horticulturae

MDPI

Article

Genetic and Seasonal Factors Influence Pungent Pepper Capsaicinoid and Vitamin C Content

Ingrid Melinda Gyalai [1,2], Lajos Helyes [1], Hussein G. Daood [1,*], Flórián Kovács [2,3], Adrienn Szarvas [2] and Ferenc Lantos [2]

[1] Doctoral School of Plant Sciences, Hungarian University of Agriculture and Life Sciences, 2100 Gödöllő, Hungary; gyalai.ingrid.melinda@szte.hu (I.M.G.); helyes.lajos@uni-mate.hu (L.H.)
[2] Faculty of Agriculture, Institute of Plant Sciences and Environmental Protection, University of Szeged, 6800 Hódmezővásárhely, Hungary; kovacs.florian@szte.hu (F.K.); szarvas.adrienn@szte.hu (A.S.); lantos.ferenc@szte.hu (F.L.)
[3] Department of Agro-Environmental Studies, Hungarian University of Agriculture and Life Sciences, 1118 Budapest, Hungary
* Correspondence: daood.hussein@uni-mate.hu

Abstract: Pungent red peppers and chilis are healthy foods and crucial ingredients of modern diets due to their content of bioactive phytochemicals such as carotenoids, tocopherols, capsaicinoids, polyphenols, and vitamin C. The production of pungent peppers with outstanding nutritional properties is influenced by genetic factors and their interaction with the environments where they are cultivated. This study was conducted to investigate the effects of genotype and climate conditions on the response of capsaicinoids and vitamin C. The extracts of pungent materials and vitamin C were separated and qualitatively determined by HPLC alone or hyphenated with mass spectroscopy. Four genotypes were selected and cultivated under traditional agricultural practices in southern Hungary. The yield properties and the contents of capsaicinoids and vitamin C were significantly influenced by genetic factors and, to a high extent, by the interaction between genotype × environment. The highest yield of 2.86 ± 0.59 kg/m^2 was recorded for the CS variety. The yield was significantly decreased under high precipitation and low air temperature. The highest concentration of capsaicin of 1586–1734 µg/g dwt was found in the BHJ variety. The lowest level of 514 µg/g dwt of capsaicin was determined in the CS variety, in which the content significantly increased to 772 µg/g dwt with the increase in rainfall and decrease in air temperature in the warmer season of 2022. In most cultivars examined, capsaicin content negatively responded to the change of the climate toward higher precipitation and lower temperature except for SZ178, in which the concentration of capsaicinoids stayed insignificantly varied. The amount of vitamin C ranged between 570 and 135 µg/g dwt, with the highest content being in the BHJ and the lowest in the REK variety. In most varieties except the REK, vitamin C positively responded to a high and negatively to the reduced levels of precipitation. In conclusion, the phytochemical components of hot peppers respond differently to climatic factors—such as high rainfall, elevated air temperature, and intense sunlight—depending on the genotype's ability to adapt to environmental changes.

Keywords: capsaicinoids; vitamin C; pungent pepper; chili; *Capsicum* sp.; climate; genotype

check for updates

Academic Editors: Shivani Kathi and Catherine R. Simpson

Received: 3 February 2025
Revised: 1 March 2025
Accepted: 4 March 2025
Published: 6 March 2025

Citation: Gyalai, I.M.; Helyes, L.; Daood, H.G.; Kovács, F.; Szarvas, A.; Lantos, F. Genetic and Seasonal Factors Influence Pungent Pepper Capsaicinoid and Vitamin C Content. *Horticulturae* **2025**, *11*, 286. https://doi.org/10.3390/horticulturae11030286

1. Introduction

Plant-based meals rich in bioactive phytochemicals are fundamentally beneficial to human health. Sweet and pungent types of peppers are among the plant-based functional foods or food ingredients due to their considerably high content of bioactive phytochemicals such as carotenoids, tocopherols, polyphenols, capsaicinoids, and vitamin C [1–3]. Capsaicinoids are responsible for the pungent flavor of chilis and other hot peppers [4]. Capsaicinoids deserve considerable attention for their positive pharmacological effects, but only in the last decades has extensive research been conducted to determine their biological and physiological effects, such as antioxidant [5], anti-inflammatory [6], anticancer [7,8], antibacterial [9], gastric mucosal [10], and fat accumulation prevention [11].

Recent studies have shown that capsaicinoid accumulation is influenced not only by the pepper genotype but also by various environmental factors, including light, temperature, soil nutrients, water, hormones, and pathogens [12]. These factors can influence the biosynthetic pathways of capsaicinoids through gene transcription factors [13,14]. The genotypes studied by Souza et al. [15] differed not only in capsaicinoid content but also in the ratio of capsaicin to dihydrocapsaicin. The capsaicinoid content of chili pepper within the same variety showed fluctuations at different harvest times following a normal distribution pattern, while another chili pepper exhibited a skewed distribution. This observation suggests a potential association with both genotype and harvesting time [16].

Vitamin C is an essential micronutrient for humans, and its pleiotropic functions are related to its electron-donating capacity. It is a powerful antioxidant and cofactor of a family of biosynthetic and gene-regulatory enzymes. It contributes to immune protection by supporting the various cellular functions of the innate and adaptive immune system, as well as supporting epithelial protection against pathogens [17].

Like capsaicinoids, vitamin C content has also been found to be influenced by genetic and environmental factors [18]. Bae et al. [17], Anitra et al. [19] observed highly significant interactions between cultivar, growing season, and maturity stage for key bioactive compounds, including ascorbic acid, capsaicinoids, flavonoids, and phenolics. Studies by Lekala et al. [20] showed that the interaction of pepper cultivars and growing environmental conditions affected the accumulation of different antioxidant compounds. They found that cultivation in a temperature-controlled foil greenhouse resulted in higher yields and promoted the accumulation of carotenoids, ascorbic acid, and vitamin C. Among the pre-harvest factors, light intensity and temperature are the factors most likely to affect final vitamin C content of the crops [21]. Furthermore, Tripodi et al. [22] stated that phenotypic variation in crops is the result of the genotype $\times$ environment (G $\times$ E) interaction, defined as the variation of the relative performance of genotypes (G) in different environments (E). The estimation of the G $\times$ E effect helps select the genotypes adapted to a single or a range of environmental conditions.

Maturity has been found to affect the vitamin C and capsaicinoid content of hot peppers. Deepa et al. [23], Gnayfeed et al. [24], and Jifon et al. [25] found that genetics, agronomic characteristics, and other factors can alter the bioactive properties of plant crops. However, little information is available on the effect of environmental factors and cultivation technology on the bioactive phytochemicals of hot or sweet peppers.

The main objective of the present work was to investigate the effect of climatic conditions on the vitamin C and capsaicinoid content of four different hot pepper varieties.

2. Materials and Methods

2.1. Experimental Design

In Hungary's Pusztaföldvár (GPS coordinates: 46.535054, 20.800754), open-field, mulch-free growing was the setting for the studies in 2022 and 2023. Chernozem meadow makes up the soil in the area. The results of a soil analysis completed in March 2022 are displayed in the Supplemental Materials (Table S1). The planting was conducted on 13 May in 2022 and 7 May in 2023. The first harvest in 2022 was on 9 September, and the second was on 20 September. In 2023, the first harvest was on 30 August, and the second was on 16 September. The four distinct types received identical treatment in terms of fertilization, irrigation, and plant protection. Drip irrigation was also used to apply nutrients and water supply. Nutrient solution was prepared with Solinure 5 general purpose complex fertilizer. From the post-flowering period, Haifa MKP fertilizer, Tradecrop Bentley microelement fertilizer, and Fitohorm Ca 40 calcium solution were used as a foliar fertilizer. Solinure 5 G was applied, as a basic fertilizer, at a dose of 1.5 kg/1000 L. Trade Corp Bentley was used as a microelement supplement in an amount of 50 g/1000 L, as well as 250 mL/1000 L nitric acid to adjust the pH. During the fruit development period, mono-potassium phosphate (Haifa MKP) was applied as a supplement in the amount of 1 kg/1000 L. The pH of the nutrient solution was on average between 5.5–6, and its EC was between 2–3 mS/cm.

The amount of irrigation was applied according to the needs of the plant and the rainfall. If the average daily temperature is multiplied by 0.2, it gives the daily water requirement in mm, in case of rain-free weather [26]. This amount of irrigation water was between 400 and 600 L for the entire plant population, on occasion.

A randomized block design was used for experiments, with 30 plant replicates per variety within a block and a total of 120 plants per variety (a total of 480 plants for each experiment. The varieties studied were the Bhut Jolokia (*Capsicum chinense* × *Capsicum frutescens*) chili pepper, the Cserkó (*Capsicum annuum* L. var. *cerasiforme*) cherry pepper, the Szegedi 178 (*Capsicum annuum* L. var. *longum grossum*) spice pepper, and the Rekord (*Capsicum annuum* L.) table pepper. All these varieties produce pungent (hot) fruits that turn red at biological ripeness. For each variety and for each replication, all ripe fruits were harvested and overripened, and one kilogram of each was dried and tested separately.

2.2. Measurements During Vegetation

Daily measurements of solar radiation intensity (Voltcraft LX-10) and rainfall, maximum and minimum temperatures, and solar radiation intensity were made. Every week, the irrigation water, fertilizer solution, pH (Milwaukee PH600), and EC (Adwa AD32) were measured.

2.3. Preparation of Samples

The fruits were stored in boxes for a week at ambient conditions in shade after each harvest before being sliced and dried. This step is necessary for spice peppers to reach the technological ripeness (over-ripeness), at which the quality parameters are accomplished. The drying process was performed in a Memmert UF55 drying cabinet for 24 h at 60 °C. The dried samples were vacuum-foiled and milled by a coffee mill immediately before analytical determination to avoid subsequent oxidative deterioration of vitamin C and capsaicinoids.

2.4. Analytical Measurements

For the determination of capsaicinoid content, 0.5 g of well-homogenized ground powder from each sample was weighed into an Erlenmeyer flask, and 50 mL of HPLC grade methanol was added. The flasks were placed in a 300 rpm rotating mechanical shaker (GFL 3005) (LAUDA DR. R. WOBSER GMBH & CO. KG, Lauda-Königshofen, Germany) for 20 min followed by a 5 min ultrasound sonication using Raypa (Barcelona, Spain) water bath ultrasonic device. The samples were then filtered through 0.45 μm Whatman no.1 filter paper (Dassel, Germany). The filtered extracts were diluted 10 times with HPLC grade methanol and further cleaned up by passing through Chromofil hydrophobic PTFE syringe filter before injection into HPLC instrument.

The capsainoid extracts were separated to their individual compounds on Purospher Star C18, 2.7 μ, 125 mm reversed phase column (Merck Life and Science Ltd., Budapest, Hungary) with isocratic elution of 48:52 water–acetonitrile at a flow rate of 0.7 mL/min according to a previous work [27]. Capsaicinoids were detected by fluorescence detector (Merck Hitachi, Darmstadt, Germany) at Ex:285 nm and Em: 320 nm. The affluents were identified by comparing their retention time and total ion from a previously described HPLC-MS/MS protocol [27]. Quantification was based on calibration of standard nordihydrocapsaicin, capsaicin, and dihydrocapsaicin from Sigma-Aldrich via Merckgroup Life and Science Ltd., Budapest, Hungary.

For the determination of vitamin C content, 30 mL of cooled 3% metaphosphoric acid was added to 0.5 g of ground powder of each sample in a stoppered flask. The mixtures were shaken at 300 rpm by rotating mechanical shaker (GFL 3005) (LAUDA DR. R. WOBSER GMBH & CO. KG, Lauda-Königshofen, Germany) for 20 min. The mixtures were then transferred to centrifuge tubes and centrifuged at 5000 g for 5 min. The supernatants were taken and cleaned up by passing through a 0.22 μ, 25 mm nylon syringe filter before injection into the HPLC instrument.

Separation of L-ascorbic acid (vitamin C) from other organic acid and polar phenolic compounds was performed on Nautilus C18 aqua, 3μ, 150 mm column (Machery Nagel, Darmstadt, Germany) with gradient elution of acetonitrile in 0.01 M potassium dihydrogen phosphate buffer at a flow rate of 0.7 mL/min. Detection was carried out at 244 nm using diode array detector (DAD). L-ascorbic acid C was identified by comparing retention time and spectral characteristic of the sample peak with those of standard material (Sigma-Aldrich, via Merckgroup Life Science Ltd., Budapest, Hungary), from which standard curve was made for quantification.

A Hitachi Chromaster instrument consisting of a Model 5440 diode-array detector, a Model 5210 autosampler, a Model 5440 Fluorescence detector, and a Model 5110 gradient pump was used. The instrument was operated, and the chromatograms were evaluated using EZChrome Elite Software 3.5.1.

LC-MS/MS analysis was performed on an Agilent 1100 HPLC equipped with a degasser (G1322A), a binary pump (G1312A), an autosampler (G1313A), a column thermostat (G1316A) and a fluorescent detector (G1314A). The mass spectrometry detection was carried out with an Applied Biosystems API 2000 triple quadrupole. Data acquisition and evaluation were performed using Analyst software SCIEX OS 2.0.1. Positive electrospray ion source was used with the following parameters: IS 5000, CUR 20, TEM 550, GS1 60, GS2 70, CAD 7. Nitrogen was used as collision gas for collision-induced dissociation (CID), curtain, nebulizing, and drying gas. The product ion spectra were acquired at 25 and 70 eV from 30 to 600 m/z according to Daood and co-workers [27]

2.5. Statistical Evaluation

All analyses were performed using R version 4.2.1 (RStudio Team, 2020) at a 95% significance level. Capsaicinoids and vitamin C concentrations were assessed across years, harvests, and varieties using Multivariate Analysis of Variance (MANOVA), with treatment efficacy evaluated via Wilks' lambda (λ). In the MANOVA model, years, harvests, and varieties were included as factors, while capsaicinoids and vitamin C concentrations were considered dependent variables. Homogeneity of covariances was verified using Box's test, while variance homogeneity for individual variables was checked using Levene's test. Residual normality was confirmed using the Shapiro–Wilk and Kolmogorov–Smirnov tests. Graph values show means $\pm$ standard deviation (SD). Partial eta-squared (η^2) was used to determine effect size, indicating the probability of detecting a significant effect, ranging from 0 (independence) to 1 (deterministic relationship).

3. Results and Discussion

3.1. Temperature and Precipitation in the Two Years Under Study

The two cultivation seasons under study varied substantially in the amount of precipitation and temperature. The average temperatures for the two months of June and September showed significant differences ($p < 0.05$) (Figures 1 and 2). In 2022, the number of days at which the maximum temperature exceeded 40 °C and the average temperature exceeded 30 °C was substantially higher than what was measured in 2023, particularly 3 weeks before the first and second harvests at each season. The longer sunshine period in 2022 manifested itself in a higher intensity of solar irradiation (Figure 3), which may influence the photosynthetic activity of the plants.

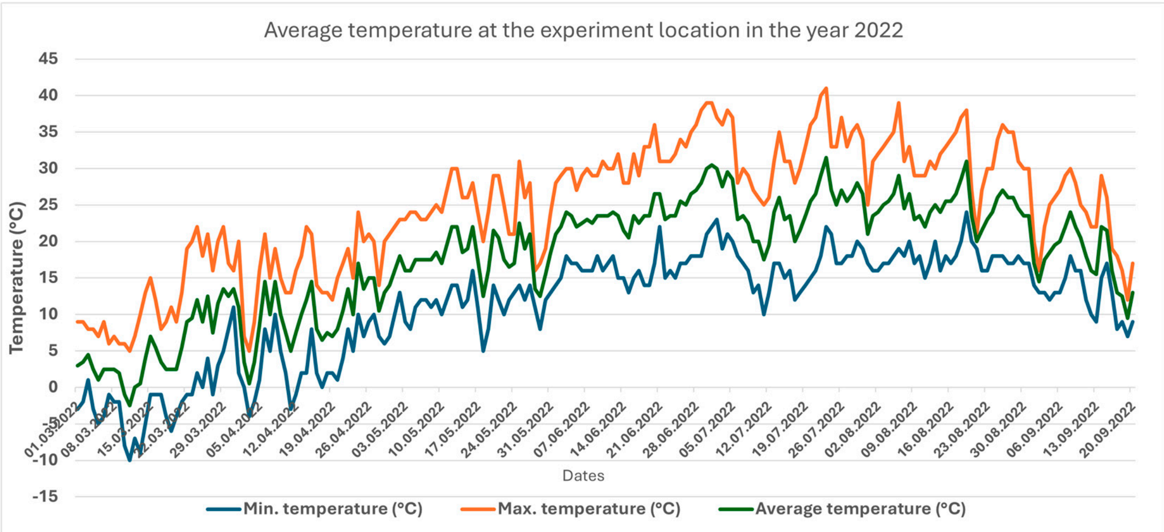

Figure 1. Air temperatures measured during the 2022 season in the experiment location.

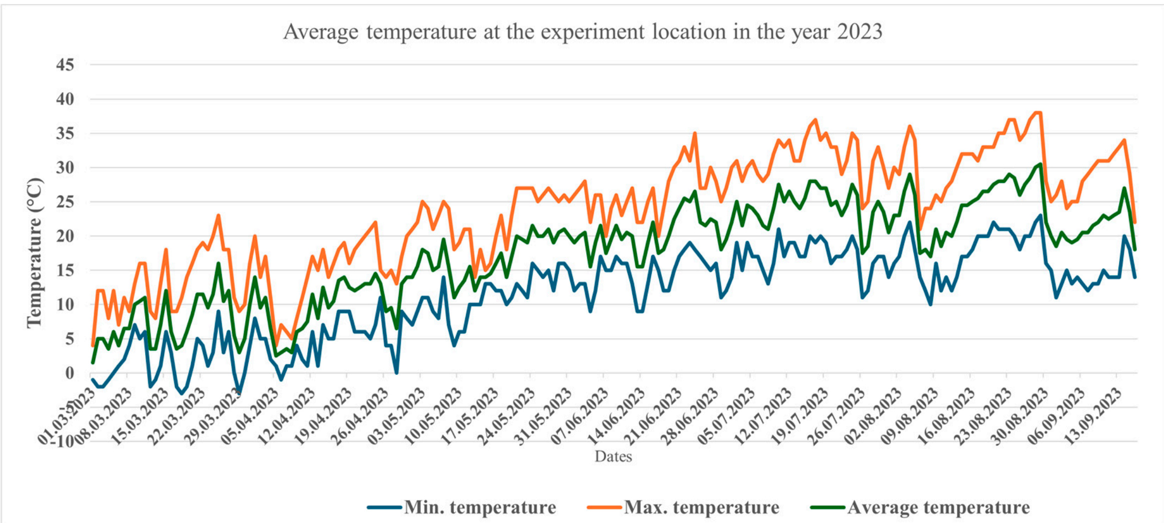

Figure 2. Air temperatures measured during the 2023 season in the experiment location.

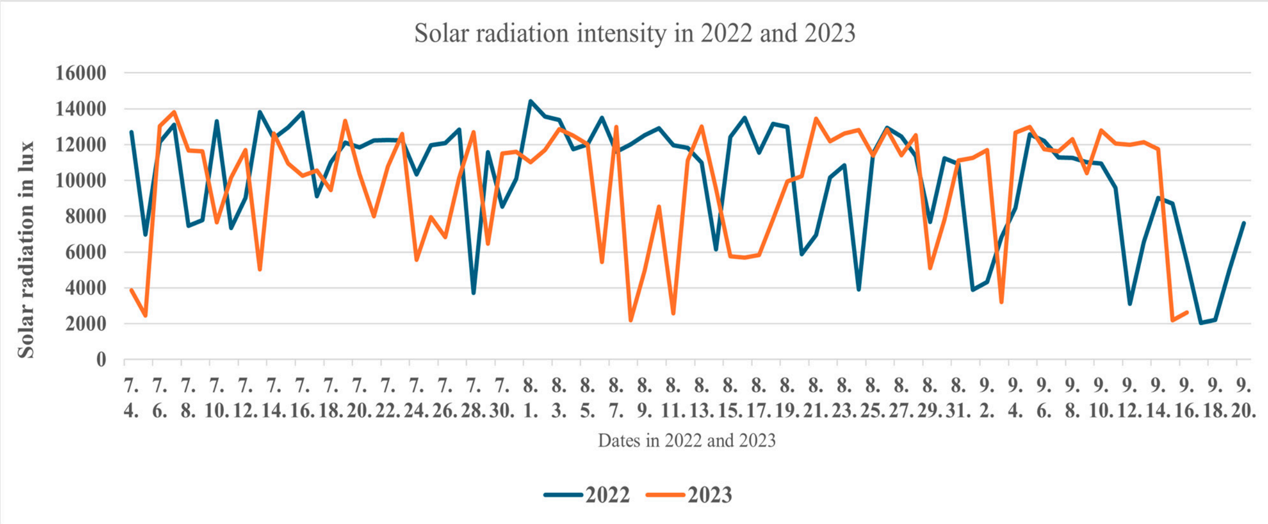

Figure 3. Solar radiation measured between July and September in 2022 and 2023 in the experiment location.

Regarding precipitation, the least amount (a total of 154 mm) was measured between May and September of the growing season of 2022, while in 2023, the total precipitation was 437 mm in the same period (Figure 4). However, in the last month of cultivation, the amount of precipitation was significantly higher in 2022 than in 2023.

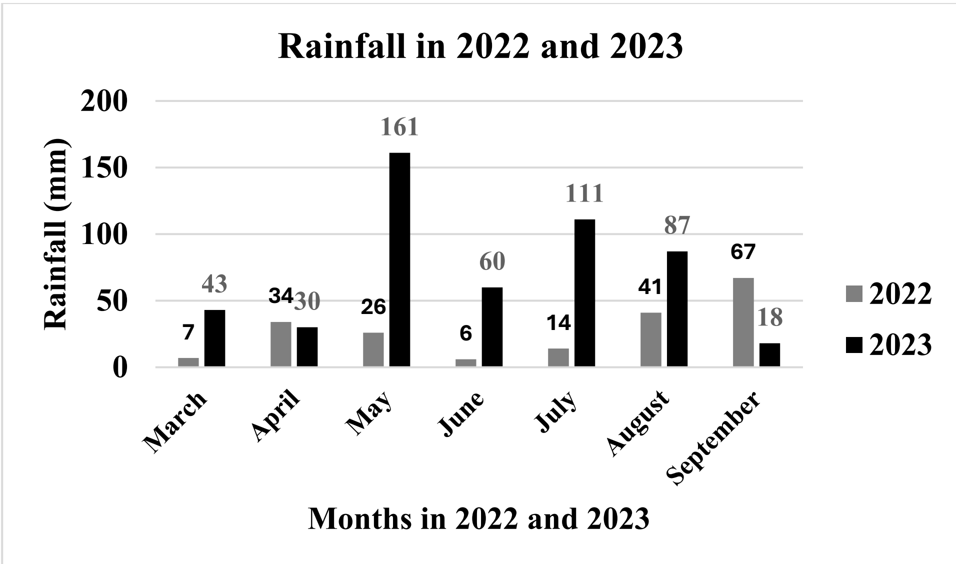

Figure 4. Amounts of rainfall (mm) measured between March and September in 2022 and 2023 in the experiment location.

3.2. Properties of Pepper Fruits Yield

Table 1 summarizes the changes that took place in a number of fruits/plants and the average weight of fruit as a function of changes in climate conditions in the two cultivation seasons. The different genotypes varied significantly in the yield properties as well as in the response of the yield to changes in climate conditions. The highest yield was found in the CS variety, followed by REK and the standard pungent variety SZ178.

Table 1. Average fruit number, fruit weight, and yield for different genotypes in 2022–2023.

Year	Variety	Fruits/Plant	Fruit Weight	
			(g)	Fruit Yield kg/m^2
2022	Bhut Jolokia	18 ± 1.98	8.7 ± 0.99	0.579 ± 0.19 ^{AXa}
2022	Cserkó	24 ± 2.55	32.3 ± 1.19	2.86 ± 0.59 ^{AXb}
2022	Szegedi 178	17 ± 1.11	19.8 ± 0.89	1.24 ± 0.27 ^{AXc}
2022	Rekord	15 ± 2.12	22.00 ± 1.45	1.21 ± 0.14 ^{AXc}
2023	Bhut Jolokia	22 ± 1.74	9.00 ± 1.36	0.73 ± 0.11 ^{AXa}
2023	Cserkó	21 ± 3.21	28.2 ± 1.63	2.19 ± 0.39 ^{AXd}
2023	Szegedi 178	15 ± 1.08	19.1 ± 3.99	1.06 ± 0.21 ^{AXc}
2023	Rekord	13 ± 1.55	15.8 ± 4.11	0.75 ± 0.12 ^{BXa}

Uppercase letters (AB) show differences between varieties between harvests; lowercase letters indicate differences between varieties in the same years. Means within a column followed by the same letter are not significantly different (Tukey's HSD test, $p < 0.05$). X shows the variation due to years

In the context of the response to climate factors, it was indicated that the high temperature and lower precipitation amount in 2022 caused a marked increase in the number of fruits/plants and fruit weight compared to fruits harvested in 2023, in which the precipitation and air temperature were opposite. An exception was that the number of fruits/plants of the BHJ variety positively responded to low temperature and high precipitation during the season of 2023. The obtained results demonstrate that the response of pepper fruits is influenced by the interaction between genetic and agroclimatic factors. The better properties, in terms of the number and weight of fruits in warmer cultivation seasons, may be attributed the higher solar radiation intensity (from long sunshine period), which has been reported to lead to a better yield (in terms of size, length, and weight of fruit) of pungent Habaneros produced in the open fields compared to fruits of the same genotype produced inside a greenhouse [28].

3.3. Impact of Climate Conditions on Capsaicinoids

The HPLC protocol used for the determination and identification of capsaicinoids gave an excellent separation of eight capsaicin-derived compounds that contributed to the pungency of such crops (Figure 5). The detected and identified capsaicinoids are nor-nor-dihydrocapsaicin (NNDC), nor-dihydrocapsaicin (NDC), capsaicin (CAP), dihydrocapsaicin (DC), homo-capsaicin (HCAP), an isomer of DC (iDC), homo-dihydrocapsaicin-1 (HDC-1), and homo-dihydrocapsaicin-2 (HDC-2). The HPLC profile of capsaicinoids is like that found for most Hungarian pungent spice red peppers [26] and some Italian genotypes (Gioffrida et al., 2014) [29]. As a result of genetic factors, the different pungent peppers showed significant variation in the content and proportion of the individual and total capsaicinoids.

All three main factors—Year, Harvest, and Variety—had significant effects on the measured parameters (Year: Wilks' $\lambda = 0.024$, $F(6,43) = 292.38$, $p < 0.001$, partial $\eta^2 = 0.97$; Harvest: Wilks' $\lambda = 0.25$, $F(6,43) = 21.03$, $p < 0.001$, partial $\eta^2 = 0.74$; Variety: Wilks' $\lambda = 0.00037$, $F(18,122) = 103.52$, $p < 0.001$, partial $\eta^2 = 0.92$). The two-way interactions Year × Harvest (Wilks' $\lambda = 0.34$, $F(6,43) = 13.94$, $p < 0.001$, partial $\eta^2 = 0.66$), Year × Variety (Wilks' $\lambda = 0.0013$, $F(18,122) = 63.54$, $p < 0.001$, partial $\eta^2 = 0.89$), and Harvest × Variety (Wilks' $\lambda = 0.231$, $F(18,122) = 13.94$, $p < 0.001$, partial $\eta^2 = 0.38$) were also significant. Furthermore, the three-way interaction among Year, Harvest, and Variety was significant as well (Wilks' $\lambda = 0.20$, $F(18,122) = 5.11$, $p < 0.001$, partial $\eta^2 = 0.41$). The greatest effects were observed for the differences between years and varieties, with both having the highest partial η^2 value.

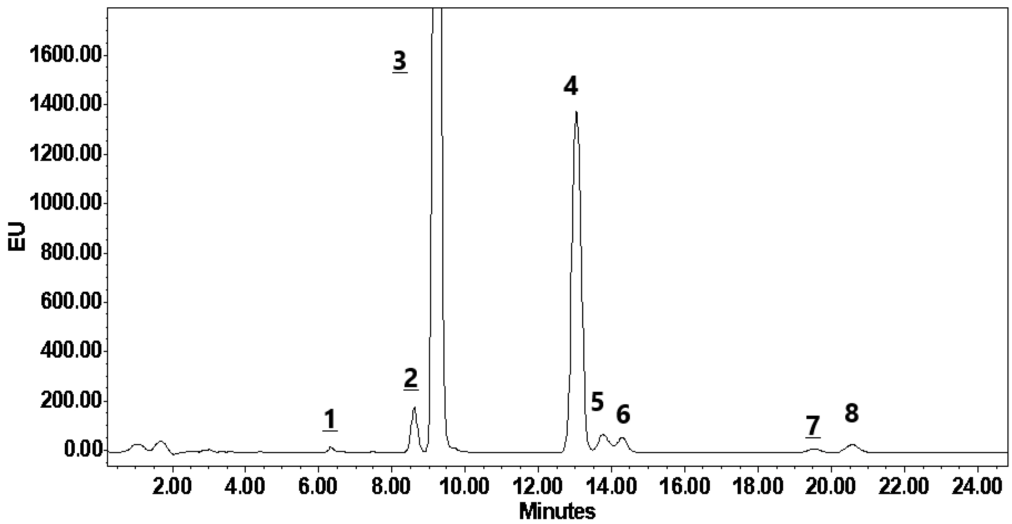

Figure 5. HPLC profile of capsaicinoids separated on Purospher Star column with isocratic elution of water–acetonitrile.

The concentration of the dominant CAPS and DC in BHJ, CS and SZ178 is close to that found in highly pungent cultivars such as Corneto sottile and Numex cultivated in Italy in two different locations [29]. In terms of Scoville Hotness Unit (SHU), a range between 36,292 and 40,293 was obtained for the three cultivars mentioned before. The REK cultivar has a SHU of 16,740, which makes it a moderately pungent pepper. Usama et al. [30] reported similar concentration for the sum of CAPS and DC contents in AVPP0002 and AVPP0805 varieties cultivated in Bangladesh, and Shams et al. [31] determined slightly higher contents for the same capsaicinoids in Maras and Habanero varieties than that determined in REK genotype in the present study. Schmidt et al. [32] studied, among others, Jalapeño (*C. annuum*) and Bhut Jolokia (*C. chinense*) peppers grown in Austria and found the CAP and DC content of the fruit placenta and seeds to be 296 $\pm$ 27.3 µg/g and 233 $\pm$ 18.3 µg/g dry base, respectively. The concentration of the major capsaicinoids achieved in our study is much lower than that reported for extremely pungent varieties such as Naga King chili, which distributed 2.54 $\pm$ 0.58, 0.50 $\pm$ 0.17, and 0.02 $\pm$ 0.005 g/100 g dry matter of CAPS, DC, and NDC, respectively [33]. Duah et al. [34] and Souza et al. [15] investigated the capsaicinoid content in different hybrids including the highly pungent Unijol. None of the varieties investigated in this work showed as high a content of all capsaicinoids reported for Unijol.

Table 2 shows the differences between harvest dates and genotypes in the content of the six most important capsaicinoids found in the examined varieties in 2022. The examined types of Capsicum species differed significantly in their capsaicinoid content as well as in the proportion (%) of each, particularly in that of the major compounds. Regarding the content, the REK variety contained the least amount of all capsaicinoids, particularly CAPS, in the season of 2022, while the highest content of CAPS was recorded for BHJ (chili pepper) in both the first and second harvests. As for DC, the significantly highest level was found in SZ178 followed by CS in both harvesting times of the same season.

Table 2. Content of capsaicinoids as affected by genotype and climate conditions at harvesting times in 2022.

Harvest	Variety	N DC	CAPS	DC	HCAPS	HDC-2
1st	BHJ	36.11 ± 5.36 ^{AXa}	1586.36 ± 123.35 ^{AXc}	640.13 ± 85.04 ^{AXa}	35.04 ± 4.86 ^{AXb}	5.27 ± 0.52 ^{AXa}
	CS	246.31 ± 25.12 ^{AXd}	1339.94 ± 73.36 ^{AXbc}	1133.67 ± 83.22 ^{AXb}	9.87 ± 0.90 ^{AXa}	92.25 ± 6.84 ^{AXd}
	REK	82.39 ± 3.50 ^{AXb}	514.24 ± 38.25 ^{AXa}	522.76 ± 26.40 ^{AXc}	10.37 ± 0.81 ^{AXa}	29.41 ± 1.26 ^{AXb}
	SZ178	195.02 ± 22.33 ^{AXc}	1200.74 ± 124.39 ^{AXb}	1263.53 ± 123.30 ^{AXb}	30.33 ± 4.12 ^{AXb}	65.38 ± 6.30 ^{AXc}
2nd	BHJ	37.88 ± 1.10 ^{AXa}	1734.01 ± 44.41 ^{AXc}	646.98 ± 7.70 ^{AXa}	10.05 ± 1.08 ^{AYa}	10.16 ± 0.37 ^{BXa}
	CS	268.84 ± 16.28 ^{AXc}	1319.94 ± 145.89 ^{AXb}	1171.11 ± 103.56 ^{AXb}	8.28 ± 0.70 ^{AYa}	104.98 ± 6.78 ^{AXd}
	REK	129.41 ± 21.22 ^{AXb}	771.99 ± 147.49 ^{AXab}	744.30 ± 125.36 ^{AXab}	14.15 ± 2.10 ^{AXa}	43.10 ± 5.67 ^{BYb}
	SZ178	239.06 ± 32.32 ^{AXc}	1401.93 ± 132.62 ^{AXb}	1448.39 ± 161.53 ^{AXc}	37.91 ± 5.04 ^{AXb}	81.01 ± 10.60 ^{AXc}

Uppercase letters show differences between harvest across years, as well as between harvests (XY); lowercase letters indicate differences between varieties in the same years. Means with the same letters are not significantly different (Tukey's HSD test, $p < 0.05$).

Since the climate factors of the second harvest in 2022 substantially differed from those prior to the first one, significant variation in the response of the genotypes to climate changes in the two harvesting times was expected (Table 3). CAPS' positive response to climate change was noticeable in all genotypes investigated, except REK, which showed a negative response. In the case of NDC and DC, their concentrations tended to increase, but only in REK and SZ178. The minor capsaicinoid isomers in CS and BHJ showed an opposite response to that exhibited by CAPS and DC as the climate changed towards the second harvest, while in the REC and SZ178 genotypes, the minors showed a similar response to that of the majors. The first interesting and remarkable alteration was that the CAPS concentration dropped extremely in all genotypes tested except in the SZ178 variety, in which CAPS was interestingly stable (unchanged). Regarding the response of DC, the genotypes showed an interesting variation. In the variety CS, a dramatic decrease took place in the content of the isomer DC (decreased from 1133.67 ± 83.22 and 1171.11 ± 103.56 ug/g dry base in 2022 to 289.00 ± 32.97 and 267.50 ± 63.36 ug/g dry base in 2023 at the first and second harvests, respectively. In contrast to the BHJ and REK varieties, DC responded negatively to the climate changes, and in SZ178, it stayed unchanged. The hydrated capsaicin (HCAPS) in BHJ and CS varieties responded negatively (decreased) as the weather changed in 2023, whereas in REK and SZ-178, its concentration increased; in the latter, it was twofold higher than that in 2022. The changes in the levels of the homo-derivatives were not clearly understood due to there being variable intermediates.

In 2022, the temperature dropped from 42 °C before the first harvest to 23 °C prior to the second harvest, while precipitation increased from 41 mm to 67 mm (Figure 4). The change in such parameters was the opposite in 2023, when the temperature slightly decreased from 32 °C to 29 °C, and the precipitation dramatically dropped from 87 mm to 18 mm. Therefore, there was a considerable variation in the content and response of capsaicinoids between the genotypes cultivated and harvested in 2022 and 2023. In 2022, a significant change in the major capsaicinoids was found between the two harvests only in the CS variety, while in the others, the levels did not significantly change. The HCAPS response to change in climate conditions was genotype dependent. In BHJ and CS, the content of the capsaicin isomer significantly ($p < 0.05$–0.01) decreased toward the second harvest, with that in BHJ being dramatic. In REK and SZ178, there was a slightly significant increase in the content of HCAS. Concerning HDC-1, its response was not understood in all genotypes examined, whereas the climate change was favorable and had a positive impact (significant at $p < 0.01$) on the content of HDC-2 isomer in all genotypes studied.

Table 3. Content of capsaicinoids as affected by genotype and climate conditions at harvesting times in 2023.

Harvest	Variety	NDC	CAPS	DC	HCAPS	HDC-2
1st	BHJ	73.04 ± 9.72 BXa	919.09 ± 26.91 BXb	854.83 ± 38.27 BXb	25.58 ± 11.99 AXb	18.98 ± 3.23 BXa
	CS	38.72 ± 3.58 BXa	246.03 ± 38.21 BXa	289.00 ± 32.97 BXa	5.56 ± 1.65 BXa	22.76 ± 1.35 BXa
	REK	48.61 ± 9.55 BXa	241.38 ± 38.18 BXa	337.19 ± 51.57 BXa	12.01 ± 1.38 AXab	29.57 ± 4.49 BXa
	SZ178	208.28 ± 31.47 AXb	1233.90 ± 218.18 AXc	1417.39 ± 224.81 AXc	63.04 ± 12.52 BXc	81.22 ± 11.50 BXb
2nd	BHJ	51.03 ± 23.31 AXa	786.38 ± 490.60 BXb	775.94 ± 72.51 BXa	17.23 ± 1.78 AXa	22.90 ± 1.56 AXa
	CS	38.28 ± 5.49 BYa	202.29 ± 59.04 BYa	267.50 ± 63.36 BXb	4.08 ± 0.92 BYa	20.59 ± 2.05 BYab
	REK	81.18 ± 10.84 BYa	558.50 ± 154.48 AYab	664.62 ± 98.34 AYa	13.66 ± 1.54 AXa	39.77 ± 4.62 AYab
	SZ178	47.26 ± 54.49 BYa	1430.46 ± 236.65 BYab	1425.24 ± 218.95 AXc	15.18 ± 1.78 BYb	76.42 ± 9.64.57 BYb

Each value represents the mean ± standard deviation (SD) of $n = 4$. Different: uppercase letters (AB) show differences between varieties across years, as well as between harvests (XY).; lowercase letters indicate differences between varieties in the same years. Means within a column followed by the same letters are not significantly different (Tukey's HSD test, $p < 0.05$).

In the cultivation year of 2023, the climate factors prior to harvesting time were characterized by relatively higher temperature (29 °C) and extremely lower precipitation (18 mm) as compared to those recorded in 2022. The response of capsaicinoids to the climate condition a few weeks before harvest was influenced mostly by genetic factors. For instance, NDC in BHJ and SZ178 showed a decreasing tendency, while REK exhibited a significant increase ($p < 0.01$) as a response to high temperature and low precipitation. The response of the major compounds (CAPS and DC) was negative in the BHJ and CS varieties and positive in REK.

It seems that the response of pungent materials in peppers to the environment is affected by the interaction between the impacts of two abiotic factors (air temperature and precipitation). The air temperature has been found to influence the expression of capsaicin biosynthesis genes in various peppers. A study by González-Zamora et al. [35] on the effect of air temperature on the capsaicinoid content in various varieties found that with rising temperatures, the levels of CAPS and DC increased. Yang and co-workers [12] reviewed several studies, in which high temperature led to an increase in the content of capsaicinoids in many but not all hot pepper genotypes. Additionally, Naves et al. [36] emphasized the positive effect of high air temperature on the accumulation of capsaicinoids for some varieties, while in other varieties, the high temperature had a negative impact.

The air temperature in the cultivation location is associated with the sunshine period and the intensity of radiation coming from sunlight. Although the effect of light intensities on capsaicinoid content has been demonstrated to be inconsistent, many studies indicated that optional light intensity is required to regulate the biosynthesis of capsaicinoids and increase their content in chili peppers [37,38]. Furthermore, exposure to elevated light intensity has been reported to increase significantly the total capsaicinoids in wild genotypes [39]. In the present study, the high concentration of CAPS and DC in 2022 when the maximum temperature before the first harvest exceeded 40 °C for some days together with a high radiation intensity supported the aforementioned facts. The other climate variable that may stand beyond the low content of capsaicinoids in 2023 is the high amount of precipitation. It has been demonstrated that in some cases, excessive watering or high precipitation with certain genotypes of hot peppers may lead to a reduced capsaicinoid content [40]. Other studies indicated that water deficit or mild drought increased the activity of the enzymes involved in the regulation and biosynthesis of capsaicinoids, while the overwatering had the opposite impact on such enzymes [16,41].

One of the analytical parameters that assists in a better understanding of the changes in capsaicinoids as a matter of genetic variation and as a response to climate changes is the ratio of CAPS to DC. Table 4 shows the effects of harvesting time, climate condition and genotype on the CAPS/DC ratio. The highest values (2.4) for the ratio were recorded for BHJ in both cultivation years and harvest. A similar ratio has been reported by Tupoz and Ozdemir [42] for some Capsicum annum cultivars (1245F1, Amazon F1, Serademre 8, and Kusak 295 F1) cultivated in Turkey.

Table 4. Caps/DC ratio as affected by genetic and environmental factors 3 weeks before harvests of 2022 and 2023.

Harvests	**Variety**	**CAPS/DC**	
		2022	**2023**
First harvest	BHJ	2.48 ± 0.33 ^{BXa}	1.08 ± 0.03 ^{BXa}
	CS	1.18 ± 0.06 ^{BXb}	0.58 ± 0.04 ^{BXb}
	REK	0.98 ± 0.07 ^{BXc}	0.72 ± 0.11 ^{BXc}
	SZ178	0.95 ± 0.12 ^{BXc}	0.87 ± 0.12 ^{BXc}
Second harvest	BHJ	2.03 ± 0.05 ^{BXa}	1.01 ± 0.08 ^{BXa}
	CS	1.13 ± 0.10 ^{BXb}	0.76 ± 0.13 ^{BXcb}
	REK	1.04 ± 0.20 ^{BXb}	0.84 ± 0.10 ^{BXb}
	SZ178	0.97 ± 0.16 ^{BXb}	1.01 ± 0.11 ^{BXa}

Each value represents the mean ± standard deviation (SD) of n = 4. Different: uppercase letters (AB) show differences between harvests; lowercase letters indicate differences between varieties in the same years. Means within a column followed by the same letters are not significantly different (Tukey's HSD test, $p < 0.05$). X shows the variation due to years.

The highest values achieved for the ratio in BHJ is close to the values determined in most conventional varieties of pungent spice red pepper genotypes widely cultivated in Hungary [27]. This revealed that the recently developed BHJ cultivar of Capsicum annum might originate from one traditional Hungarian variety via breeding-based biotechnological protocol. The values determined by Duelund et al. [43] for proportion of CAPS and DC in hot peppers in Denmark were slightly lower than what we found for BHJ. The values found for other varieties ranged between 0.97 and 1.04 and are close to those found for some accessions of hot peppers harvested at green or fully ripe stages [44].

The seasonal variation in climate parameters caused the ratio to decrease in BHJ variety from 2.4 in 2022 to 1.08 in 2023. The change in the climate between first and second harvests in 2022 did not result in a significant change in the ratio in all genotypes studied, while in 2023, the climate conditions of the second harvest caused the ratio to significantly increase ($p < 0.05$) in all genotypes examined. The change in the CAP/DC is associated with the level of precipitation prior to harvesting times rather than with the changes in the air temperature. Such findings supported the fact that high water supply diminishes the activity of the enzymes regulating biosynthesis of capsaicinoids in certain landraces of hot peppers or chilis. Of the enzymes of the biosynthesis of capsaicinoids, hydrogenases catalyze the synthesis of dihydro derivatives of capsaicin [31].

The drastic dropping in the level of precipitation before the second harvest of 2023 caused the ratio to increase significantly compared to the first harvest, confirming the positive effect of mild drought (low water supply) on the activity of the enzymes catalyzing capsaicin synthesis (capsaicin synthase) and diminishing, to some extent, the activity of dihydrocapsain synthase.

3.4. Content and Response of Vitamin C

The recent analytical protocol applied to determine L-ascorbic acid, the abundant biological form of vitamin C in nature, allowed for the efficient resolution of the vitamin from the accompanying organic acids and water-soluble phenolics (Supplement Figure S1). It is of special interest that the dried and freshly milled powders contain considerable amounts of vitamin C despite the detrimental effect of thermal drying at 60 °C for 24 hr. This agrees with previous research, in which drying of ripe spice peppers caused either no change or a significant increase in the content of vitamin C in the dry matter [45].

Figure 6 shows the vitamin C content of pungent peppers affected by genetic factors, seasonal variation, and climate conditions before harvesting times. The results showed that BHJ variety had the highest vitamin C content (570 mg/100 g dry base) in 2022 compared to other varieties tested. The lowest concentration of 130 mg/100 g dry base was recorded for REK. The range found between the highest and the lowest concentration determined for the four hot peppers under study is in the range reported for vitamin C in several varieties cultivated in Italy [22]. The obtained range of vitamin C in all varieties examined is much higher than that reported by Tupoz and Ozdimer [42] for some cultivars of *Capsicum annuum* cultivated in Turkey. Furthermore, Kantar et al. [46] studied the vitamin C content of different types of pepper cultivars, including some Hungarian-bred varieties like BHJ, Fehérözön, and Szegedi óriás, which, when cultivated in North America, contained less vitamin C than the varieties investigated in the present work. In another study [47], chilis and spicy hot peppers contained between 90 and 130 mg/100 g vitamin C, which is close to the range measured for the four varieties of our study. Nagy et al. [48] determined 248.8 and 368.4 mg/100 g dry matter for Beibiehong and Fire Flame cultivars of chili peppers, respectively, which is less than that found in all varieties examined in this research work. The range between 130 and 570 mg/100 g of dry matter (18–80 mg/100 g fresh weight) may contribute to 20–26% to 88–114% of RDI for men and women, respectively. According to Yuni et al. [49] and Olatunji et al. [50], the recent RDI for vitamin C in men and women is 90 and 75 mg/day, respectively.

Concerning the impact of climate conditions at harvesting time, in all genotypes, it was found that there was a significant increase in the concentration of the vitamin, as the precipitation significantly increased ($p < 0.01$) at the second harvest of the first cultivation season, particularly for the CS variety, in which the increment was approximately threefold, making the level of vitamin C in this variety rank second after that of vitamin C in BHJ. In 2023, when the precipitation decreased at the second harvest, the vitamin C content tended to significantly decrease for all genotypes under study except REK, in which the level of the vitamin surprisingly increased almost twofold. Such findings confirm that the biosynthesis of vitamin C in peppers with a high pungency is significantly affected by increasing water supply via precipitation or irrigation, while in cultivars with low or medium pungency, the reduced water supply or mild drought promotes regulation and accumulation of vitamin C. The positive correlation between water supply and content of vitamin C does not agree with the inverse relation between water supply and vitamin C level reported previously by Duah et al. [51]. As a matter of fact, the increasing amount of rainfall leads to a rise in the moisture content of the soil that has been stated to correlate positively with accumulation of vitamin C in chili peppers [52].

The exposure to high sunlight intensity in 2022, in which the air temperature exceeded 40 °C for some days, might assist in increasing the biochemical factors that regulate vitamin C synthesis. It has been found that high air temperature caused by high radiation intensity increases the content of vitamin C in several cultivars of sweet peppers [53].

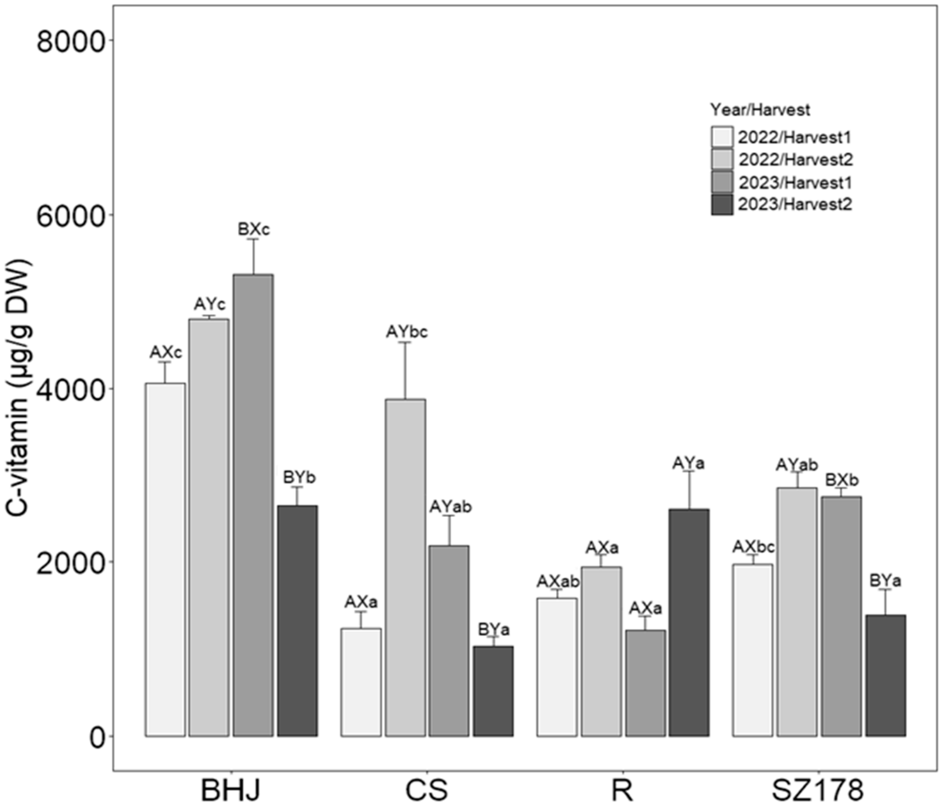

Figure 6. Response of vitamin C to changes in the climate conditions before harvests of pungent peppers in two cultivation seasons. Uppercase letters (AB) show differences between varieties across years, as well as between harvests (XY); lowercase letters indicate differences between varieties in the same years. Means within a column followed by the same letter are not significantly different (Tukey's HSD test, $p < 0.05$).

4. Conclusions

In the present study, it was found that the content variation and response to environmental factors of capsaicinoids and vitamin C in chili and pungent spice peppers is associated with the effect of genotype in addition to the significant effect of interaction between genotype and environment (G × E). The findings achieved could highlight how the genotype is a ruling aspect to the climate conditions in the variation of capsaicinoid and vitamin C content. It could be concluded that the response of phytochemicals in hot peppers to the changes in the climate factors such as high precipitation, high air temperature, and intensive radiation of sunlight would be positive or negative depending on the genotype adaptation to the changes in the environmental conditions. Accordingly, the BHJ and CS varieties are recommended as crucial sources of capsaicinoids and vitamin C due to their high adaptation to climate conditions. It is also concluded that all varieties examined are a potential source of capsaicinoids and can contribute efficiently to the recommended dietary intake of vitamin C and good raw materials for pharmaceutical industries even when subjected to unexpected changes in the climate conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae11030286/s1. Figure S1: HPLC profile for separation of L-ascorbic acid and some polyphenols from paprika powder using Nautilus aqua column and gradient elution of acetonitrile in 0.01M KH_2PO_4; Table S1: Components of the fertilizers used in the experiment, expressed as a percentage; Table S2: The effect of the year, harvest and variety and their interactions on the response variables. Results showing the tests of between-subject effects (UNIANOVA).

Author Contributions: I.M.G. and F.K.: Analysis, Methodology, Data curation. H.G.D.: Conceptualization, Writing original draft, Correspondence. A.S.: Investigation, Statistical Analysis. L.H.: Validation, Supervision. F.L.: supervision, data curation. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Flagship Research Groups Programme (2024–2027) of the Hungarian University of Agriculture and Life Sciences.

Data Availability Statement: All of the data are incorporated in the manuscript.

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

Abbreviations

The following abbreviations are used in this manuscript:

BHJ	Bhut Jolokia variety
CS	Cserkó variety
REK	Rekord variety
SZ178	Szegedi178 variety
RWI	Recommended Dietary Intake

References

1. Dludla, P.V.; Cirilli, I.; Marcheggiani, F.; Silvestri, S.; Orlando, P.; Muvhulawa, N.; Moetlediwa, M.T.; Nkambule, B.B.; Mazibuko-Mbeje, S.E.; Hlengwa, N.; et al. Bioactive Properties, Bioavailability Profiles, and Clinical Evidence of the Potential Benefits of Black Pepper (Piper nigrum) and Red Pepper (*Capsicum annuum*) against Diverse Metabolic Complications. *Molecules* **2023**, *28*, 6569. [CrossRef] [PubMed]
2. Hernández-Pérez, T.; Gómez-García, M.; Del, R.; Valverde, M.E.; Paredes-López, O. *Capsicum annuum* (hot pepper): An ancient Latin-American crop with outstanding bioactive compounds and nutraceutical potential. *A Rev. Comp. Rev. Food Sci. Food Safe.* **2020**, *19*, 2972–2993. [CrossRef]
3. de Sá Mendes, N.; de Andrade Gonçalves, É.C.B. The role of bioactive components found in peppers. *Trends Food Sci. Technol.* **2020**, *99*, 229–243. [CrossRef]
4. Reyes-Escogido, M.L.; Gonzalez-Mondragon, E.G. Vazquez-Tzompantzi, E. Chemical and Pharmacological Aspects of Capsaicin. *Molecules* **2011**, *16*, 1253–1270. [CrossRef] [PubMed]
5. Chen, K.S.; Chen, P.N.; Hsieh, Y.S.; Lin, C.Y.; Lee, Y.H.; Chu, S.C. Capsaicin protects endothelial cells and macrophage against oxidized low-density lipoproteininduced injury by direct antioxidant action. *Chem-Biol. Interact.* **2015**, *228*, 35–45. [CrossRef]
6. Kim, C.S.; Kawada, T.; Kim, B.S.; Han, I.S.; Choe, S.Y.; Kurata, T.; Yu, R. Capsaicin exhibits anti-inflammatory property by inhibiting IkB-a degradation in LPS-stimulated peritoneal macrophages. *Cell Signal* **2003**, *15*, 299–306. [CrossRef]
7. Lin, C.H.; Lu, W.C.; Wang, C.W.; Chan, Y.C.; Chen, M.K. Capsaicin induces cell cycle arrest and apoptosis in human KB cancer cells. *BMC Complem. Altern. Med.* **2013**, *13*, 1–9. [CrossRef]
8. Meral, O.; Alpay, M.; Kismali, G.; Kosova, F.; Cakir, D.U.; Pekcan, M.; Yigit, S.; Sel, T. Capsaicin inhibits cell proliferation by cytochrome c release in gastric cancer cells. *Tumor Biol.* **2014**, *35*, 6485–6492. [CrossRef] [PubMed]
9. Wang, X.; Yu, L.; Li, F.; Zhang, G.; Zhou, W.; Jiang, X. Synthesis of amide derivatives containing capsaicin and their antioxidant and antibacterial activities. *J. Food Bioch.* **2019**, *43*, e13061. [CrossRef]
10. Park, J.S.; Choi, M.A.; Kim, B.S.; Han, I.S.; Kurata, T.; Yu, R. Capsaicin protects against ethanol-induced oxidative injury in the gastric mucosa of rats. *Life Sci.* **2000**, *67*, 3087–3093. [CrossRef]
11. Lee, G.R.; Shin, M.K.; Yoon, D.J.; Kim, A.R.; Yu, R.; Park, N.H.; Han, I.S. Topical application of capsaicin reduces visceral adipose fat by affecting adipokine levels in high-fat diet-induced obese mice. *Obesity* **2013**, *21*, 115–122. [CrossRef] [PubMed]
12. Yang, Y.; Gao, C.; Ye, Q.; Liu, C.; Wan, H.; Ruan, M.; Zhou, G.; Wang, R.; Li, Z.; Diao, M.; et al. The influence of different factors on the metabolism of capsaicinoids in pepper (*Capsicum annum* L.). *Plants* **2024**, *13*, 2887. [CrossRef]
13. Zhu, Z.; Sun, B.; Cai, W.; Zhou, X.; Mao, Y.; Chen, C.; Lei, J. Natural variations in the MYB transcription factor MYB31 determine the evolution of extremely pungent peppers. *New Phytolt.* **2019**, *223*, 922–938. [CrossRef]
14. Arce-Rodríguez, M.L.; Ochoa-Alejo, N. An R2R3-MYB transcription factor regulates capsaicinoid biosynthesis. *Plant Physiol.* **2017**, *174*, 1359–1370. [CrossRef] [PubMed]
15. Souza, C.S.; Daood, H.G.; Agyemang, S.D.; Vinogradov, S.; Palotás, G.; Neményi, A.; Helyes, L.; Pék, Z. Stability of carotenoids, carotenoid esters, tocopherols and capsaicinoids in new chili pepper hybrids during natural and thermal drying. *LWT—Food Sci. Technol.* **2022**, *163*, 1–9. [CrossRef]

16. Phimchan, P.; Techawongstien, S.; Chanthai, S.; Bosland, P.W. Impact of drought stress on the accumulation of capsaicinoids in Capsicum cultivarswith different initial capsaicinoid levels. *HortScience* **2012**, *47*, 1204–1208. [CrossRef]
17. Bae, H.; Jayaprakash, G.K.; Crosby, K.; Yoo, K.S.; Leskovar, D.I.; Jifon, J.; Patil, B.S. Ascorbic acid, capsaicinoid, and flavonoid aglycone concentrations as a function of fruit maturity stage in greenhouse-grown peppers. *J. Food Compos. Anal.* **2014**, *33*, 195–202. [CrossRef]
18. Orellana-Escobedo, L.; Garcia-Amezquita, L.E.; Olivas, G.I.; Ornelas-Paz, J.J.; Sepulveda, D.R. Capsaicinoids content and proximate composition of Mexican chili peppers (*Capsicum* spp.) cultivated in the State of Chihuahua. *CyTA-J. Food* **2013**, *11*, 179–184. [CrossRef]
19. Anitra, C.C.; Maggini, S. Vitamin C and immune function. *Nutrients* **2017**, *9*, 1211. [CrossRef]
20. Lekala, C.S.; Saber, M.K.H.; Phan, A.D.T.; Maboko, M.M.; Fotouo, H.; Soundy, P.; Sultanbawa, Y.; Sivakumar, D. Cultivar-specific responses in red sweet peppers grown under shade nets and controlled-temperature plastic tunnel environment on antioxidant constituents at harvest. *Food Chem.* **2019**, *275*, 85–94. [CrossRef]
21. Lee, S.K.; Kader, A.A. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharv. Biol. Technol.* **2000**, *20*, 207–220. [CrossRef]
22. Tripodi, P.; Cardi, T.; Blanchi, G.; Migliori, C.A.; Schiavi, M.; Rotino, G.L.; Scalzo, R.L. Genetic and environmental factors underlying variation in yield performance and bioactive compound content of hot pepper varieties (*Capsicum annuum*) cultivated in two contrasing Italian locations. *Eur. Food Res.Technol.* **2018**, *244*, 1555–1567. [CrossRef]
23. Deepa, N.; Kaur, C.; George, B.; Singh, B.; Kapoor, H.C. Antioxidant constituents in some sweet pepper (*Capsicum annuum* L.) genotypes during maturity. *LWT-Food Sci. Technol.* **2007**, *40*, 121–129. [CrossRef]
24. Gnayfeed, M.H.; Daood, H.G.; Biacs, P.; Alcaraz, F. Content of bio-active compounds in pungent spice paprika as affected by ripening and genotype. *J. Sci. Food Agric.* **2001**, *81*, 1580–1585. [CrossRef]
25. Jifon, J.L.; Laster, G.; Stewart, M.; Crosby, C.; Leskovar, D.I. Fertilizer use and functional quality fruits and vegetables. *Fertil. Crops Improv. Hum. Health A Sci. Rev.* **2012**, *2*, 21–24.
26. Helyes, L. *A Paradicsom Termesztése (Tomato cultivation)*; Mezőgazda Kiadó: Budapest, Hungary, 2000; pp. 75–76, ISBN 9789630053280.
27. Daood, H.G.; Halasz, G.; Palotás, G.; Palotás, G.; Bodai, Z.; Helyes, L. HPLC Determination of Capsaicinoids with cross-linked C18 column and buffer-free eluent). *J. Chromatogr. Sci.* **2015**, *53*, 135–143. [CrossRef]
28. Camposeco-Montejo, N.; Flores-Naveda, A.; Ruiz-Torres, N.; Álvarez-Vázquez, P.; Niño-Medina, G.; Xochitl Ruelas-Chacón, X.; Torres-Tapia, M.M.; Rodríguez-Salinas, P.; Villanueva-Coronado, V.; Josué, I.; et al. Agronomic Performance, Capsaicinoids, Polyphenols and Antioxidant Capacity in Genotypes of Habanero Pepper Grown in the Southeast of Coahuila, Mexico. *Horticulturae* **2021**, *7*, 327. [CrossRef]
29. Giuffrida, D.; Dungo, P.; Torre, G.; Bignardi, C.; Cavazza, A.; Corradini, C.; Dugo, G. Evaluatioon of carotenoid and capsaicinoid content in powder of red chili peppers during on year of storage. *Food Res. Int.* **2014**, *65*, 163–170. [CrossRef]
30. Usman, M.G.; Rafii, M.Y.; Ismail, M.R.; Malek, A.; Latif, M.A. Capsaicin and dihydrocapsaicin determination in chili pepper genotypes using ultra-fast liquid chromatography. *Molecules* **2014**, *19*, 6474–6488. [CrossRef]
31. Shams, M.; Yuksel, E.A.; Agar, G.; Ekinci, M.; Kul, R.; Turan, M.; Yildirin, E. Biosynthesis of capsaicioids in pungent peppers under salinity stress. *Physiol. Plantar.* **2023**, *175*, e13889. [CrossRef]
32. Schmidt, A.; Fiechter, A.; Fritz, E.-M.; Mayer, H.K. Quantitation of capsaicinoids in different chilies from Austria by a novel UHPLC method. *J. Food Compos. Analy.* **2017**, *60*, 32–37. [CrossRef]
33. Ananthan, R.; Subhash, K.; Longvah, T. Capsaicinoids, amino acid and fatty acid profiles in different fruit components of the world hottest Naga king chilli (*Capsicum chinense* Jacq). *Food Chem.* **2018**, *238*, 51–57. [CrossRef] [PubMed]
34. Duah, A.S.; Souza, C.S.; Daood, H.G.; Pék, Z.; Neményi, A.; Helyes, L. Content and response to ɣ-irradiation before over-ripening of capsaicinoid, carotenoid, and tocopherol in new hybrids of spice chili peppers. *LWT-Food Sci. Technol.* **2021**, *147*, 111555. [CrossRef]
35. González-Zamora, A.; Sierra-Campos, E.; Luna-Ortega, J.G.; Pérez-Morales, R.; Ortiz, J.C.; Garcia-Hornández, J.L. Characterization of different *Capsicum* varieties by evaluation of their capsaicinoids content by high performance liquid chromatography, detection of pungency and effect of high temperature. *Molecules* **2013**, *18*, 13471–13486. [CrossRef] [PubMed]
36. Navez, E.R.; de Ávil Silva, L.; Sulpice, R.; Araújo, W.L.; Nunes-Nesi, A.; Peres, L.E.P.; Zsögön, A. Capsaicinoids: Pungency beyond *Capsicum*. *Trend Plant Sci.* **2019**, *24*, 109–120. [CrossRef]
37. Jeeatid, N.; Techawongstien, S.; Suriham, B.; Bosland, P.W.; Techawongstien, S. Light intensity affects capsaicinoids accumulation in hot peppers (*Capsicum* Chinese Jscq) Cultgivars. *Hortic. Environ. Biotechnol.* **2017**, *58*, 103–110. [CrossRef]
38. Mawarni Saputri, M.; Qory Oktaria, Q.; Alnia Junaidi, A.M.; Agis Ardiansyah, M.A. The effect of light intensity and sound intensity on the growth of various types of chilies in indoor system. *J. Pen. Pend. IPA* **2023**, *8*, 6330–6336. [CrossRef]
39. Gurung, T.; Techawongstien, S.; Suriharn, B.; Techawongstien, S. Impact of environments on the accumulation of capsaicinoids in *Capsicumm* spp. *HortScience* **2011**, *461*, 1576–1581. [CrossRef]

40. Sung, Y.; Chang, Y.Y.; Ni-lun, T. Capsaicin biosynthesis in water stressed hot pepper fruits. *Bot. Bull. Acad. Sin.* **2005**, *46*, 35–42.
41. Estrada, B.; Pomar, F.; Díáz, J.; Morino, F.; Bernal, M.A. Pungency level in fruits of the pedron pepper with different water supply. *Sci. Hortic.* **1999**, *81*, 385–396. [CrossRef]
42. Tupoz, A.; Ozdemir, F. Assessment of carotenoids, capsaicinoidsand ascorbic acid composition of some selected pepper cultivars (*Capsicum annuum* L.) grown in Turkey. *J. Food Comp. Anal.* **2009**, *20*, 596–602. [CrossRef]
43. Duelund, L.; Mouritsen, O.G. Contents of capsaicinoids in chilies grown in Denmark. *Food Chem.* **2017**, *221*, 913–918. [CrossRef]
44. Guijarro-Real, C.; Adalid-Martínez, A.M.; Pires, C.K.; Ribes-Moya, A.M.; Fita, A.; Rodríguez-Burruezo, A. The effect of the varietal, ripening stage, and growing conditions in *Capsicum* peppers. *Plants* **2023**, *12*, 231. [CrossRef]
45. Daood, H.G.; Palotás, G.; Somogyi, G.; Pek, Z.; Helyes, L. Carotenoid and antioxidant content of ground paprika from indoor-cultivated traditional varieties and new hybrids of spice red pepper. *Food Res. Inter.* **2014**, *65*, 231–237. [CrossRef]
46. Kantar, M.B.; Anderson, J.E.; Lucht, S.A.; Mercer, K.; Bernau, V.; Case, K.A.; Le, N.C.; Frederiksen, M.K.; DeKeyser, H.C.; Wong, Z.-Z.; et al. Vitamin Variation in *Capsicum* spp. Provides Opportunities to Improve Nutritional Value of Human Diets. *PLoS ONE* **2016**, *11*, e0161464. [CrossRef] [PubMed]
47. da Silveira Agostini-Costa, T.; da Silva Gomes, I.; de Melo, L.A.M.P.; Reifschneider, F.J.B.; da Costa Ribeiro, C.S. Carotenoid and total vitamin C content of peppers from selected Brazilian cultivars. *J. Food Compos. Anal.* **2017**, *57*, 73–79. [CrossRef]
48. Nagy, Z.; Daood, H.; Ambrózy, Z.; Helyes, L. Determination of Polyphenols, Capsaicinoids, and Vitamin C in New Hybrids of Chili Peppers. *J. Analyt. Meth. Chem.* **2015**, *5*, 102125. [CrossRef]
49. Yuni, W.; Ana-Rosa, B.; Enny, S.; Raoul, B.J.; Arnaud, B.G. Secondary metabolites of *Capsicum* species and their importance in the human diet. *J. Nat. Prod.* **2013**, *76*, 783–793.
50. Olatunji, T.L.; Afolayan, A.J. The suitability of chili pepper (*Capsicum annuum* L.) for alleviating human micronutrient dietary deficiencies: A review. *Food Sci Nut.* **2018**, *6*, 2239–2251. [CrossRef]
51. Duah, S.A.; e Souza, C.S.; Nagy, Z.; Pék, Z.; Neményi, A.; Daood, H.G.; Vinogradov, S.; Helyes, L. Effect of Water Supply on Physiological Response and Phytonutrient Composition of Chili Peppers. *Water* **2021**, *13*, 1284. [CrossRef]
52. Mahendran, S.; Bandara, D.C. Effects of Soil Moisture Stress at Different Growth Stages on Vitamin C, Capsaicin and P-Carotene Contents of Chilli (*Capsicum annuum* L.) Fruits and their Impact on Yield. *Trop. Agric. Res.* **2000**, *12*, 95–106.
53. Selahle, K.M.; Sivakumar, D.; Jifon, J.; Soundy, P. Postharvest response of red and yellow sweet peppers grown under photo-selective nets. *Food Chem.* **2015**, *173*, 951–956. [CrossRef] [PubMed]