Article

Determination of Color and Heat Level of Some Resistance Sources and Improved Pepper Genotypes

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ABSTRACT

Background: In this study, the capsaicin content, heat level and color of nine pepper genotypes and varieties belonging to *Capsicum annuum* and *Capsicum chinense* were described at different ripening stages. Heat level and color is a desirable feature for Turkish pepper consumers. Therefore, knowledge on the heat level and of Turkish pepper genotypes is important for a future breeding project aiming variety development.

Methods: Turkish materials (Alata 10, 15 and 111) were improved from the pepper collection of Alata Horticultural Research Institute-Turkey. The Rwandan variety Pili-Pili is consumed in Rwandan cuisine. The capsaicin content of the Alata collection and Pili-Pili were identified both at immature and red ripening stages. In addition, some accessions carrying various resistances to diseases were evaluated: namely Er-Fu-Tou (Cucumber Mosaic Virus), C29 (*Phytophthora capsici* and Potato Virus Y), PI 260429 (Tobacco Mosaic Virus) and PI 152225 (Tomato spotted wilt virus); for these accessions, sampling was done at red ripening stage. High-performance liquid chromatography (HPLC) was used for identifying capsaicinoids and extraction was performed by sodium acetate saturated ethanol. ASTA 20.0 method was applied to measure the extractable color of improved lines.

Results:

- Results demonstrated that there was considerable variation among genotypes.
- The highest capsaicin content and heat level were detected in the genotype PI 152225. For dihydrocapsaicin content, Er-Fu-Tou variety was the first and followed by PI 260429 and PI 152225.
- Heat level in pepper involves the relationship between capsaicin and dihydrocapsaicin and therefore, it is important to convert to the

Scoville Heat Unit (SHU). The most pungent genotype PI 152225 was followed by Pili-Pili variety (red stage), Er-Fu-Tou, Rwandan Pili-Pili variety (immature stage), Alata 111 (red stage) and PI 260429.
- The highest ASTA value was detected in mature fruits of Er-Fu-Tou genotype with 217 ASTA color value and it was followed by improved line Alata 111 from Kahramanmaraş pepper population with 173 ASTA.

Conclusions: At the end of study, Er-Fu-Tou (CMV resistant) and PI 152225 (TSWV resistant) lines were found to be useful to improve varieties having both quality properties and resistance to diseases from the Kahramanmaraş red pepper population via short term breeding program while they were not suitable to improve sweet pepper with rapid breeding program. Rwandan variety Pili-Pili was not found appropriate to improve varieties producing high quality dried pepper.

KEYWORDS: *Capsicum*; heat level; breeding; capsaicin; ASTA

INTRODUCTION

Peppers are known as a good source of important vitamins (ascorbic acid-vitamin C, carotenoids-vitamin A, tocopherols-vitamin E), minerals [Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg), Phosphorus (P), Sulphur (S), Manganese (Mn), Iron (Fe), Copper (Cu), Zinc (Zn) and Selenium (Se)], antioxidants, flavonoids and capsaicinoids [1,2]. Therefore, they are one of the most produced and consumed vegetables in the world. Peppers ranked 44th and 69th in terms of production and area respectively among 178 primary products in FAOSTAT [3] database with a total production of 36,092,631 tons in an area of 1,987,059 ha [4].

The origin of *Capsicum* is Bolivia with between 20 and 27 *Capsicum* species [5–8]. Among *Capsicum* species, *C. chinense* cv. Red Savina was recorded as the hottest pepper with 577,000 Scoville Heat Unit (SHU) in 2006 [8,9]. After that "Bhut Jolokia" obtained via crossing *C. chinense* and *C. frutescens* was identified to be hottest with 879,953–1,001,304 SHU by Bosland and Baral [10]. However, the hottest pepper recorded in the world is Trinidad Moruga Scorpion in 2012 [11]. Capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin have been identified as naturally occurring capsaicinoids; however, the most abundant ones are capsaicin and dihydrocapsaicin [2] and the heat level of peppers has been determined by using a formula containing these two compounds. Capsaicinoids are specific to the genus *Capsicum* and are responsible for the hot or spicy flavor. They are known with health beneficial properties such as anti-inflammatory, antioxidant and anticarcinogenic activity [2]. Many researchers reported that the epidermal cell of the placenta is the main origin of capsaicinoid synthesis and accumulation; however other parts of the plant (leaves and stem) contain capsidiol [12–14]. Together with capsaicinoid synthesis has started after 20–50 days anthesis in the pepper fruits, it increases with maturity [14]. It is known that the heat level of peppers can vary due to different factors such as genotype, growing condition and maturity stage [8].

Pepper consumption is very common in Turkey and peppers are consumed in various forms such as fresh raw, fresh-cooked, dried, pepper paste, sauce, pickle and red pepper powder. Especially pungent peppers are popular in Turkish cuisine.

Changes in carotenoid biosynthesis during ripening in pepper depend on varieties. The lutein and neoxanthin decrease during maturation and disappear completely at maturity. Beta-carotene, antheraxanthin and violaxanthin levels increase and synthesis of zeaxanthin, beta-cryptoxanthin, capsanthin, capsorubin, capsanthin-5,6-epoxide, and cucurbitaxanthin start. Zeaxanthin level increases considerably with maturation and there is an inverse relationship between the ratio of red pigments to yellow pigments and capsanthin zeaxanthin ratio [15]. Powdered or ground pepper processed from fully ripened and partially dried pepper fruits contains the highest extractable color substances. Spice peppers obtained from this type of fruits reach to 194 ASTA (The American Seed Trade Association) units. Green pepper obtained from fully immature peppers has 50 ASTA units [16].

The capsaicin content, heat level and color of nine pepper genotypes and varieties which are important as disease resistance sources to improve pepper varieties belonging to *Capsicum annuum* and *Capsicum chinense* from different origins were described at different ripening stages in this research. The aim of the study was to determine quality criteria of resistance sources and susceptible donors using to improve pepper cultivars that could be brought together resistance and fruit quality.

MATERIALS AND METHODS

Materials

Plant material of the study was selected from two different long-term breeding programs which were started in 2015 to improve CMV, Potyviruses, TSWV and *Phytophthora* blight resistant pepper cultivars. Turkish pepper materials (Alata 10, 15 and 111) were selected from the pepper collection of Alata Horticultural Research Institute (ALATA, Mersin, Turkey). The Rwandan variety Pili-Pili belonging to *C. chinense* is one of the well-known and popular pepper varieties in Rwandan cuisine (Figure 1). Pepper genotypes selected from ALATA collection are consumed both at mature and immature stages while genotypes selected from Kahramanmaraş are consumed as dried red paprika flake and powder. The capsaicin content of the Alata collection and Pili-Pili was identified both at immature and red ripening stages. In addition, some accessions carrying various resistances to diseases were evaluated: Er-

Fu-Tou (Cucumber Mosaic Virus), C29 (*Phytophthora capsici* and Potato Virus Y), PI 260429 (Tobacco Mosaic Virus) and PI 152225 (Tomato spotted wilt virus); for these accessions, sampling was done at red ripening stage. The species and types of pepper genotypes and varieties were given in Table 1.

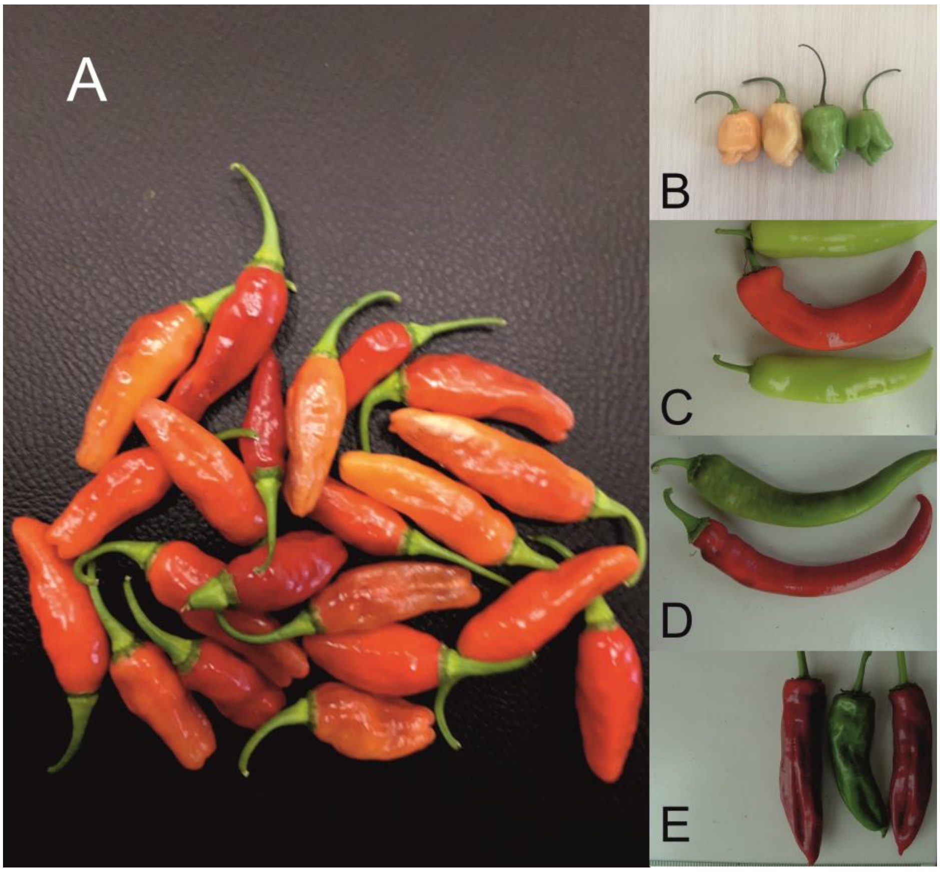

Figure 1. Fruit type of selected pepper genotypes. **A**: PI 152225; **B**: Rwandan variety Pili-Pili; **C**: Alata 10; **D**: Alata 15; **E**: Alata 111.

Methods

Extraction of capsaicin and dihydrocapsaicin

The whole fruits were dried at 50 °C for 2 days and ground to a fine powder. One gram of dried and ground fruit was stirred in 10 mL sodium acetate saturated ethanol for 3 h at 60 °C. The mixture was filtrated and analyzed.

Determination of capsaicin and dihydrocapsaicin content

Capsaicinoid content was detected by using High-Performance Liquid Chromatography (HPLC). HPLC is currently used in the identification and quantification of capsaicinoids [8].

HPLC (Model LC-20 Shimadzu, Kyoto, Japan) consisting of a high-pressure pumps, column (C18 100-5 250 × 4.6 mm^2), oven (35 °C) and UV-VIS detector (at 280 nm) was used to determine capsaicinoid content.

Isocratic mode (methanol 48.4%, water 30.2% dioxane 13.3%, acetonitrile 7.9% and perchloric acid 0.2% (2% v/v perchloric acid/water)) was performed at 1.5 mL·min^{-1} of flow rate with 20 μL sample volume [17]. Standards of capsaicin and dihydrocapsaicin were purchased from Sigma-Aldrich Co (St. Louis, MO, United States) (Figure 2). Capsaicin and dihydrocapsaicin content were converted to SHU (Scoville Heat Unit) multiplying total capsaicinoids by 15.

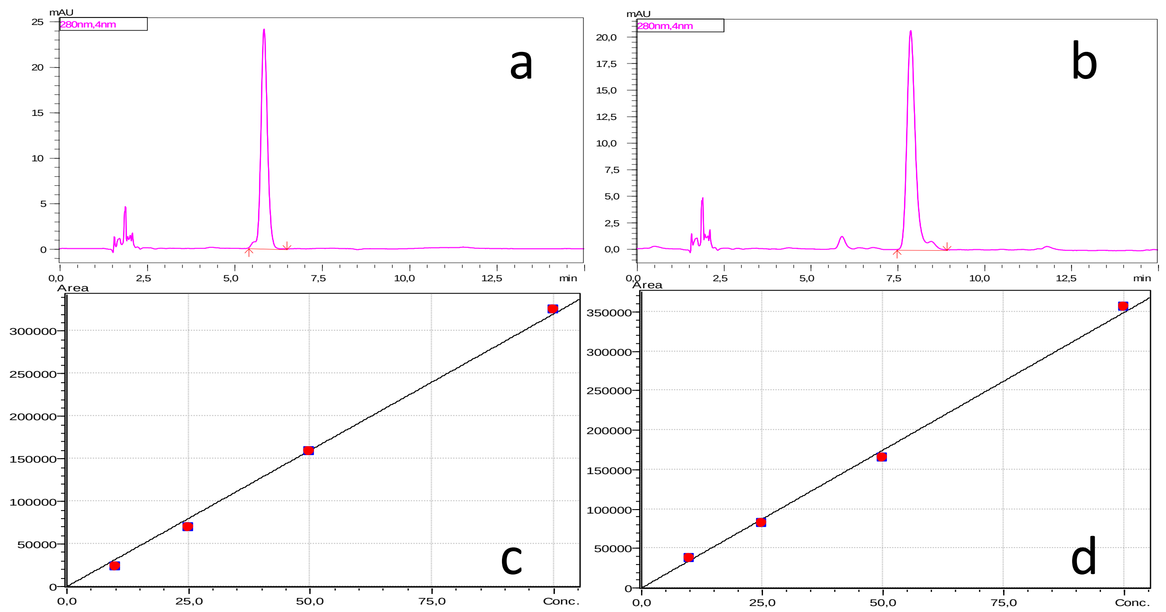

Figure 2. Chromatogram of standards: (**a**) capsaicin and (**b**) dihydrocapsaicin. The concentration of capsaicin and dihydrocapsaicin standards was 100 ppm. Calibration curves of standards: (**c**) capsaicin and (**d**) dihydrocapsaicin. The excitation and emission wavelengths were 280 and 325 nm.

Extractable color measurements

The extractable color was measured according to ASTA 20.0 method. An amount of 1 g of dried red pepper powder was transferred in acetone added 100 mL volumetric flask and shaken in the dark condition for 16 h. The absorbance of the extract was measured at 460 nm using a Thermo Scientific Multiskan GO UV/VIS spectrophotometer.

$$\text{ASTA color} = \frac{\text{Absorbance of the sample extract} \times 16.4 \times I_f}{\text{Sample weight in grams}}$$

I_f is a correction factor that is calculated by the absorbance of a standard solution of potassium dichromate, ammonium sulfate and cobalt sulfate (ASTA [18]).

RESULTS

In this study, the level of the capsaicin and dihydrocapsaicin was not sufficient to be detected in the Alata 10 and Alata 15 genotypes belonging to capia and Turkish-sivri pepper types, respectively (Table 1). We know that varieties of capia types are generally not pungent. Some of Turkish-sivri type varieties are pungent, while others are not. The highest capsaicin content and heat level were detected in genotype PI 152225 belonging to *C. chinense* with 105,236 SHU and 90,722 mg·kg^{-1} capsaicin. For dihydrocapsaicin content, Er-Fu-Tou variety was the first (20,668 mg·kg^{-1}) and followed by PI 260429 (18,758 mg·kg^{-1}) and PI 152225 (17,700 mg·kg^{-1}) (Figure 3). In our study, the SHU value of *C. annuum* varieties and genotypes varied between 9574 and 105,236, except Alata 10 and 15 genotypes. Our capsaicin and dihydrocapsaicin results varied between 2949 and 90,722, 53 and 20,668 mg·kg^{-1}, respectively.

Pungency involves the relationship between capsaicin and dihydrocapsaicin and therefore, it is important to convert to the Scoville Heat Unit. The most pungent genotype PI 152225 was followed by Pili-Pili variety (red stage), Er-Fu-Tou, Rwandan Pili-Pili variety (immature stage), Alata 111 (red ripening stage) and PI 260429 (Table 1).

The capsaicin-dihydrocapsaicin content and SHU value of our *C. chinense* variety Rwandan Pili-Pili at the red ripening stage were 3590 mg·kg^{-1}, 430 mg·kg^{-1} and 59157 SHU, respectively.

Table 1. Capsaicin-Dihydrocapsaicin content, heat level and extractable color (ASTA) of pepper genotypes-varieties tested in this study.

Genotype	**Botanical name**	**Type**	**Maturity stage**	**Capsaicin (mg·kg^{-1})**	**Dihydrocapsaicin (mg·kg^{-1})**	**SHU**	**ASTA**
Pili-Pili	*C. chinense*	Scotch Bonnet	Immature	2949	Not detected	44,241	7
Pili-Pili	*C. chinense*	Scotch Bonnet	Mature	3590	430	59,157	10
Alata 10	*C. annuum*	Capia	Immature	Not detected	Not detected	0	39
Alata 10	*C. annuum*	Capia	Mature	Not detected	Not detected	0	89
Alata 111	*C. annuum*	Turkish-Kahramanmaraş	Immature	Not detected	Not detected	0	32
Alata 111	*C. annuum*	Turkish-Kahramanmaraş	Mature	1805	53	27,741	173
Alata 15	*C. annuum*	Turkish-Sivri	Immature	Not detected	Not detected	0	41
Alata 15	*C. annuum*	Turkish-Sivri	Mature	Not detected	Not detected	0	145
Sena	*C. annuum*	Turkish-Kahramanmaraş	Mature	6453.6	3806	9574.4	84
PI152225	*C. chinense*	Wild	Mature	90,722	17,700	105,236	63
Er-Fu-Tou	*C. annuum*	Long type for dried	Mature	28,083	20,668	45,032	217
C29	*C. annuum*	Kahramanmaraş for dried	Mature	6453	3805	9574	Not tested
PI260429	*C. annuum*	Wild	Mature	7906	18,758	23,288	Not tested

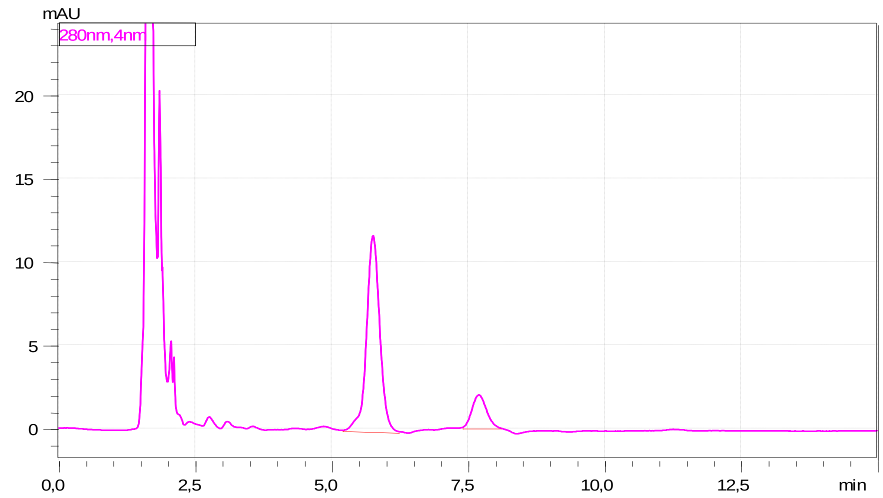

Figure 3. Capsaicin and dihydrocapsaicin chromatogram of PI 152225. The excitation and emission wavelengths were 280 and 325 nm.

Another important quality criterion for dried pepper fruits is color. The red color of ripe pepper fruit contains capsanthin, capsorubin, cryptoxanthin and zeaxanthin pigments which are related to each other in the form of fatty acid esters in the fruit. The most important of these pigments is capsanthin, which constitutes 30–60% of the total carotenoids in the fruit, and capsorubin, which is the isomer of the capsanthin, which constitutes 6–18%. The intensity of the red color is formed by the effect of these two pigments [19]. ASTA is a unit to identify the color degree of the powdered chilies measured using a spectrophotometer extracted by the solvent. The highest ASTA value was identified from mature fruits of Er-Fu-Tou genotype with 217 ASTA color value. Pili-Pili genotype had the lowest ASTA value in both mature and immature fruit stages. The ASTA value of the Rwandan Pili-Pili variety almost did not increase during maturation. Improved line Alata 111 from Kahramanmaraş pepper population had 173 ASTA color intensity, while registered dried red pepper variety Sena had 84 ASTA. While the ASTA value of mature fruit of capia type Alata 10 was 89 ASTA, it was 145 in Alata 15.

DISCUSSION

Capsaicinoid content and heat level vary at different ripening stages and are increasing with maturity. Similarly, higher results were obtained in the red ripening stage in this study. González-Zamora et al. [2] found it between 13704.8 and 483089.3 in their genotypes. They are between 0.17 and 15.36 $mg \cdot g^{-1}$, 0.61 and 13.39 $mg \cdot g^{-1}$ respectively in the study of González-Zamora et al. [2]. In other research carried out in varieties of *C. annuum* by Gougoulias et al. [20], seven red hot peppers varieties were evaluated in terms of their polyphenols, capsaicin content and antioxidant properties. Capsaicin content varied between 539.2 and 1775 $\mu g \cdot g^{-1}$ fresh weight in their studies. Genotype, fruit maturity and climate factors have an effect on the concentration of capsaicinoids [2]. All these factors alone or together may be the cause of different results.

In a study carried out by González-Zamora et al. [2], capsaicinoids content of eight different varieties of *C. annuum* was determined by a validated chromatographic procedure (HPLC-DAD). SHU values were recorded between 13,704.8 ± 2275.7 and 483,089.3 ± 54,336.8 in the *C. annuum* varieties. Total capsaicinoids were between 1.46 ± 0.08 and 31.84 ± 0.75 mg·g^{-1} dry weight. Dihydrocapsaicin was found to be higher than capsaicin in the research (capsaicin: (0.17 ± 0.01)–(15.36 ± 1.67), dihydrocapsaicin (0.61 ± 0.11)–(13.39 ± 1.60)). They also realized that total capsaicinoids was increased with high temperature in some varieties, while capsaicinoids and heat level were decreased by the effect of temperature in De'arbol and Jalapeño varieties.

When the literature regarding with *C. chinense* is examined, in a study carried out by Canto-Flick et al. [8], heat level of Habanero pepper accessions were identified by high-performance liquid chromatography (HPLC) technique in whole fruit, placenta, and pericarp. The samples used in total capsaicinoids analyses were harvested at the completely ripe stage. Results showed variations among accessions and different parts of the plant. When evaluated in terms of SHU, capsaicinoid content was found to be between (145,950 ± 2591.1)–(892,719 ± 9.57), (2545.143 ± 472.8)–(4616.628 ± 18,252.4) and (180,228 ± 675.5)–(6090.84 ± 34.6) in whole fruit, placenta, and pericarp, respectively. Similarly, as mg·g^{-1}, it was detected to be between (9.73 ± 0.17)–(595,146 ± 0.64) mg·g^{-1}, (1,696,762±0.03)–(30,7752 ± 1.22) mg·g^{-1} and (12.0152 ± 0.05)–(92.1926 ± 0.28) mg·g^{-1} in whole fruit, placenta, and pericarp, respectively. Their results showed that important variability among accessions in the heat level due to genetic and environmental factors. Habanero peppers are one of the hottest pepper groups in the world. Guillen et al. [14] determined quantification of capsaicinoids and heat level of *C. chinense* Jacq., and *C. baccatum* var. *pendulum* in a different part of the plant (pericarp, seeds, placenta and interlocular septa). They found that capsaicinoid content and heat level varied related to the fruit parts and the species. The highest values were obtained from the placenta with 79% and 51% in *C. chinense* and *C. baccatum*, respectively. Results showed that *C. chinense* contained more capsaicinoids and heat level. Also, data obtained from this study indicated that the major component of capsaicinoids was capsaicin (4399 μg·g^{-1} of the dry weight in *C. chinense* and 1.582 μg·g^{-1} in *C. baccatum*) when compared with dihydrocapsaicin and nordihydrocapsaicin.

The researchers working on capsaicinoid content and heat level have not carried out studies on pepper fruits alone. Dang et al. [21] determined concentrations of the major capsaicinoids (capsaicin and dihydrocapsaicin) in red pepper products in South Korea by using HPLC. The extraction of capsaicinoids was carried out by using 95% methanol. The highest capsaicinoids content was recorded in red pepper powder with 4.18–139.4 mg 100 g^{-1} and it was followed by gochujang (0.93–23.20 mg 100 g^{-1}), kimchi (0.05–1.16 mg 100 g^{-1}), and sliced kimchi (0.06–0.88 mg 100 g^{-1}).

Yoon et al. [22] reported that ASTA color values of 523 pepper genotypes from Korea pepper germplasm were 81.6 on average and maximum value was reached to 233.1. Zaki et al. [23] found that ASTA color values of genotypes selected from the population grown locally for spice pepper production in Morocco varied between 80 and 170. It is known that the amount of carotenoid content varies according to the light intensity, temperature, maturity of the fruit, the location where the plant is grown and genotype. In the study conducted by Krajayklang et al. [24], the ASTA value of PS72285 sweet powder pepper pungent Caysan SPS705 cultivars increased from 50 to 194 and from 25 to 120 with the ripening, respectively. In another study carried out by Derera et al. [25], it was determined that the ASTA values of peppers grown in Hungary and used in the production of ground pepper reached approximately to 130 units and that ASTA extractable color values could be obtained in the range of 180–250 from the same varieties in Australia. The researchers aimed to develop a kind with extractable color content of more than 200 ASTA values in their studies and succeeded in developing lines showing 274 ASTA values in F7 generation. Arpacı et al. [26] found the color value of Sena which numbered as 207 in their work as 69 ASTA and it was lower than this study.

CONCLUSIONS

Capsaicinoid content, heat level and color of nine pepper genotypes and varieties belonging to *C. annuum* and *C. chinense* were determined in this study. The results showed that there was considerable variation among genotypes. Also, capsaicin-dihydrocapsaicin content and SHU value increased with the maturity of fruits in the genotypes containing capsaicinoids. Hot peppers are desirable and popular in Turkish cuisine. Therefore, knowing the capsaicin content and heat level of pepper genotypes is important for future breeding projects, which will be performed in Turkey. In particular, obtaining reliable data from disease-resistant genotypes is important for breeders. The plant materials including both high capsaicinoid content and disease resistance will be priceless for the planning of future projects. Capia and sivri type peppers were not suitable for drying related to both color and heat properties, while improved lines from Kahramanmaraş population were suitable.

When these findings were evaluated, it can be concluded that CMV and TSWV resistant genotypes were found to be useful to improve both quality properties and resistance to disease of the Kahramanmaraş red pepper population.

AUTHOR CONTRIBUTIONS

BBA and DK selected and culture the plant materials. BBA, DK, HT, EK, GB and ÜHE designed and carried out the experimental work. HT and GB analyzed the results. HT, BBA and DK obtained funding for the work. HT and BBA drafted the manuscript. all authors read and corrected the manuscript.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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