%, carbohydrates at 60.6%, fibers at 4.8%, and an energy value of 346 Kcal/100 g, while cowpea has crude protein at 24.1%, lipids at 1%, carbohydrates at 54.5%, fiber at 3.8%, and an energy value of 323 Kcal/100 g; the mineral composition is also alike [25]. Having in view the similarity in nutritional characteristics between bean and cowpea, the latter could be an alternative legume crop to face climate change implications for agriculture. In this respect, an evaluation of the field performance of local cowpea landraces as a biodiversity source becomes of prime importance.

Landraces are crop populations in distinct geographical regions that have naturally evolved with conserved natural genetic diversity, variable phenology, high nutritional content, moderate yields, and good adaptation to stressful environments. They are regarded as a primary gene pool for crop improvement [32], as breeding for high productivity using elite genotypes leads to diversity diminution and could be at the expense of lower stress resistance [20]. Local landraces are well-adapted to soil and climatic conditions of a given area, and they have developed some resistance to locally spread diseases and pests, with consequently higher yield stability. In addition, local forms could be a source of diversity for selection purposes [33,34]. The cowpea collection of the Institute of Plant Genetic Resources (IPGR) in Sadovo preserves about 302 accessions, mainly of foreign origin, including 75 accessions of local origin, mainly from the southern parts of the country.

With global warming, the lack of rainfall during the last decades, and drought during summer in June, July, and August, cowpea has shown good adaptability to the warm climate, which is related to its African origin, as well as a more stable yield compared with common bean production. In agreement with the world's tendencies toward better utilization of plant genetic resources [35,36], the complex evaluation of cowpea's genetic resources to understand the morphological, agro-biological, and agronomic characteristics of each accession will give an opportunity for better utilization of local cowpea landraces.

The aim of the present study was to assess the diversity of selected accessions of cowpea landraces originated from central, southern, and southeast regions of Bulgaria by comparing in field conditions plant performance, yield, and seed biochemical composition. Higher-yielding, earlier-maturing plants producing seeds with different morphological and biochemical characteristics were found. The collected seeds were analyzed for energy value, content of proteins, fats, carbohydrates, fibers, ash, tannins, phenols, and antitrypsins. Based on the results of our study, certain landraces have been distinguished as promising genetic material for research and breeding purposes.

2. Materials and Methods

2.1. Plant Material

Fourteen accessions of local populations and one Bulgarian cultivar were included in this study. They were selected on the basis of preliminary screening for differences in antitrypsin content, and all except 1 originated from the south and southeastern parts of the country—Petritch (1 accession), Smolyan (1), Pazardjik (2), the region of Haskovo (Svilengrad, Yerussalimovo, Kap. Andreevo, 9 accessions), and 1 accession from Kavarna. Common to the origins of the accessions are local climatic conditions with a hot and dry summer period. More information about accessions is given in Supplementary Table S1. “Hrisi” is the only Bulgarian cowpea cultivar at present, a selection of IPPG with low antitrypsin content, consistent yield, and good nutritive qualities.

2.2. Field Environmental Conditions

2.2.1. Location and Soil Characteristics

Field trials were carried out at the experimental field of the Institute of Plant Genetic Resources (IPGR), Sadovo, Bulgaria, situated in the central southern part of the country between 24°57'00" E longitude and 42°09'00" N latitude, at an elevation 153 m asl. Field experiments were carried out during a period of three years (2021–2023). Cowpea is cultivated successfully in acidic to neutral soils. The plants were grown on cinnamon forest soil with a neutral pH and relatively shallow water-retaining soil layer without irrigation. During the vegetation cycle, cowpea can be infested by white fly and bruchus. We applied standard plant protection in each phase of plant development.

2.2.2. Weather Conditions

During the experimental period, the average temperatures were different, particularly in 2022, when the temperatures in May and June was higher compared with the same period of the other years and with multi-year values (Figure 1a). There is variation by month and by year in average temperatures and the monthly amount of precipitation, which is important for plant growth and plant development and affected grain yield production. The average temperature from March to August for the experimental period showed higher values compared with multi-annual data at 16.8, 16.9, and 17.3 °C against 16.6 °C. The monthly amount of rainfall from each year was equal to or higher than amount of rainfall from multi-annual data at 276.6 mm (2021), 302.1 mm (2022), and 323.7 mm (2023) against 279.6 mm (Figure 1b).

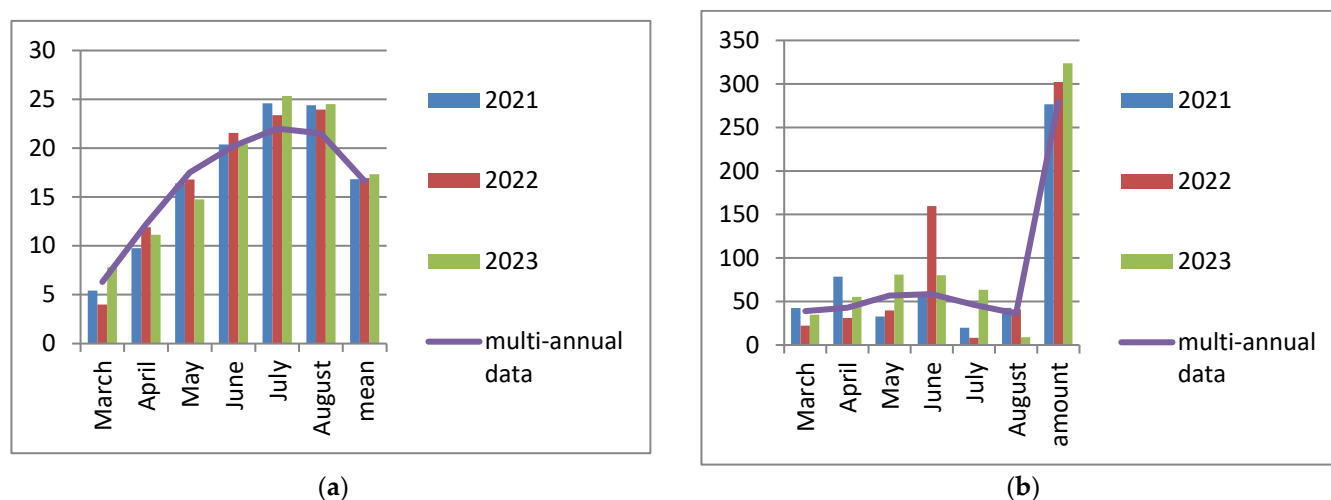


Figure 1. (a) Monthly average temperature (°C) in Sadovo during the experimental period. (b) Monthly amount of rainfall (mm) during cowpea vegetation cycle for years 2021, 2022, and 2023.

2.3. Experimental Design

Varietal experiments were performed in a randomized complete block design with three replications in a plot of 5.6 m². Each accession was sown manually in late April in a plot consisting of four rows 2 m in length and 0.7 m interrow with 50 seeds per row. Plants reached maturity in July–August. Ten individual plants per replication were randomly chosen for biometric measurements. For seed harvest, pods were picked, crushed, and cleaned manually.

2.4. Morphological and Phenological Measurements

Observations were made for 19 agro-biological and morphological characters following the International Cowpea descriptor [37] (Table 1).

Table 1. Descriptions of morphological characteristics.

No.	Character	Abbreviation	No.	Character	Abbreviation
1	Height of plant	hPl	11	Pod width	PW
2	Leaf length	LL	12	Pod thickness	PT
3	Leaf width	LW	13	Number of seeds per pod	NSP
4	Weight of plant	WPL	14	Seed length	SL
5	Weigh of plant without pods	WPL/P	15	Seed width	SW
6	Number of branches	NBR	16	Seed thickness	ST
7	Height of the 1st pod	h1st pod	17	Weight of seeds per plant	WSPL
8	Number of pods per plant	NPPL	18	Weight of 100 seeds	W100S
9	Weight of pods per plant	WPPL	19	Yield per plot	YPL
10	Pod length	PL			

The accessions were harvested after 80 to 90 days after sowing when 95% of pods matured. Standard agronomic traits, such as the total weight of the plant, the 100 seed weight, and the seed yield/plot, were recorded from ten plants randomly selected from the middle row in each plot. The morphological characteristics of the pods and the seeds, like the pods' length, the pods' width, the pods' thickness, the pods' weight, the seeds' size, and the weight of the seeds per plant, were based on 10 pods and 10 seeds randomly selected from the 10 plants used for morphological characterization.

2.5. Seed Biochemical Analyses

Collected seeds from field plots were ground in a mill to a fine powder and analyzed for water content, crude protein content, lipids, carbohydrates, insoluble fiber, ash, tannins, and energy value according to AOAC standard methods, as previously described [24]. Moisture in air-dried seeds (for 72 h at room temperature) was determined according to AOAC (1995) [38]. The crude protein content was estimated using the Kjeldahl method on the basis of seed nitrogen content using a factor of 6.25 [39]. For lipid content, the oil in the seeds was determined gravimetrically after extraction with n-hexane in a Soxhlet apparatus for 8 h, and removal of the solvent was performed in a rotary vacuum evaporator [40]. Carbohydrate content was determined after treatment of the plant material with alcoholic KOH solution and acid hydrolysis of starch into glucose and then oxidation of glucose with a bivalent copper [41]. Insoluble fiber was determined gravimetrically [38]. For evaluation of the ash content, samples were incinerated for 6 h at 550 °C in a muffle furnace [38]. The tannins content was determined according to [41]. The energy value was calculated following [42].

Additionally, the soluble sugar and starch content, free amino acids, phenols, and flavonoids were estimated in 80% of ethanol extracts of seed flour through spectrophotometric methods according to López-Hidalgo et al. [43]. Anthrone reagent was used for the analysis of soluble sugar content in the supernatant and the starch in the pellet (after hydrolysis with 30% perchloric acid) using the standard curve with glucose [44]. Ninhydrin reagent was applied for estimation of amino acids content with a calibration curve prepared with L-proline and L-glycine 1:1 standard solutions [45]. Folin-Ciocalteu reagent was applied for determination of phenolic substances according to [46] using caffeic acid for the standard curve; results are expressed as caffeic acid equivalents. The flavonoid content was determined using the aluminum chloride method described by Huang et al. [47] and a standard curve with quercetin. Trypsin inhibitory activity (TIA) in the seed material was measured after extraction with 10 mM of NaOH by applying the method of Kakade et al. [48]. One unit of TIA was defined in the extract volume as giving 50% inhibition of trypsin activity measured with substrate benzoyl-DL-arginine-p-nitroanilide.

2.6. Statistical Analysis

Data collected in field trials were analyzed through analysis of variance (ANOVA) with the statistical software SPSS 19 [49]. Biochemical data were analyzed by statistically applying the multifactor ANOVA incorporated in StatGraphicsPlus software (Version 2.1) at *p* level 0.05. Data are given as the mean and standard deviation from 3–4 replicates of independent extractions. Statistically significant differences are designated by different letters after mean values $\pm$ standard deviations.

3. Results

3.1. Phenological Observations

The studied genotypes had varying days to emergence, flowering, and maturation. The number of days needed for plants' emergence was from 16.5 to 20.3, with mean value of 18.4 days. During sowing time, the average temperature in April was lower than 10 °C in 2021 and lower than 15 °C in May in 2023 compared with multi-annual data (Figure 1a). It is important to mention that the amount of rainfall during April in 2022 and in May during 2021 and 2022 was lower compared with multi-annual data (Figure 1b). Accessions A8E0563 and BOE0035 started flowering 52.7 and 54 days after emergence. These accessions also matured early (80 and 86 days) compared with the rest of the studied accessions. Early maturation can be considered an advantage for the production of grains. Accession A9E1105 was the latest to flower (57.7 days), with the maximum number of days to maturity (89 days). The mean value of days to flowering was recorded as 55.1, while the average number of days to reach maturity was 87.1 (Figure 2).

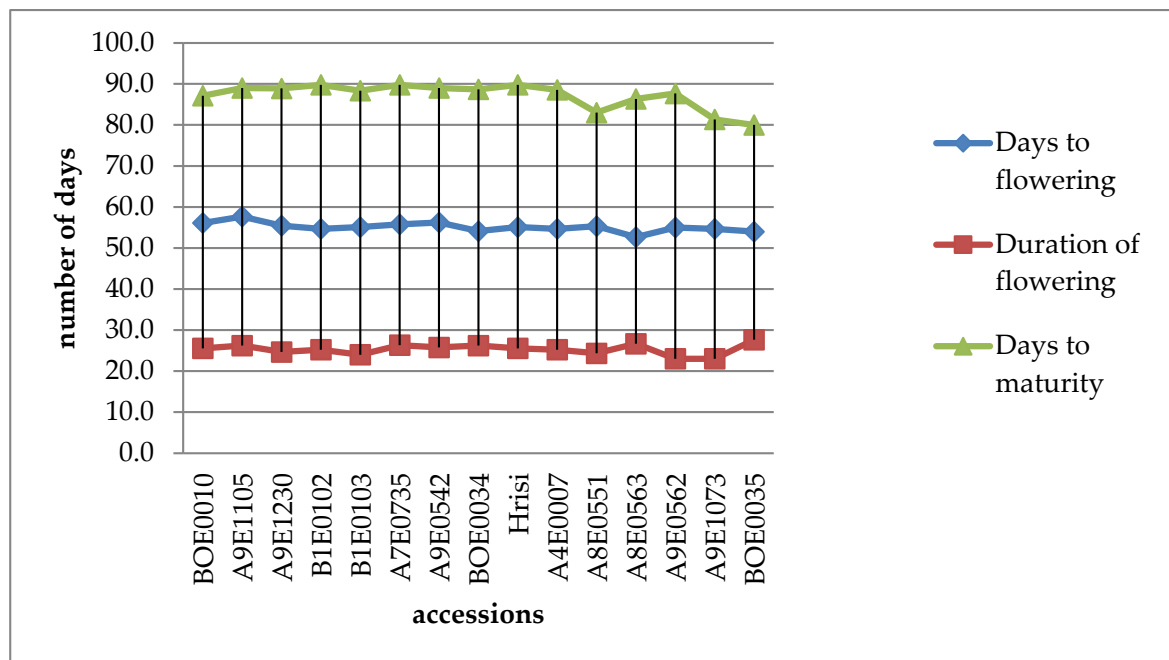


Figure 2. Phenological observations of cowpea genotypes.

3.2. Morphological Characterization

The earliest accessions had relatively short plant heights of 52.8 and 53.4 cm (Table 2). This trait ranged from 52.8 for accession A9E0562 to 119.2 cm for A8E0551. The mean value of plant height was 79.4 cm). The greatest weight of the plants (g) was recorded in accession A8E0551 at 127.2 g, followed by accession A9E1073 at 123.2 g and cultivar “Hrisi” with 122.8 g of dry biomass weight of the plant. The leaf length and the leaf width had the highest values in accession A8E0563 (LL 10.9 cm and LW 8.2 cm), followed by accession B1E0102, with LL 10.3 and LW 10.1 cm. The variability in morphological traits was expressed by the coefficient of variation (CV%) of different traits. The highest level of variability was observed in the weight of the plant without pods (CV 32%), followed by the weight of the plant (CV 28.2%). The number of branches and the height of the first pod had similar variations of 14.2 and 13.4% (CV%). The number of branches ranged from 2 to 3.7, and the highest value was counted in the cultivar “Hrisi”.

The number of pods per plant and the weight of the pods per plant showed a high coefficient of variation compared with the height of the plant and the height of the first pod at 20.6% and 27%, respectively. The longest pod belongs to accessions B1E0103 and BOE0034, at 15.6 cm, while the shortest pods were A8E0551 and BOE0010 at 11.2 and 11.8 cm. The variation in the pod length was 10.6% (CV%), while the pod width and the pod thickness varied at the same level, with CV 6.9%. The hundred seed weight ranged from 13.1 to 22.7 g, as the biggest seeds were found in accession B1E0103. The highest level of variation was recorded in the weight of seeds per plant, which ranged from 8.9 to 28.3 g, with a variation of 27.6% (CV%) (Table 3).

Table 2. Mean agronomic traits of 15 cowpea genotypes.

No.	Cat. No.	hPl (cm)	LL (cm)	LW (cm)	WPL (g)	NBR	WPL/P (g)	h1stP (cm)	NPPI	WPPI (g)
1	BOE0010	76.0	9.1	8.5	74.6	2.7	56.3	34.2	14.3	17.5
2	A9E1105	74.8	8.6	6.7	86.6	3.1	61.2	34.5	16.0	24.3

Table 2. Cont.

No.	Cat. No.	hPl (cm)	LL (cm)	LW (cm)	WPL (g)	NBR	WPL/P (g)	h1stP (cm)	NPPI	WPPI (g)
3	A9E1230	92.1	9.3	7.8	87.9	3.0	62.7	37.9	14.5	24.2
4	B1E0102	89.9	10.3	10.1	90.4	3.0	66.9	35.2	14.6	22.0
5	B1E0103	78.1	10.2	7.9	113.6	3.5	79.3	31.8	17.6	32.5
6	A7E0735	86.8	7.8	6.1	109.1	3.3	73.9	33.6	17.9	32.5
7	A9E0542	79.9	10.1	7.1	102.8	3.1	76.5	34.3	16.0	25.1
8	BOE0034	86.4	10.5	7.7	116.3	3.4	79.6	33.3	18.9	35.2
9	Hrisi	83.3	9.1	7.4	122.8	3.7	89.0	31.5	18.6	32.1
10	A4E0007	87.6	10.2	6.8	94.6	3.0	68.0	34.9	14.9	25.0
11	A8E0551	119.2	10.0	7.4	127.2	2.1	106.7	47.7	10.2	19.0
12	A8E0563	62.3	10.9	8.2	63.9	3.3	39.0	35.6	12.6	24.0
13	A9E0562	52.8	9.1	7.2	67.7	2.9	51.4	35.8	12.5	18.0
14	A9E1073	68.5	9.8	7.0	123.2	3.0	99.5	47.0	13.7	22.9
15	BOE0035	53.4	9.1	7.1	32.0	2.2	21.5	34.0	8.2	11.3
	Min.	52.8	7.8	6.1	32.0	2.1	21.5	31.5	8.2	11.3
	Max.	119.2	10.9	10.1	127.2	3.7	106.7	47.7	18.9	35.2
	Mean	79.4	9.6	7.5	94.2	3.0	68.8	36.1	14.7	24.4
	Std. Error	4.3	0.2	0.2	6.9	0.1	5.7	1.2	0.8	1.7
	Std. Dev.	16.6	0.8	0.9	26.6	0.4	22.0	4.8	3.0	6.6
	CV%	20.9	8.6	12.5	28.2	14.2	32.0	13.4	20.6	27.0

hPl = height of plant; LL = leaf length; LW = leaf width; WPL = weight of plant; NBR = number of branches; WPL/P = weight of plant without pods; h1stP = height of the 1st pod; NPPI = number of pods per plant; WPPI = weight of pods per plant.

Table 3. Pod and seed morphological characteristics.

No.	Cat. No.	PL (cm)	PW (cm)	PT (cm)	NSP	SL (cm)	SW (cm)	ST (cm)	WSPL (g)	W 100S (g)	YPL (g)
1	BOE0010	11.8	0.6	0.6	10.8	0.8	0.6	0.5	13.6	13.1	197.5
2	A9E1105	12.7	0.6	0.6	10.8	0.8	0.6	0.5	19.5	17.4	285.9
3	A9E1230	13.2	0.7	0.7	9.8	0.9	0.7	0.5	19.4	19.1	269.2
4	B1E0102	12.9	0.7	0.7	10.5	0.9	0.7	0.5	17.5	18.5	262.3
5	B1E0103	15.6	0.7	0.7	10.9	1.0	0.7	0.6	26.7	22.7	232.5
6	A7E0735	14.4	0.6	0.6	10.3	0.9	0.7	0.6	27.4	21.8	308.4
7	A9E0542	12.4	0.7	0.7	11.1	0.8	0.7	0.5	19.1	14.1	419.8
8	BOE0034	15.6	0.7	0.7	9.8	1.0	0.8	0.6	28.3	21.4	327.1
9	“Hrisi”	14.0	0.7	0.7	11.2	0.9	0.7	0.5	25.2	19.5	306.7
10	A4E0007	15.0	0.7	0.7	10.2	1.0	0.7	0.6	20.6	19.8	190.2
11	A8E0551	11.2	0.7	0.7	5.7	0.8	0.7	0.6	15.7	19.1	287.4
12	A8E0563	11.5	0.6	0.6	9.0	0.8	0.7	0.5	19.9	20.1	263.2
13	A9E0562	12.5	0.6	0.6	8.6	0.9	0.7	0.5	15.0	19.1	206.8

Table 3. Cont.

No.	Cat. No.	PL (cm)	PW (cm)	PT (cm)	NSP	SL (cm)	SW (cm)	ST (cm)	WSPL (g)	W 100S (g)	YPL (g)
14	A9E1073	14.4	0.7	0.7	9.1	1.0	0.7	0.5	18.5	19.8	393.3
15	BOE0035	13.7	0.6	0.6	11.2	0.8	0.6	0.5	8.9	19.6	256.9

PL = pod length; PW = pod width; PT = pod thickness; NSP = number of seeds per pod; SL = seed length; SW = seed width; ST = seed thickness; WSPL = weight of seeds per plant; W 100S = weight of 100 seeds; YPL = yield per plant.

3.3. Cluster Analysis (CA)

The dendrogram for 15 cowpea accessions formed three main clusters. The first two clusters were divided in two subclusters, and the last two accessions were grouped together in one cluster (Figure 3). The dendrogram showed that the genotypes were derivatives of genetically similar genotypes that clustered together. Cluster 1 contains eight accessions, and cluster 2 contains five accessions. The accessions with narrow genetic bases are shown in Table S2. Two of the total number of accessions were located out of the two main groups/clusters in cluster C3, which showed their wide genetic distance (Table S3). The first two clusters were divided into two subclusters. The main morphological traits that separated accessions in cluster 1 into two subclusters (A and B) were days to flowering, days to maturity, plant height, and yield per plot. Cluster 1, subcluster A contains four accessions, and, among them, B1E0103 differed from the others in the same group based on the weight of the plant, the weight of the plant without pods, the weight of the seeds per plant, and the weight of 100 seeds. This accession showed the highest values for these traits among all accessions in the group. Cluster 1, subcluster B contains four accessions. Accession A8E0551 is different from others with regards to days to maturity, height of the plant, weight of the plant and weight of the plant without pods, weight of the pods, and seeds per plant. Cluster 2 contains two subclusters (C and D), with five accessions. The first three accessions in subcluster C were similar in the number of days to reach maturity, the number of pods per plant, the weight of the seeds per plant, and the yield per plot, while the accessions in subcluster D differed from others in the group by the weight of the seeds per plant, the weight of 100 seeds, and the yield per plot. The last cluster, E, contains only two accessions, A9E0542 and A9E1073, and both had the highest yield per plot (Figure 3).

3.4. Traits' Associations

The results of the correlation analysis showed significant and positive correlations between the duration of flowering and days to emergence, the total weight of the plant and the plant height, and the number of branches and days to maturity at the 0.01 level, as presented in Table 4. The traits related to grain yield showed significant positive correlations between the number of pods per plant and days to maturity and the weight of pods per plant and the number of branches at the 0.01 level. The weight of the pods per plant showed significant positive correlations with the weight of the plant and the number of branches. Pod width and pod thickness were positively correlated with the weight of the plant (WPL) and the weight of the plant without pods (WPL/P). A significant negative correlation was expressed between the number of seeds per pod (NSP) and the height of the first pod (h1stP). Breeding new cowpea varieties with optimal height of the first pod is important for mechanical harvesting. Seed length (SL) had a significant positive association with pod length (PL), while seed width (SW) was correlated with the weight of the plant (WPL), the weight of the plant without pods (WPL/P), as well as the pod width (PW) and the pod thickness (PT) (Table 4). The weight of the seeds per plant (WSPL) showed significant positive correlations (at level 0.001) with the weight of the pods per plant (WPPL), followed by the number of pods per plant (NPPL), the number of branches (NBR), and the weight of the plant (WPL) (at level 0.001). The influence of the investigated factors (year, genotype, yearx genotype interaction) on the weight of the seeds per plant

showed that the strongest, very well proven factor was the genotype (η -48.3%), followed by the influence of the interaction of year $\times$ genotype (η -0.4%) and the influence of the year (η -0.2%). The influence of the first factor, the genotype, was statistically significant at $\alpha = 0.001$, while the last two factors, the year and the interaction year $\times$ genotype, were statistically non-significant (Table 4). To study the influence of factors on the grain yield per plot, analysis of variance was performed (Table 5). The strongest influence on the yield per plot was shown by the year (η -61.6%), followed by the interaction of the year $\times$ genotype (η -17.2%) and the genotype (η -9.3%). All three factors were statistically significant at $\alpha = 0.001$.

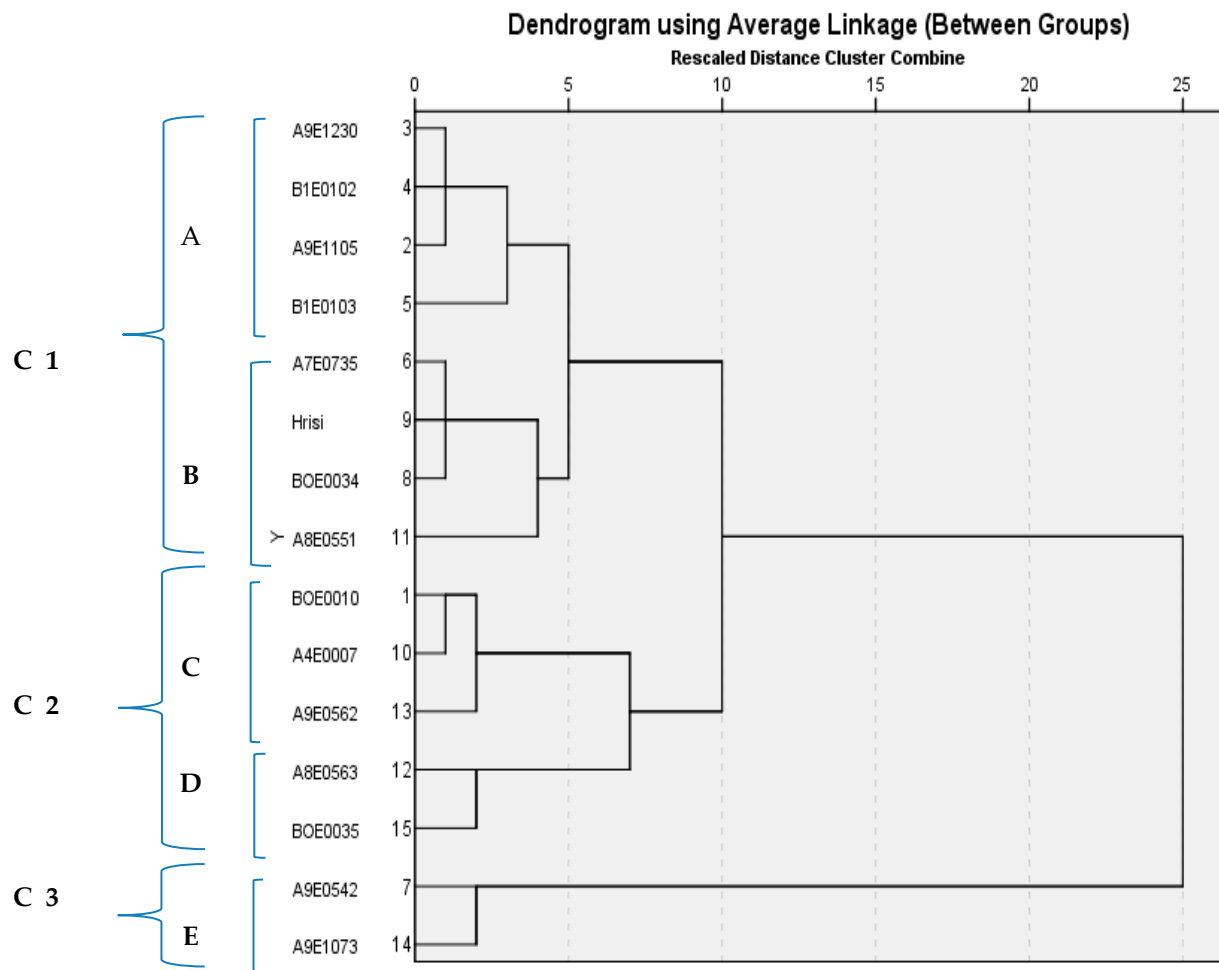


Figure 3. Dendrogram illustrating similarities among 15 cowpea accessions.

Table 4. Influence of factors on the weight of the seeds per plant.

Source of Variation	SS	df	MS	F exp	F tab	η , %	Sign.
Year	11.8	2	5.9	0.14	7.5	0.2	n.s.
Genotype	3713.9	14	265.3	6.07	2.9	48.3	***
Interaction Year $\times$ Genotype	28.5	28	1.0	0.02	2.4	0.4	n.s.
Error	3936.5	90	43.7			51.2	
Total	7690.8	134				100.0	

SS—sum of squares; df—degree of freedom; MS—variance; F exp.—F experimental; F tab.—F tabular; η —force of influence of the factor (%); n.s.—non significant, ***—significance proven at $\alpha = 0.001$.

Table 5. Influence of factors on the yield per plot.

Source of Variation	SS	df	MS	F exp	F tab	η , %	Sign.
Year	3,569,189.1	2	1,784,594.5	232.6	7.5	61.6	***
Genotype	539,737.8	14	38,552.7	5.0	2.9	9.3	***
Interaction Year x Genotype	998,938.4	28	35,676.4	4.6	2.4	17.2	***
Error	690,523.3	90	7672.5			11.9	
Total	5,798,388.6	134				100.0	

SS—sum of squares; df—degree of freedom; MS—variance; F exp.—F experimental; F tab.—F tabular; η —force of influence of the factor (%); ***—proven at $\alpha = 0.001$.

3.5. Seeds' Biochemical Composition

Proximate analysis of seeds collected from the field trials showed results of seed moisture content in the range of 10.2–12% with a mean value of $11.5 \pm 0.3\%$ and an energy value between 344 and 350 kcal/100 g, on average 347.9 ± 1.2 kcal/100 g, without statistically significant differences upon comparing accessions. The significant differences are presented in Table 6. The crude protein content was in the range of 22.5–27%, with a mean value among accessions of $24.53 \pm 0.98\%$, the lipid content varied between 1.6% and 2.55% (on average, $2.01 \pm 0.24\%$), carbohydrates were in the range of 56.5–61.4% ($57.9 \pm 1.2\%$ average), the ash content was 3.8–4.3% (mean value of $4.01 \pm 0.13\%$ for the studied accessions), and the fiber content was 3.1–4.5% ($3.86 \pm 0.44\%$). The carbohydrate content had insignificant variations for most of the genotypes, except accessions A9E1230 and A8E0542, which had the highest values. Starch contributes most to the seeds' carbohydrate content, varying between 458.9 and 855.7 mg of starch per g FW (Table 7), with accession A8E0562 having the highest starch level, followed by B0E0035, while accessions B0E0034 and A8E0551 had the lowest. The lowest value of lipids in seeds was found in accession B0E0035 and the highest was found in accession A4E0007. Dietary fibers were lowest in accession B1E0102 and highest in accession A8E0542. The total protein content was lowest in accessions A9E1230 and A8E0542 and highest in accessions A8E0562, A8E0563, and B0E0010. In this respect, landrace A9E1073 presented both high levels of soluble sugars and free amino acids, whereas in landrace B0E0034 there were low levels of both indices.

Table 6. Crude protein, lipid, ash, fibers, tannins, and carbohydrate content (in percentage) in seeds harvested in field trials, with mean values and standard deviations of three replicates.

No.	Cat. No.	Proteins	Lipids	Ash	Fibers	Tannins	Carbohydrates
1	BOE0010	27.0 ± 0.3^h	1.60 ± 0.03^{ab}	4.1 ± 0.01^{ce}	4.4 ± 0.14^{de}	16.4 ± 0.7^a	56.5 ± 0.4^a
2	A9E1105	24.9 ± 0.5^{cde}	2.05 ± 0.07^{cde}	4.0 ± 0.14^{bc}	3.2 ± 0.07^{ab}	19.5 ± 0.7^{defg}	57.5 ± 1.2^a
3	A9E1230	22.4 ± 0.4^a	2.1 ± 0.03^{cde}	3.9 ± 0.07^{ab}	4.4 ± 0.14^{de}	20.4 ± 0.4^{ghi}	60.1 ± 0.8^{bc}
4	B1E0102	25.3 ± 0.4^{fg}	2.1 ± 0.14^{cde}	4.1 ± 0.07^c	3.1 ± 0.07^a	17.5 ± 0.7^b	57.0 ± 0.6^a
5	B1E0103	25.7 ± 0.1^g	2.3 ± 0.28^{efg}	4.0 ± 0.07^{bc}	3.5 ± 0.42^{ab}	18.8 ± 0.1^{cd}	56.7 ± 2.1^a
6	A7E0735	25.6 ± 0.1^{def}	1.85 ± 0.07^{bc}	3.8 ± 0.07^a	4.3 ± 0.14^{de}	20.7 ± 0.4^{hi}	57.2 ± 0.9^a
7	A8E0542	22.5 ± 0.1^a	1.95 ± 0.07^{cd}	4.1 ± 0.07^c	4.5 ± 0.35^e	17.9 ± 0.6^{bc}	61.4 ± 0.8^{cd}
8	BOE0034	24.2 ± 0.3^{bc}	2.40 ± 0.03^{fg}	4.3 ± 0.03^d	4.0 ± 0.28^{cd}	19.7 ± 0.3^{defgh}	57.4 ± 1.1^a
9	"Hrisi"	24.4 ± 0.4^{bcd}	2.25 ± 0.07^{ef}	4.1 ± 0.07^c	3.6 ± 0.14^{bc}	19.1 ± 0.2^{de}	57.6 ± 1.3^a
10	A4E0007	24.7 ± 0.3^{cde}	2.55 ± 0.07^g	4.3 ± 0.14^d	3.6 ± 0.21^{bc}	20 ± 0.7^{efghi}	56.5 ± 0.6^a
11	A8E0551	24.8 ± 0.2^{cde}	1.95 ± 0.07^{cd}	3.8 ± 0.07^a	4.3 ± 0.03^{de}	21.0 ± 0.6^i	57.8 ± 0.9^{ab}

Table 6. Cont.

No.	Cat. No.	Proteins	Lipids	Ash	Fibers	Tannins	Carbohydrates
12	A8E0563	25.1 ± 0.1 ^{efg}	2.15 ± 0.07 ^{def}	3.8 ± 0.07 ^a	3.6 ± 0.14 ^{bc}	19.3 ± 0.1 ^{def}	57.2 ± 1.1 ^a
13	A8E0562	25.4 ± 0.4 ^{efg}	1.85 ± 0.21 ^{bc}	3.8 ± 0.14 ^a	3.3 ± 0.07 ^{ab}	20.2 ± 0.4 ^{fghi}	57.3 ± 1.2 ^a
14	A9E1073	23.9 ± 0.4 ^b	1.65 ± 0.21 ^{ab}	4.0 ± 0.14 ^{bc}	4.0 ± 0.28 ^{cd}	22.1 ± 0.1 ^j	58.4 ± 1.9 ^{abc}
15	B0E0035	22.5 ± 0.1 ^a	1.45 ± 0.07 ^a	3.8 ± 0.07 ^a	4.3 ± 0.07 ^{de}	19.2 ± 0.4 ^{def}	60.6 ± 1.0 ^{cd}

Statistically significant differences at *p* level 0.05 are designated by different superscript letters after each value.

Table 7. Content of soluble sugars, starch, phenols, flavonoids, free amino acids, and trypsin inhibitory activity in the harvested seeds.

No.	Cat. No.	Sol. Sugars mg.g ⁻¹ FW	Starch mg.g ⁻¹ FW	Phenols mg.g ⁻¹ FW	Flavonoids mg.g ⁻¹ FW	Amino Acids mg.g ⁻¹ FW	TIA U. mg ⁻¹ FW
1	BOE0010	44.6 ± 5.3 ^a	589.5 ± 50.8 ^{ab}	2.18 ± 0.09 ^{abcd}	2.19 ± 0.03 ^{ab}	0.677 ± 0.009 ^a	0.723 ± 0.173 ^a
2	A9E1105	58.9 ± 1.5 ^{abcde}	500.9 ± 6.8 ^a	1.48 ± 0.03 ^a	2.09 ± 0.23 ^{ab}	0.759 ± 0.043 ^{ab}	2.450 ± 0.071 ^f
3	A9E1230	70.4 ± 3.8 ^{cdef}	622.5 ± 52.4 ^{ab}	3.04 ± 0.07 ^{defg}	2.67 ± 0.02 ^{cd}	0.724 ± 0.032 ^a	2.466 ± 0.284 ^f
4	B1E0102	65.4 ± 1.4 ^{bcdef}	621.8 ± 17.0 ^{ab}	3.24 ± 0.37 ^{efg}	2.48 ± 0.28 ^{bcd}	1.403 ± 0.078 ^d	2.229 ± 0.175 ^{ef}
5	B1E0103	57.9 ± 3.6 ^{abcd}	592.3 ± 49.7 ^{ab}	1.58 ± 0.27 ^{ab}	2.07 ± 0.33 ^{ab}	0.776 ± 0.060 ^{ab}	1.540 ± 0.056 ^{bc}
6	A7E0735	57.5 ± 3.2 ^{abc}	583.9 ± 74.6 ^{ab}	3.36 ± 0.45 ^{fg}	2.89 ± 0.33 ^{de}	0.769 ± 0.009 ^{ab}	1.827 ± 0.094 ^{cd}
7	A8E0542	61.7 ± 11.2 ^{bcdef}	528.9 ± 7.7 ^a	4.39 ± 0.18 ^h	3.70 ± 0.16 ^f	0.649 ± 0.052 ^a	2.001 ± 0.047 ^{de}
8	BOE0034	50.9 ± 3.2 ^{ab}	458.9 ± 22.4 ^a	1.31 ± 0.06 ^a	1.85 ± 0.11 ^a	0.652 ± 0.011 ^a	1.964 ± 0.253 ^{de}
9	“Hrisi”	58.9 ± 4.8 ^{abcde}	538.8 ± 3.12 ^a	1.65 ± 0.17 ^{ab}	2.15 ± 0.15 ^{ab}	0.757 ± 0.065 ^{ab}	1.345 ± 0.487 ^b
10	A4E0007	77.8 ± 7.1 ^f	564.8 ± 24.5 ^{ab}	1.91 ± 0.07 ^{abc}	2.19 ± 0.14 ^{ab}	0.933 ± 0.165 ^{abc}	1.795 ± 0.114 ^{cd}
11	A8E0551	59.8 ± 2.3 ^{abcde}	483.5 ± 25.9 ^a	3.53 ± 0.47 ^{gh}	3.15 ± 0.26 ^e	0.765 ± 0.028 ^{ab}	1.778 ± 0.015 ^{cd}
12	A8E0563	68.6 ± 8.0 ^{cdef}	572.9 ± 44.0 ^{ab}	1.56 ± 0.13 ^{ab}	2.13 ± 0.03 ^{ab}	1.037 ± 0.030 ^{bc}	1.534 ± 0.006 ^{bc}
13	A8E0562	73.9 ± 5.6 ^{def}	855.7 ± 102 ^c	2.16 ± 0.42 ^{abcd}	2.33 ± 0.36 ^{bc}	1.089 ± 0.306 ^c	2.527 ± 0.037 ^f
14	A9E1073	75.2 ± 7.2 ^{ef}	727.0 ± 51.4 ^{bc}	2.36 ± 0.46 ^{bcde}	2.42 ± 0.19 ^{bc}	1.412 ± 0.007 ^d	2.055 ± 0.124 ^{de}
15	B0E0035	50.5 ± 2.8 ^{ab}	790.3 ± 145 ^c	2.64 ± 0.44 ^{cdef}	2.47 ± 0.19 ^{bcd}	0.742 ± 0.035 ^{ab}	2.450 ± 0.071 ^f

Statistically significant differences at *p* level 0.05 are designated by different superscript letters after each value.

Despite the observed differences in proximate composition comparing landraces, the overall nutritive quality in all studied genotypes was good enough and corresponded to the expected range for cowpea as a species.

The content of phenols and flavonoids in the seeds of the landraces under study varied in the range of 1.3–4.4 mg per g FW, and that of flavonoids was 1.85–3.7 mg per g FW, with accession BOE0034 having the lowest values and accessions A8E0542 and A8E0551 the highest values of these secondary metabolites. In our study, trypsin-inhibiting activity in cowpea seeds ranged from 0.7 to 2.5 units per mg FW; it was lowest in BOE0010, variety “Hrisi”, and B1E0103 and highest in B0E0035, A9E1230, and A8E0562. Tannins (Table 6) had values from 16 to 22% in the harvested seeds, depending on the accession, with the lowest content in BOE0010 and B1E0102 and the highest in A9E1073 and A8E0551.

The analysis of seeds’ biochemical composition revealed cowpea landrace variations in proximates and other phytochemicals and discerned some interesting landraces with differences in nutritive qualities and bioactive compounds, which could be considered in future breeding programs.

4. Discussion

Evaluation of the genetic diversity in cowpea local germplasm is indispensable for efficient breeding. A diversity pattern can be obtained through systematic landrace evaluation to be used in breeding for enhanced yield and adaptation to climate change [20,32]. Key areas of improvement in cowpea selection are the more efficient manipulation of crop

duration, with an emphasis on high grain yield plants with early to medium maturity, erect growth habits and long peduncles that carry pods above the canopy to facilitate harvesting, and resistance to diseases and pests [50,51]. Cowpea breeding continues to develop dual-purpose cultivars with high yield potential and seeds with good nutritional quality, as well as adaptability to environmental conditions [52,53]. Cowpea is predominantly a crop for hot and dry areas. The environmental conditions of the experimental field of IPGR, Sadovo during the summer season, based on multi-annual climatic data, are particularly suitable for cowpea cultivation, permitting us to discern variability in desired traits among landraces. The effect of climate variations in temperature and rainfall on cowpea yield has been evaluated for a 10-year period, and a positive relationship between temperature and cowpea yield was established [54]. Although vegetative growth in cowpea is stimulated at temperatures from 21 to 33 °C, higher temperatures can cause flower abscission and poor pod set [54]. For other staple crops, such as wheat, rice, maize, and soybean, a reduction in yield by 3.1 (soybean) and 7.4 (maize) for each degree Celsius increase has been reported, and indirect temperature impacts, such as water depletion of soils and increases in weeds, pests, and disease loads, can also be expected [55].

4.1. Field Performance of Cowpea's Local Forms

The accessions with a short vegetation cycle succeeded in escaping high daily temperatures during summer and ensured high yield production [56]. Breeding cowpea for improved earliness could help minimize the risk of harsh conditions, particularly for the dry savanna ecological zones, which pose serious threats to the food and income security of millions of smallholder farmers [57]. Cowpea's short maturity is also beneficial in intercropping systems and crop rotation [58]. The genetic control of earliness in cowpea is governed by complementary and duplicate genes. The most applicable breeding approach for traits influenced by duplicate epistasis is the selection of desirable recombinants by segregating populations developed from bi-parental crosses [57]. Development of determinate early, short-duration, and high-yielding genotypes would be profitable [59]. In our study, two local landraces presented relatively early flowering and seed maturation, A8E0563 and BOE0035, while the accession A9E1105 was late-maturing.

Plant height is affected by the agro-climatic conditions, particularly the sum of rainfall during April, May, and June. Differences in plant height were found among cowpea varieties [60]. Plant height positively correlated with grain yield as a consequence of the greater number of branches, the number of racemes per plant, the number of pods per plant, the weight of the pods, and the seeds per plant [61]. The cowpea is a multipurpose crop, as it is used not only for grain, developed as dry pulse for human food/fresh vegetable pods, but also for fodder [62]. Accessions with a greater dry weight of biomass can be recommended for livestock feeding in a season when the pastures are dry. The height from the soil at the base of the plant to the first pod is a trait of key importance when using a mechanical combine harvester [63]. To minimize seed loss during harvesting, the height of the first pod has to be reasonably high, such as, for example, 12 cm in soybean and 15 cm in common bean [64]. Among the landraces studied, accession A8E0551 had the maximal plant height and plant biomass, followed by B1E0102, whereas accession A9E0562 had the shortest stem. Our results indicated that there was greater diversity of these characteristics in cowpea, which can be used in the direct breeding process to increase yield production. Variability in the number of pods per plant and the seed yield per plant was also reported by Manggoel et al. [65]. According to the results of a field experiment with three cowpea lines in two locations, Mfeka et al. found that the number of pods per plant and the number of seeds per pod contributed significantly to the yield [66]. Similar correlations between seed yield per plant and related characteristics were reported by Singh et al. [24]. All of these associations indicate that these traits are important for improving cowpea's grain yield through direct selection of traits positively correlated with grain yield. These results are in agreement with the conclusions of similar studies [67,68].

4.2. Biochemical Composition of Seeds Harvested from Field Trials

Proximate analysis of seeds of the accessions included in the present study resulted in contents of crude protein, lipids, carbohydrates, ash, and fiber and energy values comparable to previously reported ones for different genotypes of cowpea [29,69–71]. Grain legumes in general, and cowpea in particular, are considered protein-rich, health-promoting functional foods and a source of high-quality protein rich in essential amino acids, covering the necessities of an adult organism, and with about 70% in vitro protein digestibility [19,29]. Cowpea content of fibers and carbohydrates, both digestible and non-digestible, confers prebiotic properties and a low glycemic index [19,27,29]. Cowpea also has low lipid content but a beneficial quantity of unsaturated fatty acids [28]. It is rich in minerals, with a high level of potassium, and it is low in sodium [19], with strong antioxidant potential due to its various bioactive substances. In a study of varietal differences in the proximate composition of cowpea among 28 cultivars, Henshaw established that the total mineral content (ash values) contributed to 71% to the differences, while the protein and carbohydrate content accounted for 25% of variance in the proximate composition [69]. Amino acids and soluble sugars in seeds are reported to be linked with germination potential; they are readily used in the germination process and are favorable for fast seedling growth and plant establishment [71]. In our study, the highest total carbohydrate content was found in accessions A9E1230 and A8E0542, while most of the genotypes had insignificant variations. The lipid content was highest in accession A4E0007. Dietary fibers were lowest in accession B1E0102 and highest in accession A8E0542. Landrace A9E1073 presented both high levels of soluble sugars and free amino acids, whereas in landrace BOE0034 there was a low level in both indices.

Apart from their nutritive quality, legumes contain various bioactive substances, such as tannins, phenols, flavonoids, and protease inhibitors, which are regarded as diminishing the nutritive value of grains [27,30,72]. Bioactive substances are a part of the natural plant's protective mechanisms against various stresses and diseases. A positive correlation has been detected between the plant's flavonoid content and resistance to aphids [20]. These components could be toxic if ingested continuously and in high doses, but they are also considered to have health-promoting properties [72]. Various processing methods, such as boiling, microwaving, and autoclaving, could partly destroy these substances and diminish the negative effects of the antinutrient components [27,29]. Phenols and flavonoids possess antioxidant and free radical scavenging activities and hence significant anti-inflammatory effects and potential synergistic interactions with conventional drugs in health problems, such as cancer, diabetes, and cardiovascular disease [29,72]. In our study, we established the lowest content of phenols and flavonoids in accession BOE0034 and the highest in accessions A8E0542 and A8E0551. Interestingly, the seed coat is considered to be the part of the seed where these substances are concentrated, and it is expected that colored seeds will present a higher quantity of phenols and flavonoids [72], but we did not observe such a dependency. Tannins are also considered antinutrients because of their properties that precipitate proteins and diminish the nutritive value of foods [73]. Antitrypsins have antinutrient properties due to the inhibition of the digestive enzymes in monogastric animals, thus diminishing nutrient absorption from food; however, these protease inhibitors also have carcinogenesis-preventive properties [74]. According to Oluwatosin et al. [75], the level of antitrypsins in seeds is mainly genetically determined, which has been confirmed through initial screening analysis of seeds from the same accessions stored at the IPPG gene bank, whereas the level of tannins is more influenced by environmental factors [75]. In our study, trypsin-inhibiting activity in cowpea seeds was lowest in BOE0010, variety "Hrisi", and B1E0103 and highest in B0E0035, A9E1230, and A8E0562. The content of tannins was lowest in BOE0010 and B1E0102 and highest in A9E1073 and A8E0551. The additional data on seeds' biochemical composition of the studied landraces could be useful for selection purposes.

5. Conclusions

Among the studied accessions of cowpea, three of them (B0E0035, A8E0563, and A9E1073) matured early, with the shortest vegetation cycle. From the climate point of view, these accessions have advantages to avoid higher temperatures and the dry period during the summer typical of the local agro-climatic conditions. The highest level of variability in the morphological traits of plants, pods, and seeds allows breeders to select plants with desired characteristics, like taller plants, higher height of the first pod, high vegetative biomass, a larger number of pods per plant, and a greater weight of seeds per plant. Based on the information regarding the morphological and agro-biological traits' variability, breeders may use the proper plant material for breeding purposes. The influence of the year, the genotype, and their interaction is significant for the grain yield per plot. The weight of the seeds per plant was influenced significantly by the genotype, while the year and the interaction of year $\times$ genotype were non-significant. The seeds' biochemical composition highlighted accessions higher in protein content (B1E0103) and enriched in lipids (A4E0007), carbohydrates (A8E0542), fibers (A8E0542), those with higher antioxidant potential (A8E0542), and those with different tannin and antitrypsin content. Such information is an added value and could be of special interest for inclusion in future breeding programs. Results on proximates confirm the equality of cowpea and bean in terms of nutritive value, supporting the idea that cowpea could be an alternative crop for future climate changes in more temperate climatic regions due to its advantageous high resilience to drought and high temperatures and, at the same time, its high nutritional and health-promoting qualities.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agriculture14122339/s1>. Table S1. Studied local forms—additional information on seeds. Table S2. Genetically closed genotypes. Table S3. Genetically distanced genotypes.

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