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Assessment of Balkan Pepper (*Capsicum annuum* L.) Accessions for Agronomic, Fruit Quality, and Pest Resistance Traits

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Abstract: To maintain the continuous genetic variation and increase the genetic gain, appreciable germplasm diversity and its comprehensive characterization is necessary to further utilize gene sources for pre-breeding. The diversity of pepper forms, cultivation traditions and diverse fruit usages are typical for Balkan countries. Considering this rich diversity, 21 pepper accessions from the Balkan region were evaluated for morphological, biochemical, and insect resistance traits during 2018 and 2019 at Maritsa Vegetable Crops Research Institute, Plovdiv, Bulgaria. Among the studied accessions, the highest productivity was observed in pumpkin shape K1115 and kapia type K1081 accessions, with 0.74 kg and 0.70 kg per plant, respectively. Concerning fruit quality, the highest total polyphenols and ferric reducing antioxidant power (FRAP) were observed in pumpkin shape K712 (203.44 mg GAE/100 g FW) and K1103A (11.49 µmol Fe^{2+}/g FW) accessions, respectively. Concerning insect resistance, 38% of studied accessions showed no infestation of green peach aphid. The kapia type K697 accession was seen as the most reliable resistance source, as it was not infested by aphids and had the least thrips (20% on plants) and cotton bollworm (6.67% on plants and 8.34% on fruit) damage. Based on examined traits, accessions were identified for enhanced fruit quality and promising insect resistance and have been included in further pre-breeding efforts.

Keywords: sweet pepper breeding; productivity; plant fruit morphology; ferric reducing antioxidant power (FRAP); polyphenols; thrips; aphids; cotton bollworm

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1. Introduction

Among the widely cultivated and economically significant vegetable crops, peppers (*Capsicum annuum* L.) are famous for the high nutritional value of their fruit, rich in many phenolic substances, vitamins, and antioxidants [1,2]. Many factors, including fruit maturity, environment, and cultivation techniques, likely affect the biochemical composition of the fruit [3–6]. However, major variation is contributed by the genetic background [7]. Within the germplasm collections, natural variation is a valuable source for genetic improvement of fruit nutrition and quality traits. Since awareness about healthy food is on the rise, so does the consumer's interest in consuming food enriched with antioxidants and nutritional quality. Considering this new niche, breeding for enhanced fruit quality is becoming an important breeding objective for most pepper breeding programs [8]. Characterization of the pepper agro-biodiversity that exists in the Balkan germplasm collections can assist breeding programs, giving more insight on the fruit quality. Parameters such as antioxidant capacity and phenolic content need to be quantified alongside traditional quality attributes, including total soluble solids and dry matter content. A comprehensive germplasm characterization provides insights on the selection of promising breeding lines that could improve the fruit quality and nutritional value of future pepper varieties.

Due to widespread production and intense cultivation, it is often seen that widely cultivated varieties are attacked by different pests throughout the growing season (from sowing to harvest), and species composition of pests seem to have increased in recent years. The most commonly occurring insect infestations are caused by thrips, aphids, and cotton bollworm. These pests primarily damage leaf, fruit, and whole plants, whereas some pests also cause secondary damage by viral infections (aphids and thrips) and mycoplasma diseases (leafhoppers) in the form of vector carriers. Due to domestication, commercially grown hot and sweet peppers have lost their resistance against thrips and have become susceptible to several thrips species. Thrips species commonly found on *Capsicum* include *Frankliniella occidentalis* (western flower thrips) and *Thrips tabaci* (onion thrips) [9,10]. *F. occidentalis* is one of the most widespread thrips species. It causes damage on leaves, flowers and developing fruit by feeding and egg deposition. Moreover, it can transmit at least five types of tospoviruses [11]. *T. tabaci* is known to occur on a broad range of hosts, including *Capsicum*, and causes damage on the foliage [9]. Controlling thrips on *Capsicum* with pesticides is difficult, and the identification of resistant accessions is necessary for successful and sustainable production of pepper in the future [12]. Besides thrips, green peach aphid (*Myzus persicae* Sulz.) is one of the major pepper pests. It causes both direct damage and serves as a carrier of virus diseases. Tolerant varieties could be an important element in the integrated control of *M. persicae*. Along this line, screening tests have been carried out to determine the response of different pepper varieties and accessions that discern resistance against aphids [13,14]. Cotton bollworm (*Helicoverpa armigera*; *Lepidoptera: Noctuidae*) is the main moth that causes serious damage in pepper when larvae feed on the leaves, flowers, and fruit; however, it has been noted that the most serious damage is to the fruit [15].

Damage caused by pests results in the production of low-quality fruit and significant yield losses. Often, intensive use of chemical pesticides lead to a building of resistance among pest populations and even species evolution that leads to the development of new species [16]. The intensive application of pesticides also poses a danger to the environment and human health. The insecticides used are not always effective enough; therefore, integrated pest management (IPM) practices have been implemented and combined with chemical and biological strategies to grow healthy crops [17,18]. The economic burden and environmental damage caused by the use of synthetic chemicals highlights the importance of finding and incorporating resistance in emerging/new cultivars that could provide an alternative approach that is economic and sustainable. Conventionally, genetic resistance against different biotic stresses has been seen to be effective; therefore, breeders have been working on finding resistance sources and subsequently introducing them in a cultivated background to develop resistance cultivars that are adapted to the local environment.

The Balkan region and Bulgaria are well known for the diversity of pepper shapes, growing traditions, end-use consumption, and development of novel pepper cultivars. The availability of a rich collection of genetic resources and comprehensive trait characterization are a prerequisite to establish successful breeding programs [19]. In recent years, we have focused on studying the pepper's genetic resources in terms of productivity, fruit morphology, quality, and resistance to different biotic stresses [20,21]. The purpose of this study was to carry out a comprehensive characterization of pepper accessions according to morphological, economic, and biochemical traits and assess pest infestation in a natural background of field conditions.

2. Materials and Methods

2.1. Germplasm Selection and Evaluation

The research was conducted in 2018 and 2019 at the Maritsa Vegetable Crops Research Institute (Maritsa VCRI), Plovdiv, Bulgaria. From the available pepper gene pool, 21 pepper accessions were selected based on their biomorphological and fruit quality attributes, including shape (Figure 1). The selection criteria that we used to select the included accessions were primarily based on how each accession performed for horticultural, fruit quality, and insect resistance. Additionally, during the accession selection, we sought those accessions that could be used to develop ideotypes with these desired traits. The selected accessions were comprised of breeding lines and local forms (landraces) and were mostly from different regions of Bulgaria (15 accessions), but also with representation from Greece (3 accessions), Albania (2 accessions), and North Macedonia (1 accession). Subsequently, selected accessions were divided into four different varietal types classified based on their shape, including corniform, blocky (dolma), pumpkin (ratund), and kapia. Corniform-type accessions formed elongated horned fruit suitable for frying and pickling (Figure 1A). The blocky type is a square or rectangular fruit with 3–5 apexes suitable for stuffing (Figure 1B). The pumpkin-shaped variety is a short but wider fruit suitable for roasting and pickling (Figure 1C). Kapia is a long triangular suitable for roasting, stuffing, and pickling (Figure 1D). A natural infestation of important pests was also studied in the tested accessions.

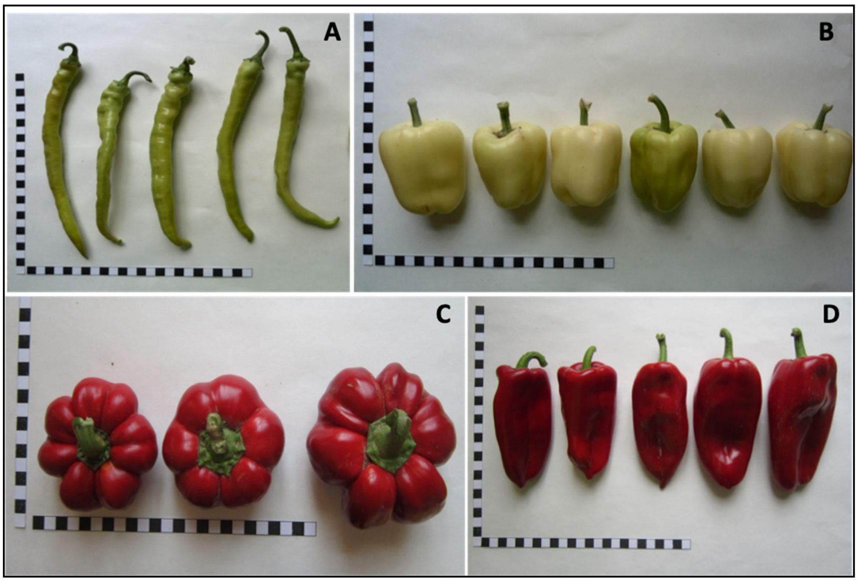

Figure 1. Pepper varietal types: corniform (**A**), blocky (**B**), pumpkin (**C**), and kapia (**D**) shapes.

2.2. Experimental Design and Field Evaluation

Seedling production was carried out in unheated greenhouses from the middle of March till the middle of May. The open field where the experimental evaluation was done had an alluvial meadow soil type. During growing season, the weather is usually warm to hot and most of the precipitation occurs in June, while it is scarce in August and September. During our experiment, in 2018 and 2019, the total rainfall was 11.93 and 12.44 inches, respectively. More details on the average temperature and rainfall received each month across during the growing season is shown in Table S1. Additionally, soil content details for the experimental field are shown in Table S2. The experiments were conducted using the randomized complete block method with 3 replications and 10 plants per replication for each accession according to the scheme 120 + 40/15 cm. All accessions presented in this study were evaluated with 3 replications. The plants were transplanted on a high bed in two rows with 40 cm distance between them, as 15 cm was the distance between the plants in a row. During the cultivation, plants were tendered as per the cultivation practices adapted for mid-early open field pepper production [22]. Throughout the growing season, all agronomic practices, including fertilization, irrigation, and plant protection, were conducted regularly and plant nutrition was conducted with mineral fertilizers based on soil analysis. Concerning the plant nutrition, before each growing season, P_{14} and K_{25} were imported with subsequent soil tillage and plant nutrition was administered thrice with soil tillage across the growing season, so the total fertilization for each year was accomplished with N_{34} P_{14} K_{42} Ca_{12}. The harvest period was two months' duration—from the end of July to the end of September. We harvested the accessions according to their consumption timeline. The fruit from corniform and blocky accessions was harvested before botanical maturity as green and greenish white, while that from ratund (pumpkin) and kapia accessions was harvested at botanical maturity as red fruit.

2.3. Trait Characterization

Morphological plant and fruit traits were assessed in the second harvest according to the IPGRI, AVRDC, and CATIE [23] descriptor definitions. Productivity (kg/plant), plant height (cm), stem height (cm) and branches at the first order (number) were assessed after the end of vegetative growth, while the productivity was the sum of all harvests of the plant. Fruit of different pepper accessions were collected according to their usage either before maturity or at maturity stages. Corniform and blocky varietal types were harvested before maturity (in an intermediate stage of ripening), while pumpkin and kapia varietal types were harvested at maturity. Evaluated morphological traits of the fruit were length (cm), width (cm), wall thickness (mm), locules (number), fruit weight (g), and edible part (%).

2.4. Fruit Compositional Quality

To assess fruit quality, collected fruit was characterized for dry matter content, total soluble solids (TSSs), total polyphenols, and ferric reducing antioxidant power (FRAP). Immediately after the harvest, fruit from each sample was washed three times with distilled water and wiped dry. Half of the sample was freshly homogenized, while the rest was frozen at −20 °C and lyophilized. Fresh samples were used for determination of dry matter content and TSSs by drying the tissue in an oven at 105 °C to a constant weight and by an OPTi® digital handheld refractometer (Bellingham & Stanley Limited, Tunbridge Wells, UK), respectively. The lyophilized material was used for analysis of total polyphenol content and determination of ferric reducing antioxidant power (FRAP). Total polyphenols (TPs) were quantified according to Singleton and Rossi [24], and results are expressed as mg GAE/100 g fresh weight (FW), whereas antioxidant activity by ferric-reducing antioxidant power (FRAP) was measured according to Benzie and Strain [25], and results are expressed as µmol Fe^{2+}/g FW.

2.5. Screening for Insect Resistance under Natural Conditions

Green peach aphid (*Myzus persicae* Sulz.): Aphid damage was measured as percentage damage to plants and degree of infestation. Damage was assessed on a scale of 0 to 4, as recommended by Leclant and Remaudiere [26]: 0—no aphids, 1—up to 5 aphids/plant, 2—from 6 to 25 aphids/plant, 3—from 26 to 50 aphids/plant, and 4—>50 aphids/plant.

Thrips (*Frankliniella occidentalis* Perg. and *Thrips tabaci* Lindeman): Thrips damage was assessed as percentage damage to plants and average number of mobile plant forms. Degree of infestation was assessed on a scale of 0 to 4, as recommended by Fery and Schalk [14]: 0—no symptoms, 1—minimal symptoms, 2—poorly expressed symptoms, 3—average expressed symptoms, 4—strongly expressed symptoms.

Cotton bollworm (*Helicoverpa armigera* Hubn.): A standard method of applied entomology was used to determine the damage from cotton bollworm. Damage readings were measured as percentage damage total plants, whereas the percentage of damaged fruit was recorded for 10 peppers per replication, so in total 30 peppers were assessed across all three replications.

2.6. Statistical Analysis

General descriptive statistics and histograms were estimated in Microsoft Excel (Version 2401). Analyses of variance for both morphological and fruit quality traits were performed for each year separately to assess the fixed effects of accessions and replication, and then both years' data were analyzed together to check the interaction between fixed effects (accession) and random effect (year). Analysis of variance of quantitative traits was performed using generalized linear model (GLM). Statistical analysis was performed with XLSTAT version 15. The manuscript structure was adapted from a previous work on the biomorphological diversity of the Balkan pepper core collection [21]. Principal component analysis of biomorphological diversity was performed using correlation matrices of a total of 14 morphological and 4 fruit quality biochemical traits using R. Eigenvalues, eigenvectors, percentage variance of different principal components, and accession by trait (A*T) biplot were estimated using ggplot2 [27], missMDA [28], FactoMineR [29], and Factoextra [30] R packages (Version 4.2.2).

3. Results and Discussion

The studied accessions selected for more detailed characterization have a sweet fruit taste and belong to corniform/horned (var. corniform), blocky (var. dolma), kapia (var. kapia), and ratund/pumpkin (var. ratundum) varietal groups. The selected accessions were selected with an intention to further utilize them in subsequent ideotype development to improve horticultural traits, enhance fruit quality, and insect resistance. With that vision, the proposed study was planned and conducted accordingly.

3.1. Inter-Varietal Grouping Variation

Concerning fixed effects of accessions, significant differences among accessions were seen for all morphological and fruit quality traits evaluated during both years, except for branches, productivity, and edible part evaluated during 2019, the last two traits, and across years (Table 1). For replications, no differences were found in either year's evaluation, whereas for the random effect of year, number of branches, productivity per plant, fruit width, and ferric reducing antioxidant power (FRAP) showed significant differences. Besides fixed and random effects, interactions between the main effects of accession and replication were significant in both years. However, when the data of each year were pooled together, the interaction between accession and replication was not significant for branches, productivity, or edible part. Similarly, the interaction between accession (fixed effect) and year (random effect) was significantly different for all morphological and fruit quality traits; however, there was no interaction between replication and year (Table 1). Overall, significant interactions between fixed (accession) and random effects (replication and year) indicated that the fixed effect was always significantly different when the interaction was significant, except in the case of number of branches evaluated in both years and productivity in 2019 and pooled across both years (Table 1).

Table 1. Analysis of variance (ANOVA) for plant and fruit morphological and quality traits of studied accessions.

	2018			**2019**			**Across Years**					
Traits	**Acces**	**Repl**	**A*R Inter**	**Acces**	**Repl**	**A*R Inter**	**Acces**	**Repl**	**Year**	**A*R Inter**	**A*Y Inter**	**R*Y Inter**
DF	2	2	4	2	2	4	2	2	1	4	2	2
Morphological Traits:												
Plant Height	3.20 **	0.01	46.95 ***	4.41 ***	0.79	7757 ***	4.99 ***	0.142	1.77	5.03 ***	3.98 ***	1.06
Stem Height	2.02 *	0.20	4487 ***	3.44 **	0.83	29.01 ***	2.71 ***	0.01	0.15	2.58 **	3.05 ***	0.03
Branches	1.88	0.36	576.38 ***	1.25	0.03	21.39 ***	1.72 *	1.01	4.98 *	1.58	1.92 *	1.88
Productivity	2.45 **	0.01	71.99 ***	1.83	0.95	1086 ***	0.86	0.01	16.7 ***	0.90	2.27 **	0.01
Fruit Length	73.94 ***	0.26	11.08 ***	106.12 ***	0.02	3.77 ***	97.67 ***	0.10	0.03	71.15 ***	64.82 ***	0.42
Fruit Width	10.23 ***	0.39	5.20 ***	25.81 ***	0.03	5.64 ***	23.29 ***	0.06	6.71 **	15.75 ***	15.39 ***	0.13
Fruit Wall Thickness	10.94 ***	0.04	12.02 ***	7.06 ***	0.36	6.85 ***	10.13 ***	3.53	0.06	9.79 ***	9.35 ***	1.26
Locules	3.44 **	0.02	21.80 ***	3.98 ***	0.32	22.10 ***	6.41 ***	0.18	0.30	5.03 ***	4.29 ***	0.67
Fruit Weight	10.32 ***	0.07	12.07 ***	8.38 ***	0.01	14.83 ***	13.10 ***	0.21	0.18	8.58 ***	8.61 ***	0.05
Edible Part	2.98 **	0.15	267.76 ***	1.71	1.86	556.17 ***	1.23	3.28	1.92	1.16	2.78 ***	2.17
Fruit Quality Traits:												
Dry Matter	13.50 ***	0.01	508.26 ***	31.30 ***	0.01	73.64 ***	24.31 ***	0.15	0.36	23.16 ***	23.68 ***	0.003
TSSs	19.82 ***	0.14	42.51 ***	32.08 ***	0.03	25.74 ***	37.28 ***	0.03	0.96	30.58 ***	33.17 ***	0.28
FRAP	1.93	0.49	145.52 ***	16.15 ***	0.03	29.70 ***	6.17 ***	0.41	17.2 ***	4.99 ***	8.30 ***	0.04
Total Polyphenols	7.65 ***	0.79	105.50 ***	37.14 ***	0.01	36.41 ***	11.36 ***	0.62	002	9.39 ***	25.99 ***	0.11

*, **, *** Significant differences among accessions at $p < 0.05$, <0.01, and <0.001, respectively. Abbreviations: A: accession, R: replication, Y: year, Inter: interaction, DF: degrees of freedom, TSSs: total soluble solids, and FRAP: ferric reducing antioxidant power.

3.2. Intra-Varietal Grouping Variation

3.2.1. Plant Traits

Concerning plant traits, no big differences among the varietal types were seen for plant height, stem height, branches of the first order, or productivity (Figure 2 and Table S3). The variation among varietal types for all plant traits was within 10% of the average across varietal groups. Among morphological traits, plants were tallest in the blocky varietal type, with 80.17 cm, while the pumpkin-shape type was the shortest, with 71.90 cm (Figure 2 and Table 2). Moon et al. reported similar averages for plant heights of *C. annuum* (138.93 cm) and *C. frutescens* (137.55 cm) accessions, but they established a wide variation between studied accessions in each group [31]. The abovementioned accessions also had the tallest stems and the highest productivity in their group. In our study, stem height was shortest in ratund or pumpkin shape (21.82 cm) and longest in the corniform type (25.25 cm). The blocky, kapia and ratund types formed 2.80, 2.79, and 2.76 primary branches, respectively, while the corniform type had 2.42 primary branches (Figure 2). The productivity per plant was from 0.50 kg/plant for the corniform type to 0.60 kg/plant for the blocky type.

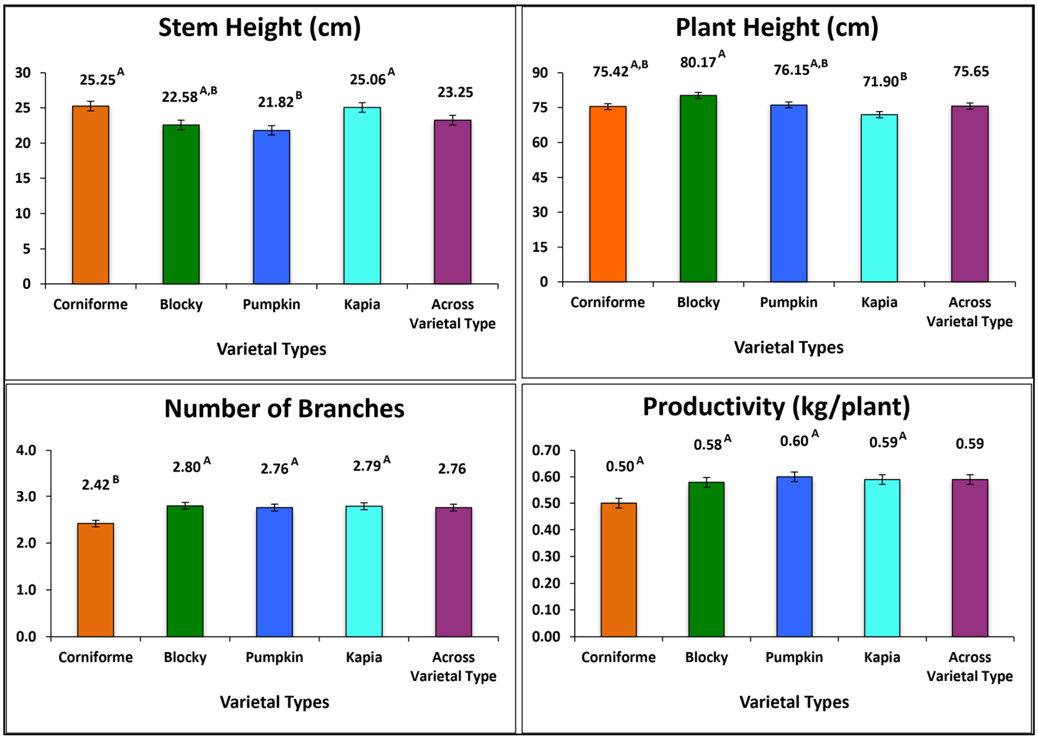

Figure 2. Bar chart depicting plant trait variation among varietal types. Mean separation of varietal groups was assessed using *t*-test, (LSD), and means with the same letters do not show significant differences among varietal groups.

Productivity and growth variables comprising plant height, stem height, and number of main branches were used in describing the pepper genotypes and determining their utility. Plant height and stem height are considered important for mechanical harvesting. Among all studied accessions, K1100 (92.08 cm), belonging to the blocky type, was the tallest, while the K1103B (57.50 cm) kapia type was the shortest (Table 2). The pumpkin-type accessions K1115 (17.92 cm) and K1103A (18.33 cm) demonstrated the lowest stem height, while kapia types K1093 (28.75 cm) and K1074 (27.92 cm) had the longest stems (Table 2). Elizondo-Cabalceta and Monge-Perez reported the biggest variation in stem height in bell peppers, ranging from 11.38 cm to 31.38 cm [32], while our results showed much smaller variation, ranging from 20.42 cm to 24.58 cm. Concerning the number of branches, we observed a range of 2.42 (genotypes K696 (corniform) and K1056 (pumpkin shape)) to 3.25 (K712 (pumpkin shape)). Our results corroborate an earlier study [33];

however, the latter reported larger variation in the number of branches (2.3 to 5.3) than we observed. Concerning productivity, K1115 (pumpkin shape) and K1112 (blocky) were the most and least productive accessions, with 0.74 kg per plant and 0.46 kg per plant, respectively (Table 2). Among the kapia-type accessions, K1081 stood out with 0.70 kg per plant, followed by K1094 (0.67 kg per plant).

Table 2. Passport data of the evaluated pepper accessions along with plant traits and productivity means and standard errors. Mean separation of the evaluated accessions was assessed using *t*-test, (LSD), and means with the same letters do not show significant differences.

Code	Accession	Name	Origin	Population Type	Plant Height (cm)	Stem Height (cm)	Branches (n)	Productivity (kg/plant)
Corniform								
G1	K696	Chorbadzhiyski Sladak	Bulgaria	Local Form	$75.42^{\text{E–H}} \pm 13.39$	$25.25^{\text{A–D}} \pm 7.56$	$2.42^{\text{E}} \pm 0.51$	$0.50^{\text{B–D}} \pm 0.15$
Dolma (Blocky)								
G2	K1086	B2E0048	Bulgaria	Local Form	$70.42^{\text{G–J}} \pm 9.64$	$20.42^{\text{E,F}} \pm 4.50$	$2.58^{\text{C–E}} \pm 0.51$	$0.66^{\text{A–C}} \pm 0.33$
G3	K1098	89601135/25466	Greece	Breeding Line	$74.17^{\text{E–I}} \pm 17.9$	$22.08^{\text{C–F}} \pm 7.82$	$2.92^{\text{A–C}} \pm 0.51$	$0.61^{\text{A–D}} \pm 0.34$
G4	K1099	89601136/25467	Greece	Breeding Line	$76.25^{\text{D–H}} \pm 13.51$	$22.08^{\text{C–F}} \pm 3.34$	$3.00^{\text{A,B}} \pm 0.00$	$0.53^{\text{B–D}} \pm 0.21$
G5	K1100	89601137/25468	Greece	Breeding Line	$92.08^{\text{A}} \pm 12.33$	$24.58^{\text{A–E}} \pm 7.82$	$3.00^{\text{A,B}} \pm 0.60$	$0.64^{\text{A–D}} \pm 0.23$
G6	K1112	B1E0372	Albania	Local Form	$87.92^{\text{A,B}} \pm 9.88$	$23.75^{\text{B–E}} \pm 6.08$	$2.50^{\text{D,E}} \pm 0.52$	$0.46^{\text{B–D}} \pm 0.11$
Pumpkin								
G7	K1053	B1E0021	Bulgaria	Local Form	$75.00^{\text{D–H}} \pm 9.53$	$26.67^{\text{A–C}} \pm 5.37$	$2.67^{\text{B–E}} \pm 0.49$	$0.50^{\text{B–D}} \pm 0.12$
G8	K1055	B1E0059	Bulgaria	Local Form	$72.08^{\text{F–J}} \pm 10.76$	$22.08^{\text{C–F}} \pm 4.98$	$2.75^{\text{B–E}} \pm 0.45$	$0.50^{\text{C,D}} \pm 0.11$
G9	K1056	B1E0061	Bulgaria	Local Form	$82.92^{\text{A–E}} \pm 12.52$	$21.67^{\text{D–F}} \pm 3.89$	$2.42^{\text{E}} \pm 0.51$	$0.59^{\text{A–D}} \pm 0.15$
G10	K1057	B1E0062	Bulgaria	Local Form	$80.42^{\text{B–G}} \pm 9.88$	$23.34^{\text{B–E}} \pm 4.44$	$2.67^{\text{B–E}} \pm 0.67$	$0.65^{\text{A–D}} \pm 0.12$
G11	K1083	B2E0040	Bulgaria	Local Form	$74.58^{\text{D–H}} \pm 10.76$	$20.42^{\text{E,F}} \pm 3.96$	$2.58^{\text{C–E}} \pm 0.51$	$0.49^{\text{B–D}} \pm 0.09$
G12	K1103A	Ruminska Sipka	Unknown	Not Applicable	$68.33^{\text{H–J}} \pm 13.87$	$18.33^{\text{F}} \pm 5.77$	$2.83^{\text{B–D}} \pm 0.58$	$0.67^{\text{A–C}} \pm 0.12$
G13	K1115	B1E0405	Albania	Local Form	$69.17^{\text{H–J}} \pm 12.94$	$17.92^{\text{F}} \pm 5.42$	$2.92^{\text{A–C}} \pm 0.51$	$0.74^{\text{A}} \pm 0.31$
G14	K712	Kambi S-34	Bulgaria	Local Form	$86.67^{\text{A–C}} \pm 11.35$	$24.17^{\text{A–E}} \pm 4.69$	$3.25^{\text{A}} \pm 0.45$	$0.64^{\text{A–D}} \pm 0.17$
Kapia								
G15	K1074	B1E0250	North Macedonia	Local Form	$77.08^{\text{C–H}} \pm 11.37$	$27.92^{\text{A,B}} \pm 4.50$	$3.00^{\text{A,B}} \pm 0.43$	$0.56^{\text{A–D}} \pm 0.20$
G16	K1081	B2E0034	Bulgaria	Local Form	$64.17^{\text{I–K}} \pm 9.25$	$23.75^{\text{B–E}} \pm 7.72$	$2.67^{\text{B–E}} \pm 0.49$	$0.70^{\text{A,B}} \pm 0.33$
G17	K1093	B1E0504	Bulgaria	Local Form	$75.00^{\text{D–H}} \pm 12.61$	$28.75^{\text{A}} \pm 5.28$	$2.83^{\text{B–D}} \pm 0.39$	$0.55^{\text{A–D}} \pm 0.12$
G18	K1094	B1E0525	Bulgaria	Local Form	$84.58^{\text{A–D}} \pm 13.73$	$26.25^{\text{A–D}} \pm 4.33$	$2.92^{\text{A–C}} \pm 0.51$	$0.67^{\text{A–C}} \pm 0.12$
G19	K1103B	Ruminska Sipka	Unknown	Not Applicable	$57.50^{\text{K}} \pm 9.17$	$20.00^{\text{E,F}} \pm 3.69$	$2.67^{\text{B–E}} \pm 0.49$	$0.54^{\text{A–D}} \pm 0.16$
G20	K1114	B1E0378	Bulgaria	Local Form	$82.08^{\text{A–F}} \pm 12.15$	$26.67^{\text{A–C}} \pm 5.37$	$2.58^{\text{C–E}} \pm 0.51$	$0.60^{\text{A–D}} \pm 0.20$
G21	K697	Kapia Sladka S-11	Bulgaria	Local Form	$62.92^{\text{J,K}} \pm 6.89$	$22.08^{\text{C–F}} \pm 3.96$	$2.83^{\text{B–D}} \pm 0.39$	$0.48^{\text{B–D}} \pm 0.11$

3.2.2. Fruit Morphology Traits

For fruit traits, noticeable differences were seen among different varietal types, wherein the corniform type had the longest fruit (22.67 cm), but was smallest in terms of width (2.12 cm), weight (29.29 g), locules (2.17), and wall thickness (2.09 mm). The blocky type had the most locules (3.35), while the pumpkin type had the shortest (3.91 cm), broadest (7.62 cm), and heaviest (124.13 g) fruit, as well as the thickest walls (5.69 mm) (Figure 3). However, the variation seen among the types was variable for each fruit trait in comparison to the average of all varietal groups, as most traits showed less than 10% variation, except on fruit length and fruit weight. The fruit trait variation among the groups was broad, with the main contributors appearing to be length (2.0–27.5 cm), width (1.70–10.40 cm), wall thickness (1.48–8.14 mm), and weight (15.5–245.0 g) (Table S4).

Accession-wise, the corniform-type K696 (22.67 cm) had the longest fruit, followed by the kapia-type K1114 (16.27 cm), while the pumpkin-shaped K1103A had the shortest (2.87 cm) fruit (Table 3). The pumpkin-shaped accessions K1055 and K1056 were the widest: 8.58 cm and 8.08 cm, respectively. These were also the heaviest in this group: 151.82 g and 148.11 g, respectively. K1055 also had the longest (4.48 cm) fruit in its group. Among all accessions, K1094 (kapia type) formed the heaviest (178.48 g) and widest (7.37 cm) fruit in this varietal type (Table 3). Fruit wall thickness was significantly greater in accession K1057 (6.66 mm), belonging to the pumpkin-shaped varietal, than other genotypes. All genotypes from the pumpkin-shaped varietal demonstrated thicker fruit walls than the rest of the studied accessions. Concerning the number of locules in the fruit of K1098 and

K1099 (blocky type), these were mostly four-lobed (3.58). Moon et al.'s comprehensive study of 380 *C. annuum* accessions reported an average 10.06 cm fruit length with a range of 1.20 cm to 22.30 cm and average fruit weight of 26.93 g with a very broad range of 0.30 g to 218.40 g. In the same study, 133 accessions of *C. frutescens* had an average fruit length of 3.96 cm with a wide range of 1.00 cm to 16.00 cm, whereas the average fruit weight was 3.47 g with a range of 0.20 g to 42.30 g [31]. Furthermore, Moon et al. observed that the fruit weight, diameter (width), and wall thickness were highly correlated with one another, ranging from 0.84–0.93 [31], and we made a similar observation. Another study also reported a significant positive correlation of yield per plant with fruit length (0.602) and fruit weight (0.642), which demonstrates that yield components are highly correlated with one another [34].

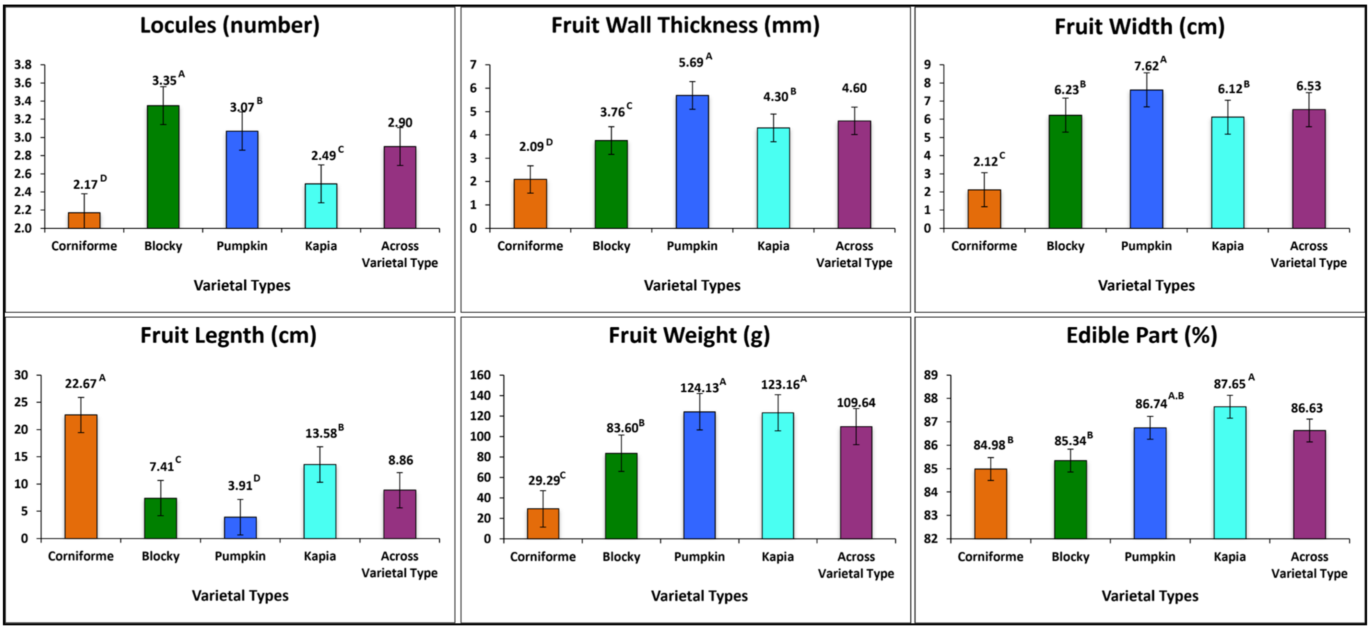

Figure 3. Bar chart depicting fruit morphology trait variation among varietal types. Mean separation of varietal groups was assessed using *t*-tests (LSD), and means with the same letters do not show significant differences among varietal groups.

Table 3. Evaluation of pepper accessions for important fruit morphology traits. Uppercase letters represent accession mean differences within each varietal group.

Code	Accession	Locules (n)	Fruit				Edible Part (%)
			Length (cm)	Width (cm)	Thickness (mm)	Weight (g)	
				Corniform			
G1	K696	$2.17^{\mathrm{H}} \pm 0.39$	$22.67^{\mathrm{A}} \pm 2.72$	$2.12^{\mathrm{G}} \pm 0.29$	$2.09^{\mathrm{J}} \pm 0.46$	$29.29^{\mathrm{J}} \pm 8.47$	$84.98^{\mathrm{D–G}} \pm 6.11$
				Blocky			
G2	K1086	$3.25^{\mathrm{A,B}} \pm 0.45$	$7.65^{\mathrm{D}} \pm 1.47$	$6.03^{\mathrm{F}} \pm 1.09$	$4.42^{\mathrm{E–G}} \pm 1.05$	$87.06^{\mathrm{H,I}} \pm 32.23$	$88.41^{\mathrm{A–C}} \pm 3.60$
G3	K1098	$3.58^{\mathrm{A}} \pm 0.51$	$7.53^{\mathrm{D}} \pm 0.67$	$6.20^{\mathrm{E,F}} \pm 0.38$	$3.48^{\mathrm{I}} \pm 0.54$	$76.38^{\mathrm{I}} \pm 14.59$	$85.29^{\mathrm{C–F}} \pm 2.37$
G4	K1099	$3.58^{\mathrm{A}} \pm 0.67$	$7.63^{\mathrm{D}} \pm 0.79$	$6.64^{\mathrm{E,F}} \pm 0.80$	$3.35^{\mathrm{I}} \pm 0.69$	$98.69^{\mathrm{G,H}} \pm 19.85$	$84.58^{\mathrm{E–G}} \pm 3.96$
G5	K1100	$3.25^{\mathrm{A,B}} \pm 0.62$	$7.44^{\mathrm{D}} \pm 0.70$	$6.23^{\mathrm{E,F}} \pm 0.57$	$3.81^{\mathrm{G–I}} \pm 1.14$	$81.27^{\mathrm{H,I}} \pm 15.04$	$83.89^{\mathrm{G–F}} \pm 2.60$
G6	K1112	$3.08^{\mathrm{B–D}} \pm 0.51$	$6.78^{\mathrm{D}} \pm 0.89$	$6.06^{\mathrm{F}} \pm 0.64$	$3.76^{\mathrm{G–I}} \pm 0.48$	$74.62^{\mathrm{I}} \pm 16.56$	$84.52^{\mathrm{E–G}} \pm 6.04$
				Pumpkin			
G7	K1053	$3.08^{\mathrm{B–D}} \pm 0.51$	$3.42^{\mathrm{E,F}} \pm 0.94$	$7.61^{\mathrm{B,C}} \pm 0.79$	$5.14^{\mathrm{D,E}} \pm 0.87$	$124.64^{\mathrm{D,E}} \pm 13.32$	$86.94^{\mathrm{A–F}} \pm 3.45$
G8	K1055	$3.25^{\mathrm{A,B}} \pm 0.45$	$4.48^{\mathrm{E}} \pm 0.94$	$8.58^{\mathrm{A}} \pm 0.89$	$5.96^{\mathrm{A,B}} \pm 1.07$	$151.82^{\mathrm{B}} \pm 45.14$	$86.84^{\mathrm{A–F}} \pm 2.90$
G9	K1056	$3.17^{\mathrm{B,C}} \pm 0.39$	$4.06^{\mathrm{E,F}} \pm 1.06$	$8.08^{\mathrm{A,B}} \pm 1.16$	$5.71^{\mathrm{B,C}} \pm 1.10$	$148.11^{\mathrm{B,C}} \pm 31.29$	$87.24^{\mathrm{A–F}} \pm 7.37$
G10	K1057	$3.08^{\mathrm{B–D}} \pm 0.67$	$4.35^{\mathrm{E}} \pm 0.92$	$7.59^{\mathrm{B,C}} \pm 0.80$	$6.66^{\mathrm{A}} \pm 1.26$	$129.59^{\mathrm{C–E}} \pm 13.72$	$85.23^{\mathrm{C–F}} \pm 3.04$
G11	K1083	$2.83^{\mathrm{C–F}} \pm 0.72$	$4.13^{\mathrm{E}} \pm 0.74$	$7.54^{\mathrm{B–D}} \pm 0.88$	$5.83^{\mathrm{B,C}} \pm 0.87$	$128.85^{\mathrm{C–E}} \pm 48.21$	$88.98^{\mathrm{A,B}} \pm 3.70$
G12	K1103A	$2.75^{\mathrm{D–F}} \pm 0.45$	$2.87^{\mathrm{F}} \pm 0.68$	$6.18^{\mathrm{E,F}} \pm 0.38$	$5.28^{\mathrm{B–D}} \pm 1.05$	$68.86^{\mathrm{I}} \pm 11.09$	$83.44^{\mathrm{G}} \pm 5.02$
G13	K1115	$3.17^{\mathrm{B,C}} \pm 0.39$	$4.08^{\mathrm{E,F}} \pm 0.92$	$7.68^{\mathrm{B,C}} \pm 0.79$	$5.65^{\mathrm{B,C}} \pm 0.97$	$120.16^{\mathrm{D–F}} \pm 17.86$	$87.39^{\mathrm{A–E}} \pm 3.50$
G14	K712	$3.25^{\mathrm{A,B}} \pm 0.45$	$3.91^{\mathrm{E,F}} \pm 0.98$	$7.69^{\mathrm{B,C}} \pm 0.72$	$5.31^{\mathrm{B–D}} \pm 1.00$	$121.01^{\mathrm{D,E}} \pm 24.19$	$87.89^{\mathrm{A–E}} \pm 4.86$

Table 3. *Cont.*

Code	Accession	Locules (n)	Fruit				Edible Part (%)
			Length (cm)	Width (cm)	Thickness (mm)	Weight (g)	
Kapia							
G15	K1074	2.33 $^{\text{H–G}}$ ± 0.49	12.83 $^{\text{C}}$ ± 1.34	6.88 $^{\text{D,E}}$ ± 1.00	4.71 $^{\text{D–F}}$ ± 1.01	131.69 $^{\text{B–D}}$ ± 27.69	89.11 $^{\text{A,B}}$ ± 2.91
G16	K1081	2.17 $^{\text{H}}$ ± 0.39	12.93 $^{\text{C}}$ ± 1.39	6.14 $^{\text{F}}$ ± 1.33	4.37 $^{\text{E–H}}$ ± 1.07	122.53 $^{\text{D,E}}$ ± 28.44	88.10 $^{\text{A–D}}$ ± 2.88
G17	K1093	2.58 $^{\text{F,G}}$ ± 0.51	13.26 $^{\text{C}}$ ± 0.90	6.26 $^{\text{E,F}}$ ± 0.72	4.07 $^{\text{F–I}}$ ± 1.28	138.60 $^{\text{B–D}}$ ± 21.49	89.57 $^{\text{A}}$ ± 2.07
G18	K1094	2.33 $^{\text{H–G}}$ ± 0.49	13.57 $^{\text{C}}$ ± 1.59	7.37 $^{\text{C,D}}$ ± 0.80	4.66 $^{\text{D–F}}$ ± 0.31	178.48 $^{\text{A}}$ ± 25.41	88.10 $^{\text{A–D}}$ ± 2.73
G19	K1103B	2.67 $^{\text{E–G}}$ ± 0.78	12.88 $^{\text{C}}$ ± 1.76	6.16 $^{\text{F}}$ ± 1.16	4.63 $^{\text{D–F}}$ ± 1.20	111.02 $^{\text{E–G}}$ ± 28.76	86.00 $^{\text{B–F}}$ ± 3.50
G20	K1114	2.33 $^{\text{H–G}}$ ± 0.49	16.27 $^{\text{B}}$ ± 3.13	5.11 $^{\text{G}}$ ± 0.44	4.02 $^{\text{F–I}}$ ± 1.02	99.67 $^{\text{F–H}}$ ± 25.77	86.29 $^{\text{A–F}}$ ± 6.07
G21	K697	3.00 $^{\text{B–E}}$ ± 0.00	13.33 $^{\text{C}}$ ± 1.09	4.92 $^{\text{G}}$ ± 0.40	3.65 $^{\text{I,H}}$ ± 0.49	80.16 $^{\text{H,I}}$ ± 9.47	86.34 $^{\text{A–F}}$ ± 2.36

3.2.3. Fruit Quality Traits

The comprehensive characterization of studied accessions included an assessment of major fruit quality traits (Table 4). Among six accessions harvested in the intermediate stage of maturity, K696 of the corniform type distinctly stood out, showing the highest values for all four quality traits, with 8.93% dry matter, 4.90 °Brix TSSs, 79.01 mg GAE/100 g FW total polyphenols, and 4.37 µmol Fe^{2+}/g FW FRAP. Among the blocky-type accessions, K1100 and K1112 had the highest values for dry matter content (7.55% and 7.53%), and TSSs (4.50 and 4.47 °Brix), while K1086 and K1098 had the highest ferric reducing antioxidant power (3.72 and 3.12 µmol Fe^{2+}/g FW) and total polyphenols (67.64 and 67.81 mg GAE/100 g FW). Predominantly, accessions that were harvested at botanical maturity had significantly higher levels of the studied traits than those that were harvested at intermediate maturity.

Table 4. Assessment of pepper accessions for fruit quality traits. Uppercase letters represent accession mean differences within each varietal group.

Code	Accession	Harvesting	Dry Matter (%)	TSSs (Brix)	FRAP (µmol Fe^{2+}/g FW)	Total Polyphenols (mg GAE/100 g FW)
Corniform						
G1	K696	before $^{\#}$	8.93 $^{\text{G}}$ ± 0.53	4.90 $^{\text{G}}$ ± 0.55	4.37 $^{\text{F,G}}$ ± 1.34	79.01 $^{\text{J}}$ ± 25.92
Blocky						
G2	K1086	before	6.64 $^{\text{I}}$ ± 0.47	4.45 $^{\text{G,H}}$ ± 0.28	3.72 $^{\text{G}}$ ± 0.67	67.64 $^{\text{J,K}}$ ± 17.43
G3	K1098	before	7.40 $^{\text{H}}$ ± 0.56	4.43 $^{\text{G,H}}$ ± 0.73	3.12 $^{\text{G}}$ ± 0.78	67.81 $^{\text{J,K}}$ ± 20.95
G4	K1099	before	6.65 $^{\text{I}}$ ± 0.56	4.13 $^{\text{H}}$ ± 0.34	2.20 $^{\text{G}}$ ± 0.54	54.66 $^{\text{K}}$ ± 10.21
G5	K1100	before	7.55 $^{\text{H}}$ ± 0.24	4.50 $^{\text{G,H}}$ ± 0.47	2.81 $^{\text{G}}$ ± 0.38	61.63 $^{\text{J,K}}$ ± 8.97
G6	K1112	before	7.53 $^{\text{H}}$ ± 0.69	4.47 $^{\text{G,H}}$ ± 0.45	2.56 $^{\text{G}}$ ± 0.51	61.47 $^{\text{J,K}}$ ± 19.10
Pumpkin						
G7	K1053	at maturity $^{\#\#}$	10.02 $^{\text{D–F}}$ ± 1.35	8.07 $^{\text{C–E}}$ ± 1.47	8.40 $^{\text{B–E}}$ ± 3.87	138.58 $^{\text{F–H}}$ ± 26.64
G8	K1055	at maturity	9.97 $^{\text{D–F}}$ ± 0.58	7.87 $^{\text{D,E}}$ ± 0.55	11.48 $^{\text{A}}$ ± 4.65	161.82 $^{\text{C–E}}$ ± 34.04
G9	K1056	at maturity	9.64 $^{\text{E,F}}$ ± 0.21	7.53 $^{\text{E,F}}$ ± 0.43	10.17 $^{\text{A–C}}$ ± 3.11	150.48 $^{\text{D–F}}$ ± 42.70
G10	K1057	at maturity	10.02 $^{\text{D–F}}$ ± 0.77	7.58 $^{\text{E,F}}$ ± 0.77	8.67 $^{\text{B–E}}$ ± 4.74	154.54 $^{\text{C–F}}$ ± 55.86
G11	K1083	at maturity	10.52 $^{\text{C,D}}$ ± 1.01	8.50 $^{\text{B,C}}$ ± 1.01	8.46 $^{\text{B–E}}$ ± 2.82	140.16 $^{\text{E–H}}$ ± 20.96
G12	K1103A	at maturity	10.23 $^{\text{C–E}}$ ± 0.70	7.80 $^{\text{D–F}}$ ± 0.73	11.49 $^{\text{A}}$ ± 5.39	189.55 $^{\text{A}}$ ± 43.79
G13	K1115	at maturity	9.56 $^{\text{F,G}}$ ± 0.55	7.22 $^{\text{F}}$ ± 0.62	8.08 $^{\text{C–E}}$ ± 2.99	175.59 $^{\text{B,C}}$ ± 53.50
G14	K712	at maturity	10.73 $^{\text{B,C}}$ ± 0.53	7.97 $^{\text{C–E}}$ ± 0.73	9.99 $^{\text{A–D}}$ ± 3.98	203.44 $^{\text{A}}$ ± 46.64
Kapia						
G15	K1074	at maturity	10.57 $^{\text{C,D}}$ ± 0.74	8.78 $^{\text{A,B}}$ ± 0.56	6.47 $^{\text{E,F}}$ ± 1.26	114.51 $^{\text{I}}$ ± 20.86
G16	K1081	at maturity	10.52 $^{\text{C,D}}$ ± 0.68	8.42 $^{\text{B–D}}$ ± 0.66	6.70 $^{\text{E}}$ ± 1.18	122.90 $^{\text{H,I}}$ ± 27.68
G17	K1093	at maturity	10.46 $^{\text{C,D}}$ ± 0.40	8.73 $^{\text{B}}$ ± 0.51	7.05 $^{\text{E}}$ ± 1.89	123.31 $^{\text{G–I}}$ ± 23.88
G18	K1094	at maturity	9.95 $^{\text{D–F}}$ ± 0.71	8.37 $^{\text{B–D}}$ ± 0.66	7.79 $^{\text{D,E}}$ ± 1.93	125.78 $^{\text{G–I}}$ ± 30.29
G19	K1103B	at maturity	10.33 $^{\text{C,D}}$ ± 0.96	7.95 $^{\text{C–E}}$ ± 0.15	8.22 $^{\text{C–E}}$ ± 1.64	138.55 $^{\text{F–H}}$ ± 27.99
G20	K1114	at maturity	11.66 $^{\text{A}}$ ± 0.60	9.38 $^{\text{A}}$ ± 0.58	7.41 $^{\text{E}}$ ± 1.97	144.97 $^{\text{D–G}}$ ± 12.77
G21	K697	at maturity	11.28 $^{\text{A,B}}$ ± 0.74	8.78 $^{\text{A,B}}$ ± 0.74	10.60 $^{\text{A,B}}$ ± 1.92	166.04 $^{\text{C,D}}$ ± 19.42

Legend: $^{\#}$ Well-ripened fruit in intermediate-maturity stage—green, pale yellow, etc.; $^{\#\#}$ well-ripened fruit at maturity—red, orange, brown, etc. Abbreviations: TSSs: total soluble solids and FRAP: ferric reducing antioxidant power.

Among the accessions harvested at maturity, pumpkin-shaped K712 showed the highest content of total polyphenols (203.44 mg GAE/100 g FW) (Table 4) and ranked first in this group for dry matter content (10.73%). Accession K1103A showed high total polyphenols (189.55 mg GAE/100 g FW) and ferric reducing antioxidant power (11.49 µmol Fe^{2+}/g FW).

Among kapia-type accessions, K697 showed the highest values for total polyphenols (166.04 mg GAE/100 g FW) and ferric reducing antioxidant power (10.60 µmol Fe^{2+}/g FW), whereas accession K1114 had the highest values of dry matter content (11.66%) and TSSs (9.38 °Brix) (Table 4). Wide variation was determined for ferric reducing antioxidant power (FRAP), from 2.20 (K1099) to 11.49 µmol Fe^{2+}/g FW (K1103A), and total phenolic content, from 54.66 (K1099) to 203.44 mg GAE/100 g FW (K712). Luitel et al. [35] reported similar variation in total soluble solids—from 5.3 °Brix to 8.5 °Brix in a collection of 55 sweet pepper genotypes. Many other studies have shown that pepper fruit is rich in phytochemicals with antioxidant properties (Howard et al. [3], Bogusz Junior et al. [36], Constantino et al. [37] and examined the natural variation that occurs among species and cultivars. Similarly, we also demonstrated varying levels of important compounds, even in a small germplasm collection. All these factors can influence our dietary considerations on consumption of different pepper types.

Variation among varietal types was seen to be higher for all fruit quality traits (Figure 4 and Table S5). Most accessions belonging to the kapia type had higher dry matter content than those of pumpkin shape. Types that were harvested in the intermediate stage had lower accumulation of phytochemicals (Figure 4), wherein average dry matter content accumulated least in blocky (7.15%) and kapia (10.68%). A similar tendency was also seen for TSSs, wherein the lowest values were observed in the blocky type (4.40 °Brix) and the highest in the kapia type (8.63 °Brix). For phenolic compounds and ferric reducing antioxidant power (FRAP), the trend continued, wherein accumulation of these compounds was higher in varietal types harvested at a mature stage (pumpkin-shaped and kapia) than varietal types (blocky and corniform) harvested at an immature stage (Figure 4); however, pumpkin-shaped accessions had higher ferric reducing antioxidant power (9.59 µmol Fe^{2+}/g FW) and total polyphenols (164.27 mg GAE/100 g FW) than kapia-type genotypes (Figure 4).

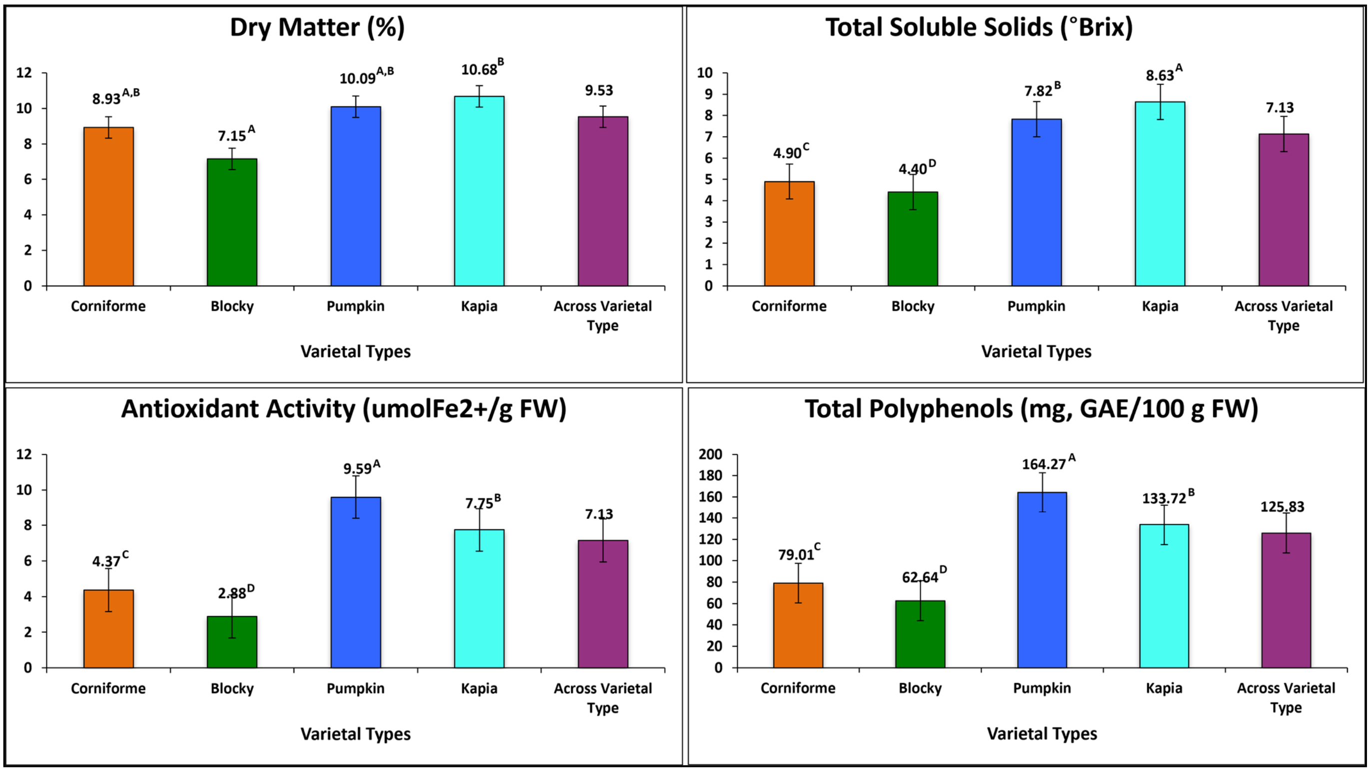

Figure 4. Bar chart depicting fruit quality trait variation among varietal types. Mean separation of varietal groups was assessed using *t*-tests (LSD), and means with the same letters do not show significant differences among varietal groups.

3.2.4. Insect and Pest Resistance

The range of pathogens affecting peppers is very broad, and includes fungi, viruses, bacteria, and insects. Considering the effect of biotic stresses limiting yield and productivity, a large number of domesticated and wild species accessions are stored in seed banks throughout the world, representing a valuable resource for breeding improvement by trait introgression and wide hybridization of traits associated with biotic stress resistance [38]. In recent years, there have been changes in the species composition and population density of insect pests, largely due to climate change, emergence of populations resistant to insecticides, and intensive commercial exchange of produce. Establishing well-adapted and resistant/tolerant sources of pest resistance is important to ensure optimal yield and increasing productivity at the producer level while maintaining food security at the global level. In this study, 21 pepper accessions were evaluated against a natural background of pest infestation of green peach aphid (*Myzus persicae* Sulz.), thrips (*Frankliniella occidentalis* Perg. and *Thrips tabaci* Lindeman) and cotton bollworm (*Helicoverpa armigera* Hubn.) in the field. During the evaluation, around 38% of accessions belonging to different varietal types showed no infestation by aphids (Table 5). A low population density of aphids was found, with the highest infestation rate reaching only 0.67, seen in K1098.

Table 5. Infestation by insect pests in pepper accessions grown in open field.

Code	Accession	Green Peach Aphid		Thrips		Cotton Bollworm	
		Damaged Plants (%)	Degree of Infestation	Damaged Plants (%)	Degree of Infestation	Damaged Plants (%)	Damaged Fruit (%)
				Corniform			
G1	K696	0.00	0.00	30.95	1.42	5.95	6.67
				Blocky			
G2	K1086	0.00	0.00	39.05	1.17	12.15	11.67
G3	K1098	4.78	0.67	32.88	1.25	14.32	16.67
G4	K1099	0.00	0.00	30.00	1.42	15.84	10.00
G5	K1100	3.13	0.50	34.59	0.92	18.96	8.34
G6	K1112	9.67	0.50	32.34	1.08	13.17	11.67
				Pumpkin			
G7	K1053	0.84	0.09	28.89	0.92	8.52	10.84
G8	K1055	2.50	0.17	41.55	1.09	12.15	11.67
G9	K1056	0.00	0.00	33.34	1.00	10.56	15.84
G10	K1057	0.00	0.00	31.10	1.09	10.27	32.50
G11	K1083	0.84	0.00	40.84	1.00	5.00	5.00
G12	K1103A	0.00	0.00	40.71	1.00	12.69	7.50
G13	K1115	2.50	0.09	38.26	1.17	13.26	8.34
G14	K712	0.00	0.00	39.93	1.00	5.61	11.67
				Kapia			
G15	K1074	2.69	0.59	30.93	0.92	11.21	5.00
G16	K1081	2.50	0.09	39.65	1.25	13.93	9.17
G17	K1093	2.50	0.17	27.98	1.67	13.57	10.84
G18	K1094	0.84	0.09	34.01	1.50	8.23	10.84
G19	K1103B	1.79	0.50	29.65	1.42	8.57	1.67
G20	K1114	3.34	0.17	28.24	0.92	7.87	11.67
G21	K697	0.00	0.00	20.00	0.75	6.67	8.34

Significant differences were found among accessions for damage rate, number of aphids per plant, and number of aphids per leaf in another screening on 21 pepper accessions for resistance to green peach aphid. No accession investigated in this study showed strong resistance against aphid colonization, while commercial cultivars bred for virus resistance appear to have strong tolerance against green peach aphid and thus suffer little damage from it. Similarly, one study that screened a diverse collection of 50 *Capsicum* accessions showed no strong resistance against green peach aphid either [13], corroborating our observation. Considering current resistance status, tolerant cultivars can be important for integrated pest management of green peach aphid [13].

Beside aphids, all accessions were infected by thrips, with damaged plants ranging from 20% (kapia K697) to 41.55% (pumpkin-shaped K1055) and the infestation rate varying from 0.75 (K697) to 1.67 (K1093). Thrips control using biological crop management appears to be inadequate; therefore, the use of thrips-resistant cultivars would be useful to increase the effectiveness of thrips control. To date, there is no commercial pepper variety with a high level of resistance to thrips; however, several wild pepper cultivars have been identified that show resistance against thrips [39]. Additionally, resistance to thrips can also delay and reduce the transmission of viruses such as tomato spotted wilt viruses (TSWV); therefore, inclusion of thrips resistance must be part of pepper breeding programs [40]. However, the number of specimens confirmed to have a high level of thrips resistance as well as the number of thrips species tested is still limited, as there is limited information available on thrips resistance mechanisms in pepper [41].

Screening for cotton bollworm showed that it infected both plants and fruit of all accessions. Low infestation was seen in K1083 (5.00% on plants and fruit), followed by K696 (5.95% on plants and 6.67% on fruit), while severe infestation was seen in K1057 (10.27% damaged plants and 32.50% damaged fruit). Among all accessions, notably K1103B showed much greater plant damage (8.57%) than fruit damage (1.67%) (Table 5).

Among all varietal types, no aphid infestation was seen in accessions belonging to the corniform type, while accessions of the blocky type showed the highest infestation, with 3.51% damage to plants. Besides aphids, thrips infestation was seen to be similar across varietal types (<5%), whereas cotton bollworm infestation was seen least in corniform-type accessions and severe among blocky-type (14.89% plant and 11.67% fruit damage) and pumpkin-shaped (9.75% plant and 12.92% fruit damage) accessions (Figure 5 and Table S6). Overall, aphid infestation was relatively lower among accessions, while thrips and cotton bollworm infestation were highest (Figure 5 and Table S6).

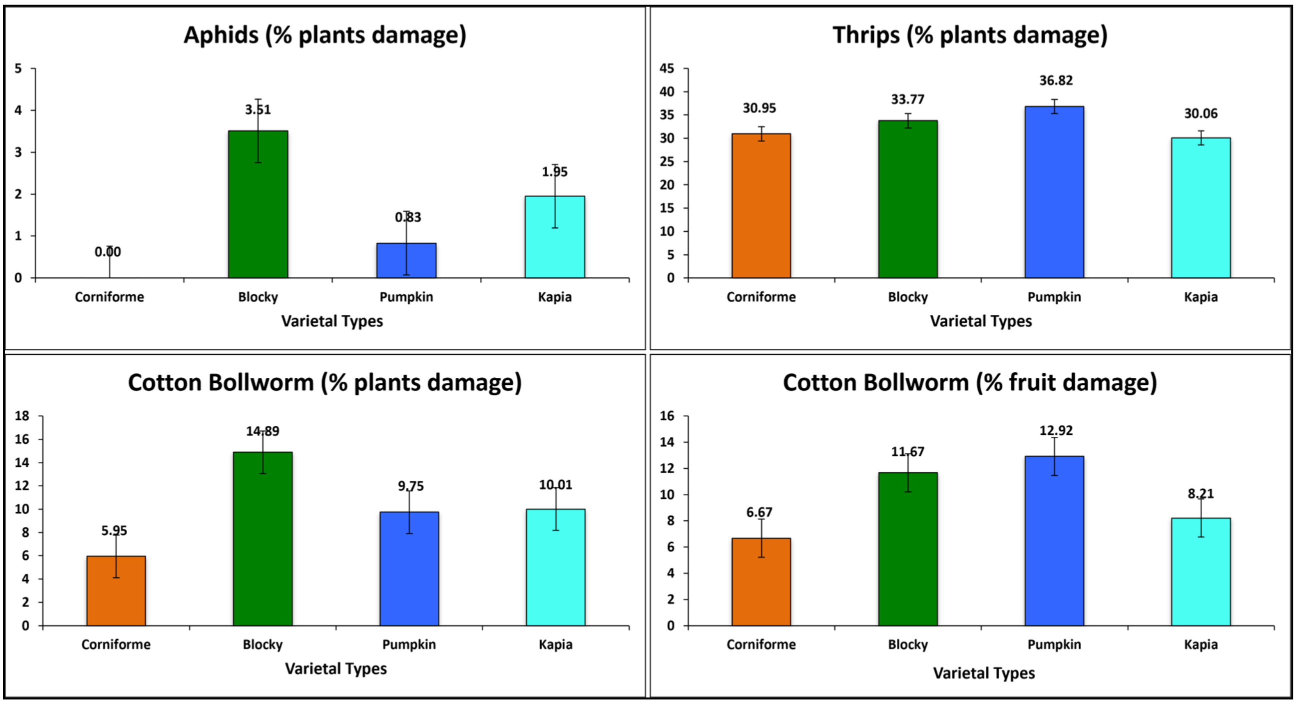

Figure 5. Bar chart depicting insect pests' infestation evaluated across varietal types.

Most breeding programs aim to develop breeding lines and cultivars that possess increased insect resistance and usually complement the integrated plant protection systems. Different studies have been set up to screen diverse pepper accessions and determine their response against pest infestation [14,39,42]. Usually, different indicators are used to evaluate the pepper accessions and provide information about the host response on one hand and its effect on the pest on the other hand. Biological parameters of population density, infection rate, relative growth, and generation time [14,43] are commonly used in host–pest interaction studies. Besides biological parameters, indicators of the degree of infestation, number of larvae or adults per plant, percentage of damaged fruit and plants are often used to assess damage [44]. Additionally, other research has also been conducted to uncover resistance mechanisms, which can be antibiosis or antixenosis. Free-choice and no-choice host tests are performed to assess the response of the plant [45].

Considering our goal of finding potential resistance sources against economically important insects to use for ideotype development, we evaluated the pepper accessions for their response to target insect pests and identified accessions that showed potential to utilize in future breeding efforts as we have utilized similar strategies in the past. Development of pepper cultivars resistant/tolerant to insects is an economic strategy, while uncovering resistance mechanisms is an important element of ecological control approaches, as pesticides have harmful effects on the environment and human health [43,44]. Therefore, alternative pest management strategies are needed in order to control pests frequently causing damage to the sweet pepper. Overall, the use of integrated techniques, including intercropping pepper with other crops, oviposition deterrents, releasing natural predators, and use of resistant cultivars as environment-friendly control methods, have been seen to be very effective [46]. The identified accessions that show promising prospects for the targeted morphological, fruit quality, and biotic stress tolerance traits could be further utilized in ideotype development and subsequently be included in trait introgression and cultivar development for an excellent combination of productivity, fruit morphology, quality, and insect resistance.

3.3. Principal Component Analysis

In order to assess the morphological and fruit quality diversity among Balkan pepper accessions, we examined qualities that reflected trait diversity. Principal component analysis (PCA) was performed on an array of pre-harvest plant and fruit morphological traits and insect screening, as well as fruit quality traits. The variation attributable to these traits can be seen in Figure 6. The variability generated for different morphological and quality traits consisted in a total of 18 principal components (PCs) (Figure S1). However, the first six PCs with eigenvalues >1 contributed around 84.76% of the total variance (Table 6 and Figure S1), wherein the first four components explained 30.74% (PC1), 21.77% (PC2), 11.46% (PC3), and 8.19% (PC4), respectively. Tsonev et al. found that the first three components accounted for 90% of the variance, wherein PC1 and PC2 explained 55.6% and 22.6%, respectively [47]. However, note that the variability explained in Tsonev et al.'s study was based on ISSR markers, which is different from the explained variance observed in our study, as it resulted from morphological and fruit quality variation. Likely the observed variance reported in our study may have been due to the genetic origin of studied germplasm as well as different uses associated with this germplasm. In the negative direction, fruit width, fruit weight, and pericarp thickness and in the positive direction, plant height were reported to be the most strongly correlated traits with the first axis and fruit length and stem height for the second. Martínez-Ispizua et al. determined 15 principal components that described around 98.86% of total variability between landraces, with the first four PCs contributing 52.71% cumulative variance [48]. Similarly to our study, Singh et al. study on chili pepper also reported that the PCs that had the highest eigenvalues contributed the most to cumulative variance explanation [49]. In this study, the first five PCs contributed almost 89% of the explained variance, and most of the PCs that explained the variation were related to plant height, primary branches per plant, days to flowering, and first harvest or picking [49]. However, fruit per plant, fruit length, and fruit width were the lowest contributors to variance, which contradicts our findings. Accession by trait (A*T) biplot between PC1 and PC2 showed that fruit wall thickness, fruit weight, edible part, dry matter, total soluble solids (TSSs), ferric reducing antioxidant power (FRAP), and total polyphenols (TP) were the primary contributing traits to PC1 variance (Figure 6 and Table 6), whereas stem height, fruit length, fruit width, locules, thrips damage and cotton bollworm damage contributed to PC2 variance (Figure 6 and Table 6). Bianchi et al. indicated that the diameter of the fruit (20.19%), height of the plant (19.46%), cup diameter (14.91%) and fruit length (14.57%) were the characters that contributed most to the total divergence (69.13%) among their evaluated accessions [50]. Tsonev et al. reported that fruit width, fruit weight and pericarp thickness in the negative direction and plant height in the positive direction were the most strongly correlated traits with the first axis, while fruit length and stem height correlated with the second axis [47]. Constantino et al. established that the flavonoid content (14.80%) contributed most to the genotype discrimination, followed by the fruit mass (12.81%) and fruit length (11.83%) while the ferric reducing antioxidant power (FRAP) contributed least to the phenotypic divergence (5.57%) of 22 *C. baccatum* accessions [37]. A biplot of PC1 and PC2 shows that the traits associated with PC1 distinctly separated accessions belonging to the kapia and pumpkin-shaped varietal types and those associated with PC2 separated accessions from blocky and corniform varietal types (Figure 6).

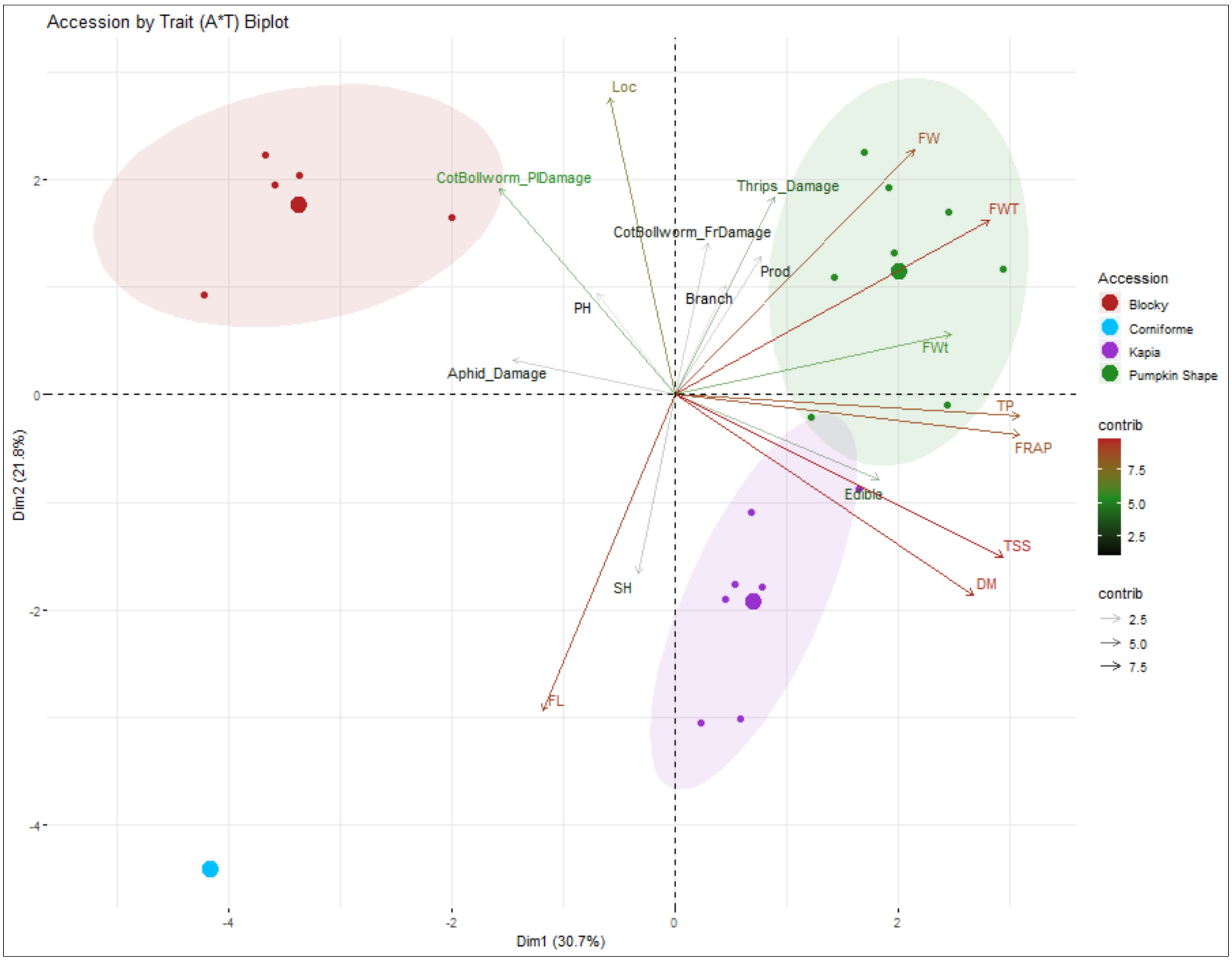

Figure 6. Accession by trait (A*T) biplot discerning accession distinctness. The A*T biplot was constructed based on the variability of biochemical and morphological traits.

Table 6. Discriminant analysis based on variable contribution, eigenvalue, eigenvector and correlation between eigenvectors of first two principal components (PCs).

Trait	Features		Corr. Coeff. (R^2)		Eigenvector		Eigenvalue	Variance (%)	Cumulative Variance (%)
	PC1	PC2	PC1	PC2	1	2			
Plant Height	0.73	1.90	−0.072	−0.05	−0.072	−0.045	5.530	30.74	30.74
Stem Height	0.16	5.95	−0.07	0.29	−0.072	0.290	3.910	21.77	52.52
Branches	0.31	2.23	0.01	−0.10	0.011	−0.098	2.060	11.46	63.98
Productivity	0.91	3.55	−0.19	0.37	−0.200	0.367	1.470	8.19	72.18
Fruit Length	2.14	18.66	0.29	−0.26	0.291	−0.257	1.180	6.56	78.75
Fruit Width	7.00	11.17	0.39	−0.17	0.398	−0.168	1.080	6.00	84.75
Fruit Wall Thickness	12.12	5.64	−0.06	−0.42	−0.061	−0.419	0.890	4.99	89.75
Locules	0.52	16.41	0.29	0.03	0.296	0.030	0.770	4.28	94.03
Fruit Weight	9.38	0.69	0.22	0.16	0.218	0.155	0.370	2.08	96.11
Edible Part	5.12	1.34	0.07	−0.16	0.067	−0.158	0.220	1.26	97.38
Dry Matter	10.95	7.48	0.28	0.33	0.279	0.330	0.180	1.02	98.40

Table 6. *Cont.*

Trait	Features		Corr. Coeff. (R^2)		Eigenvector		Eigenvalue	Variance (%)	Cumulative Variance (%)
	PC1	PC2	PC1	PC2	1	2			
Total Soluble Solids	13.20	4.88	0.26	0.39	0.259	0.389	0.130	0.72	99.13
FRAP	14.58	0.29	0.40	−0.02	0.402	−0.017	0.060	0.34	99.47
Total Polyphenols	14.54	0.08	0.39	−0.02	0.392	−0.024	0.040	0.23	99.70
Aphid Damage	3.21	0.23	−0.15	0.08	−0.150	0.076	0.020	0.15	99.86
Thrips-Damage	1.20	7.29	0.15	−0.29	0.150	−0.298	0.010	0.09	99.95
Cotton Ballworm Plant Damage	3.80	7.91	−0.24	−0.23	−0.240	−0.230	0.004	0.02	99.98
Fruit Damage	0.14	4.29	−0.01	−0.20	−0.001	−0.204	0.002	0.01	100.0

4. Conclusions

This study allowed us to comprehensively evaluate the diverse Balkan pepper collection representing pepper diversity and overcome the danger of eliminating accessions that are considered duplicates or non-significant for breeding. Additionally, the outlined comprehensive characterization also facilitated the identification of accessions that have unique fruit morphological features (highest productivity—K1115 and K1081), enhanced fruit quality (dry matter—K1114, TSSs—K697, TP—K712, and FRAP—K1103A), and noticeable resistance against green peach aphid (K1083), thrips (K1082), and cotton bollworm (K1083 and K697). These identified accessions are promising breeding resources and ideal for developing potential pepper ideotypes with high yield, enhanced fruit quality, and insect resistance that could be used in pre-breeding and breeding efforts in the foreseeable future.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae10040389/s1. Figure S1: PCA variance plot depicting variance contributed by each principal component. Blue line depicts the cumulative variance and red line the variance contributed by each principal component; Table S1: Average temperature and rainfall recorded from May to September during 2018 and 2019 across the growing season; Table S2: Soil content details for the experimental field; Table S3: Evaluation of productivity and plant traits according to a varietal type; Table S4: Evaluation of fruit traits according to a varietal type; Table S5: Evaluation of biochemical traits according to a varietal type; Table S6: Evaluation of insect pest infestation according to a varietal type.

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Conflicts of Interest: The authors declare no conflicts of interest.

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