Correlation of Carotenoids Content and ASTA Values of Pepper (Capsicum chinense) Genetic Resources

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Abstract: In this study, 226 Capsicum chinense genetic resources were analyzed using high-performance liquid chromatography and a spectrophotometer to measure and compare their carotenoid content and American Spice Trade Association (ASTA) color values, respectively. The total carotenoid content and ASTA values of the 226 pepper genetic resources were found to be in the range of 7.04–2430.85 mg/100 g dry weight (DW) and 0–221.32, respectively. Capsanthin, capsorubin, lutein, zeaxanthin, antheraxanthin, violaxanthin, \( \alpha \)-carotene, \( \beta \)-carotene, and \( \beta \)-cryptoxanthin were the individual carotenoids studied. The average content of each carotenoid (\( n = 226 \)) in mg/100 g DW was as follows: capsanthin (325.77), antheraxanthin (136.34), capsorubin (82.36), \( \beta \)-carotene (59.11), violaxanthin (46.54), zeaxanthin (43.21), \( \alpha \)-carotene (13.91), \( \beta \)-cryptoxanthin (9.67), and lutein (2.72). A strong positive correlation was observed between total carotenoid content and ASTA value (\( r = 0.965 \)). Likewise, the individual carotenoid content and ASTA value revealed a strong correlation, such as antheraxanthin (\( r = 0.964 \)), capsanthin (\( r = 0.946 \)), and capsorubin (\( r = 0.858 \)). Three genetic resources with a total carotenoid content above 2000 mg/100 g DW were obtained, such as IT261426 (2430.85 mg/100 g DW), IT183657 (2077.55 mg/100 g DW), and IT261213 (2062.54 mg/100 g DW). The findings of this study will assist in the selection of genetic resources with high carotenoid content and ASTA value that can be used to develop and breed new pepper varieties. Also, detailed results of ASTA value correlation with carotenoids in C. chinense genetic resources are provided.

Keywords: ASTA value; Capsicum chinense; carotenoid; correlation

1. Introduction

Capsicum annuum L. is a Solanaceae crop, and is one of the fruits and seasoning vegetables enjoyed in various forms in Korean cuisine. Peppers are also popular vegetables all over the world, including Korea, and have high economic value. Currently, five species are domesticated: C. annum, Capsicum baccatum, Capsicum chinense, Capsicum frutescens, and Capsicum pubescens [1,2]. C. chinense is a species from Central America with highly spicy ingredients, and its representative varieties include Butzolocia and Habanero. Habanero pepper fruit is known to be an excellent source of phytochemicals, including vitamins A and C, phenolic compounds, flavonoids, and carotenoids [3].

Phytochemicals are abundant in fresh fruits and vegetables [4]. They have health-promoting properties such as antioxidants, substances that influence blood pressure or blood sugar, and agents with immunity-boosting, anticarcinogenic, antifungal, antibacterial, antiviral, cholesterol degradation, antithrombotic, and anti-inflammatory properties [5]. These functional compound groups can comprise a wide variety of substances. Carotenoids, phenolic compounds, sulfides, protease inhibitors, and phytoestrogens are examples of anti-oxidative phytochemicals [5,6]. Carotenoids, which are mostly found in nature, are
synthesized in plants and collected in flowers, leaves, and fruits. All animals including human beings are unable to synthesize carotenoids in the intestine, therefore it should be compensated by optimizing the daily intake of fruits and vegetables [7]. Carotenoids have been shown to reduce the occurrence of chronic diseases due to their antioxidant properties and their ability to act as anticancer agents and lower the risk of cardiovascular disease [7–10].

Carotenoid is an isoprenoid compound that plays an important role in many biological processes in plants, including improving light energy absorption and protecting plants from strong light [11]. Capsicum species fruits are considered high-carotenoid, non-leafy vegetables [12]. The majority of pepper carotenoids are composed of the unique, powerful, and highly stable capsorubin and capsanthin as well as $\beta$-carotene, lutein, $\beta$-cryptoxanthin, zeaxanthin, violaxanthin, and antheraxanthin [12]. These carotenoids have a wide range of profiles and levels and are biosynthetically linked to fruit maturation stages [12]. Apart from the harvest maturity stage, genotypes, agro-climatic conditions, post-harvest handling, processing, and preparation, all influence the composition and relative content of carotenoids in Capsicum species [13–16].

The variation in genotypes that determine specific carotenoid biosynthetic enzymes has been linked to a significant variation in carotenoid profile among Capsicum species and cultivars [17]. With the exception of a few intense orange-fruited cultivars that resulted from a mixture of red pigment, capsanthin, and yellow xanthophylls, namely, $\beta$-cryptoxanthin and violaxanthin [15,18,19], the orange color of most orange pepper cultivars is due to an abundance of $\beta$-carotene. In certain yellow/orange cultivars, the absence or inactivation of the capsanthin-capsorubin synthase enzyme has blocked the synthesis of red pigments, allowing their precursors (e.g., violaxanthin) to accumulate as the major carotenoid at the fully ripe stage [20]. Red pepper is distinguished by its high red-colored xanthophyll content and composition [12].

Among the various pepper-derived products used in the industry, such as oleoresin and other enriched extracts, one of the most important is the powder known as paprika, which is used as a spice in meat products, soups, sauces, and snacks, accounting for 70% of its production [21]. The intensity of its red color is the primary quality attribute of this ingredient, which influences both consumer acceptance and commercial value [22]. There are several methods for evaluating paprika color, including surface color measurement, pigment extraction, and carotenoid profiling [22]. In international trade, paprika is classified into different quality classes based on the number of ASTA units per paprika dry weight, as determined by the ASTA (American Spice Trade Association) official analytical method [23]. Aside from being used as a condiment, providing characteristic pungency, color, and flavor, the new pepper-derived ingredients could be used for the preservation and extension of the lifespan of industrial products, as well as additives or technological ingredients with antioxidant and antimicrobial properties [22].

Currently, as people’s interest in health grows, several studies are being conducted to develop varieties containing the high functional ingredients of peppers. Therefore, the objectives of this study were to characterize useful genetic resources to help improve pepper quality, foster and develop new varieties with high carotenoid content and understand the correlation of carotenoids with the American Spice Trade Association (ASTA) color index of peppers. More or less, a similar experiment had been done before with a different species of capsicum (C. annum). However, this study provided a comprehensive result of how carotenoids correlated with ASTA color value using 226 C. chinense pepper germplasm.

2. Materials and Methods
2.1. Chemicals and Plant Materials

All chemicals used in this study, including carotenoid standards, extraction solvents, and reagents, were of analytical grade. The chemicals used in this study, including carotenoid standards (capsanthin, capsorubin, antheraxanthin, violaxanthin, lutein, zeaxanthin, beta-cryptoxanthin, alpha-cryptoxanthin), potassium hydroxide, dichloromethane,
methanol, sodium chloride, ascorbic acid, ammonium acetate, methyl tert-butyl ether, and hexane-ethyl acetate, were purchased from Sigma-Aldrich (Saint Louis, MI, USA).

A total of 226 C. chinense genetic materials from the National Agrobiodiversity Center’s (NAC) gene bank (Rural Development Administration [RDA], Jeonju, Republic of Korea) were used in this study. The number of germplasms with different fruit colors were described as follows: red: 142, orange: 35, yellow: 24, brown: 21, and deep red: 4 germplasm. Ten to twelve pepper plants were grown from each accession in the NAC greenhouses. The plants were grown in the RDA using standard agronomic practices until harvest. Pepper fruits that had fully ripened were harvested, freeze-dried, powdered, and stored in a deep freezer (−70 °C) for further analysis. The IT (introduction) number, fruit color at ripening stage, and the origins of 226 C. chinense pepper materials are summarized in the Supplementary Table S1. The graphical scheme of the study design is presented in the Supplementary Figure S1.

2.2. Extraction and Carotenoids Analysis

All of the pepper samples in this study were freeze-dried and powdered. With minor modifications, the extraction, separation, and measurement of carotenoids by HPLC were performed as described by Kim et al. [24]. Briefly, carotenoids were extracted from pepper powder (0.05 g) that had passed through a 0.7 mm sieve by adding 3 mL of ethanol containing 0.1% ascorbic acid (w/v), vortexed for 20 s, and placed in a water bath at 85 °C for 5 min. The carotenoid extract was saponified in an 85 °C water bath for 10 min with potassium hydroxide (120 µL, 80% w/v). Following saponification, samples were immediately placed on ice and 1.5 mL of cold deionized water was added. As an internal standard, β-apo-80-carotenal (0.05 mL, 25 µg/mL) was added. Carotenoids were extracted twice with hexane (1.5 mL) and centrifuged at 12,009 × g. Before HPLC analysis, aliquots of the extracts were dried under a stream of nitrogen, redissolved in 50:50 (v/v) dichloromethane/methanol, and filtered through a 0.2 µm syringe. The carotenoids were separated by HPLC (Agilent 1260/90 Infinity II, Santa Clara, CA, USA) on a C30 YMC column (250 × 4.6 mm, 3 µm; Waters Corporation, Milford, MA, USA) as mentioned above. At 450 nm, chromatograms were generated. Solvent A was a mixture of methanol and water (92:8 v/v) containing 10 mM ammonium acetate. Solvent B was made of 100% methyl tert-butyl ether. The gradient elution conditions were as follows: 0 min (83% A and 17% B), 23 min (70% A and 30% B), 29 min (59% A and 41% B), 35 min (30% A and 70% B), 40 min (30% A and 70% B), 44 min (83% A and 17% B), and 55 min (83% A and 17% B) at 1 mL/min flow rate. Calibration curves were drawn for quantification purposes by plotting four different concentrations of carotenoid standards based on peak area ratios with β-Apo-80-carotenal.

2.3. ASTA Color Index Measurement

The extractable color of the pepper powder samples was determined using the method recommended by the American Spice Trade Association [25]. After freeze-drying, 100 mg of pepper powder sample was extracted with 100 mL of 100% acetone solution. The pigment extraction of peppers was done overnight at room temperature in dark conditions for 16 h. Next, the supernatant was collected (2 mL), and the absorbance was measured using a microplate reader (Tecan Infinite 200 PRO) in the 455–460 nm range. The measurements were done in triplicate. The following formula was used to calculate the ASTA values:

\[
\text{ASTA value} = \frac{\text{Absorbance of acetone extracts} \times 16.4 \times I_F}{\text{Sample weight (g)}}
\]

\(I_F\) refers to the instrument correction factor, which is the declared absorbance of the glass reference standard. The glass filter (NIST SRM 2030 or 930) has an absorbance in the range of 0.4–0.6 at 460 nm, or equivalent, as specified by the NIST (National institute of standards and technology).
2.4. Statistical Analysis

The data were summarized using Microsoft Excel. Correlation, principal component analysis (PCA), and cluster analysis were carried out using R statistical software package (v 4.0.4).

3. Results and Discussion

3.1. ASTA Color Value

The ASTA color analysis conducted to chemically measure the degree of red pigment in peppers is used as an indicator of the quality of peppers in the international community and has high reliability [26]. The ASTA chromaticity values standardized by the U.S. Flavor Trade Union are usually below 100, with 101–130 usually classified as high and 161 and higher classified as special. The ASTA values of varieties cultivated and commercialized in Korea are distributed within a range of 67.5–145.6 [27], and 98.7–184.2 [28]. Representative resources according to the type of carotenoid composition (fingerprint) were likewise classified in the red pepper genetic resource, and ASTA values were variously distributed from 7.4 to 165.7 [29]. However, in this study, wider ASTA range values were obtained. As a result, the ASTA color value ranged from 0 to 221.32. (Table 1). This variation could be associated with the variation of pepper germplasm in fruit colors used in the study.

Table 1. Summary of carotenoid contents and ASTA value in 226 genetic resources C. chinense.

| Capsanthin | Capsorubin | Lutein | Zeaxanthin | Antheraxanthin | Violasanthin | α-Carotene | β-Carotene | β-Cryptoxanthin | Total Carotenoids | ASTA Value |
|------------|------------|--------|------------|----------------|--------------|-------------|-------------|-----------------|------------------|------------|
| Minimum    | 0.00       | 0.00   | 0.00       | 0.00           | 0.00         | 0.00        | 0.00        | 5.86            | 2430.85          | 221.32     |
| Maximum    | 1120.95    | 501.40 | 80.66      | 361.10         | 445.35       | 168.89      | 70.87       | 184.33          | 2430.85          | 221.32     |
| Average    | 325.77     | 82.36  | 2.72       | 43.21          | 136.34       | 46.54       | 13.91       | 36.90           | 719.62           | 75.12      |
| SD         | 222.80     | 80.05  | 11.16      | 46.01          | 90.40        | 31.30       | 14.01       | 10.88           | 471.42           | 45.22      |

Note: SD, standard deviation.

As presented in Figure 1, which illustrates the distribution of C. chinense pepper genetic resources according to their ASTA values, two pepper genetic resources had ASTA values greater than 200: IT261426 (221.32) and IT261213 (211.72). Among the 226 pepper genetic resources, the highest frequency count (99 pepper materials) was found with a 50–100 ASTA value, followed by 69, 44, and 12 accessions with ranges of 0–50, 100–150, and 150–200 ASTA color values, respectively. The average ASTA color value of 226 C. chinense pepper materials was 75.12 (Table 1).

Figure 1. The distribution of Pepper (Capsicum chinense) genetic resources according to the ASTA color values.
### 3.2. Composition and Content of Carotenoids

*Capsicum*’s genetic diversity results in fruits with a diverse morphology and carotenoid profile. Despite being prized for the pharmacologically active pungent capsaicinoids; carotenoids increase the nutritional value of pepper [12]. Pepper carotenoids primarily consist of the unique, powerful, and highly stable capsanthin and capsorubin, as well as β-carotene, β-cryptoxanthin, lutein, zeaxanthin, antheraxanthin, and violaxanthin [12]. Red peppers contain all the carotenoids that are typically studied with regard to diet and human health (lutein, zeaxanthin, α-carotene, β-carotene, and β-cryptoxanthin), except lycopene [30]. Capsanthin is the most abundant carotenoid in red pepper [31–33]. It was found to have the highest concentration (average 312.14 mg/100 g dry weight [DW]) and accounted for 45% of the total carotenoids in this study of 226 *C. chinense* peppers (Figure 2). Capsanthin accounts for approximately 60% of the total carotenoids in peppers [34], regardless of the various factors influencing carotenoid content in foods, such as variety, season, geographic location/climate, ripeness, and growing conditions [35]. A recent study discovered that capsanthin accounts for 84% of the total carotenoid content in Lamuyo-type sweet red peppers (*C. annuum* L.) sampled from the local market. It has been shown to have anti-obesity and insulin-sensitizing activity in animals [36], and there is growing interest in its potential benefits for humans, including an inhibitory effect on colon carcinogenesis and photo-protection [12,36,37]. Following capsanthin, antheraxanthin (average 132.51 mg/100 g DW), capsorubin (average 76.97 mg/100 g DW), and β-carotene (average 58.050 mg/100 g DW) were also abundant in the *C. chinense* pepper. Table 1 shows that the total carotenoid content ranged from 7.04 to 2430.85 mg/100 g DW. This wide variation in carotenoids could be attributed to genetic differences, despite the fact that all the tested materials belong to the *C. chinense* species.

![Figure 2](image-url). The percentage contribution of each carotenoid to the total carotenoid content in 226 *C. chinense* pepper genetic resources.

The distributions of accessions were counted and summarized based on their individual carotenoid and total carotenoid content ranges (Table 2). Based on total carotenoid content, the germplasms were distributed as follows in mg/100 g DW: 94 (500–1000), 35 (1000–1500), 13 (1500–2000), and 3 (2000–2500). The capsanthin content of 19 accessions was in the range of 600–800 mg/100 g DW, and 8 accessions were within the range of 800–1000 mg/100 g DW. Genetic factors are responsible for the variation in carotenoid content. In the previous study, the carotenoid content of *C. annuum* germplasms varied greatly [29].

| Carotenoid           | Contribution (%) |
|----------------------|------------------|
| **Lutein**           | 0.38             |
| β-Cryptoxanthin      | 1.34             |
| α-Carotene           | 1.93             |
| Zeaxanthin           | 6.00             |
| Violaxanthin         | 6.47             |
| β-Carotene           | 8.21             |
| Capsorubin           | 11.45            |
| Anthraxanthin        | 18.95            |
| **Capsanthin**       | **45.27**        |

#### Table 1

| Accession Range | Number |
|-----------------|--------|
| 500–1000        | 94     |
| 1000–1500       | 35     |
| 1500–2000       | 13     |
| 2000–2500       | 3      |
Table 2. The distribution of Pepper (C. chinense) genetic resources according to the individual carotenoid and total carotenoid content.

| Components     | Ranges in mg/100 g DW (Number of Accessions) |
|----------------|---------------------------------------------|
| Capsanthin     | 0–200 (67) 200–400 (96) 400–600 (35) 600–800 (19) 800–1000 (8) 1000–1200 (1) |
| Capsorubin     | 0–100 (172) 100–200 (29) 200–300 (19) 300–400 (5) 400–500 (0) >500 (1) |
| Lutein         | 0–50 (222) 50–100 (4) 100–150 (0) 150–200 (0) 200–250 (1) - |
| Zeaxanthin     | 0–100 (214) 100–200 (8) 200–300 (3) 300–400 (1) - - |
| Antheraxanthin | 0–100 (82) 100–200 (95) 200–300 (38) 300–400 (8) 400–450 (3) - |
| Violaxanthin   | 0–100 (215) 100–200 (11) - - - - |
| α-Carotene     | 0–50 (220) 50–100 (6) - - - - |
| β-Carotene     | 0–50 (109) 50–100 (86) 100–150 (26) 150–200 (5) - - |
| β-Cryptoxanthin| 0–20 (200) 20–40 (19) 40–60 (6) 60–90 (1) - - |
| Total carotenoid| 0–500 (81) 500–1000 (94) 1000–1500 (35) 1500–2000 (13) 2000–2500 (3) - |

Figure 2 summarizes the individual carotenoid content contributions to the total carotenoid content of 226 genetic materials. The capsanthin contribution to the total carotenoid content was higher with an average of 45.27% in 226 pepper materials (Figure 2). Capsanthin levels continue to rise as fruits ripen, accounting for between 37% and 80% of total carotenoid content [38]. Carotenoids accumulate in fruits during the ripening process, resulting in pigmentation changes [38]. Only carotenoids with two β-rings are synthesized once ripening begins, so cyclase activity is only involved in the biosynthesis of β, β-series carotenoids like zeaxanthin, violaxanthin, antheraxanthin, β-cryptoxanthin, capsanthin, and capsorubin [39–41]. Capsanthin’s relative content among these compounds may account for up to 50% of total carotenoid content [42]. The presence of high concentrations of capsanthin may contribute to the nutrition and health benefits gained from the pepper. Capsanthin is absorbed from foods such as oleoresin, powdered and fresh paprika, or red peppers and has been shown to have anti-oxidative properties as well as antitumor activity [43]. Capsanthin was followed by antheraxanthin, capsorubin, and β-carotene, which contributed 18.95%, 11.45%, and 8.21%, respectively. According to several studies, the concentration of specific carotenoids varies by species and cultivars, as well as fruit maturation stages. When compared to capsanthin, the levels of β-carotene, β-cryptoxanthin, and zeaxanthin in red chili pepper fruits are very low [44]. Lutein, β-cryptoxanthin, and α-carotene contributed the least to total carotenoid content and accounted for 0.38%, 1.34%, and 1.93%, respectively. Lutein was found in only some pepper germplasms with yellow fruits (Table S1). In this study, not all pepper germplasms with yellow fruit contained lutein. Guzman et al. [45], conducted a study with thirty-one pepper accessions with yellow fruit and found that lutein represented at least 50% of total carotenoid amounts in only nine of the accessions. They also concluded that the yellow color was not a good indicator of lutein content and that phytochemical analysis was required to determine the content of health-promoting compounds.

Table 3 shows four pepper genetic resources with high carotenoid content and ASTA values. IT261426, IT183657, IT261213, and IT261413 had total carotenoid content of 2430.85, 2077.55, 2062.54, and 1990.76 mg/100 g DW of pepper fruit powder, respectively. The rank of these germplasms according to ASTA value was similar, with the exception of IT183657, which did not match the total carotenoid content rank. Therefore, these pepper genetic resources in descending order of high ASTA value were as follows: IT261426 (221.32), IT261213 (211.72), IT183657 (196.31), and IT261413 (160.47), (Table 3). Capsanthin was the major contributor to total carotenoid content in all four pepper germplasms. It contributed 48.55%, 46.11%, 45.09%, and 43.81% of total carotenoid content in IT261413, IT261426, IT183657, and IT261213, respectively. The red fruits of C. annuum (var. lycopersiciforme rubrum) contained 37% capsanthin, 8% zeaxanthin, 7% cucurbitaxanthin, 3.2% capsorubin, and 9% β-carotene, according to HPLC analysis [46]. Capsorubin (20.63%) was the second-highest contributor in IT261426. This finding supports previous research that identified capsanthin and capsorubin as the main carotenoids in pepper. Antheraxanthin, on the
other hand, accounted for the second major percentage of contribution (20.39%, 19.99%, and 18.32%) in IT183657, IT261213, and IT261426, respectively. Figure 3 shows fruit photos of four pepper accessions with high carotenoid content.

Table 3. Four genetic resources for high content of carotenoids and ASTA value.

| Introduction Number | Capsanthin (%) | Capsorubin (%) | Lutein (%) | Zeaxanthin (%) | Antheraxanthin (%) | Viola-xanthin (%) | α-Carotene (%) | β-Carotene (%) | β-Cryptoxanthin (%) | Total Carotenoids (mg/100 g DW) | ASTA Value |
|---------------------|---------------|----------------|------------|----------------|-------------------|------------------|---------------|---------------|------------------------|-----------------------------|-------------|
| IT261426            | 46.11         | 20.63          | 0.00       | 1.55           | 18.32             | 6.95             | 1.13          | 4.95          | 0.36                   | 2430.85                    | 221.32      |
| IT183657            | 45.09         | 13.49          | 0.00       | 2.81           | 20.39             | 6.76             | 2.61          | 7.88          | 0.96                   | 2077.55                    | 196.31      |
| IT261213            | 43.81         | 16.94          | 0.00       | 1.93           | 19.99             | 6.85             | 2.41          | 7.25          | 0.83                   | 2062.54                    | 211.72      |
| IT261413            | 46.11         | 20.63          | 0.00       | 1.26           | 13.52             | 4.69             | 0.61          | 3.09          | 1.09                   | 1990.76                    | 160.47      |

Figure 3. *C. chinense* fruits of four genetic resources with the highest total carotenoid contents and ASTA color value.

3.3. Correlation of Carotenoids Content and ASTA Values

A correlation analysis was conducted to determine the relationship between carotenoids and ASTA color value (Table 4). Total carotenoids and ASTA value had a strong positive correlation (r = 0.965). This result is in line with the findings of Yoon et al. [29], who evaluated the carotenoid composition and ASTA color value in 523 *C. annum* germplasms and observed a positive correlation between total carotenoid and ASTA color value (r = 0.674). Capsanthin had a strong positive correlation with total carotenoids (r = 0.981), antheraxanthin (r = 0.952), ASTA color index (r = 0.946), and capsorubin (r = 0.927). Similarly, in *C. annum* spp., a strong positive correlation between capsanthin and ASTA color value (r = 0.723) was noticed [29]. Lutein had a negative correlation with all individual and total carotenoids (Table 4). The fact that lutein is primarily produced in yellow fruit varieties that are high in lutein and α- or β-carotene but low in capsanthin content [47]. Regarding the α-carotene, it had a strong positive correlation (r = 0.935) with β-carotene compared to other carotenoids and ASTA value. Jourdan et al. [48], reported that α-carotene has the highest significant positive correlation with β-carotene (r = 0.886 **) in carrots. β-carotene had a strong positive correlation with capsanthin (r = 0.706). Significant positive correlations (r = 0.960) were found between β-carotene and capsanthin contents in ten *C. annum* cultivars [33]. Antheraxanthin, the second most abundant carotenoid in this study (Figure 2), showed a strong positive correlation with total carotenoids (r = 0.975) and the ASTA color value (r = 0.964).
Table 4. Correlation between ASTA value and carotenoids in *C. chinense*.

| Carotenoids (mg/100 g DW) | ASTA Value |
|---------------------------|------------|
| Capsanthin                | 1.000      |
| Capsorubin                | 1.000      |
| Lutein                    | 1.000      |
| Antheraxanthin            | 1.000      |
| Violasanthin              | 1.000      |
| β-Carotene                | 1.000      |
| β-Cryptoxanthin           | 1.000      |
| Total carotenoids         | 1.000      |

The correlation between violaxanthin and total carotenoid (r = 0.936) and ASTA value (r = 0.931) was strongly positive. Further to that, violaxanthin showed a strong positive correlation with antheraxanthin (r = 0.955), capsanthin (r = 0.920), and capsorubin (r = 0.860). Capsorubin had a strong positive correlation with ASTA color value (r = 0.858). β-Carotene and α-carotene had a moderately strong positive correlation with ASTA color (r = 0.792 and 0.649, respectively). The ASTA color value was positively correlated with β-cryptoxanthin (r = 0.408) and zeaxanthin (0.541), which is consistent with the previous study [29]. Unlike the other individual carotenoid, lutein had a negative correlation with ASTA color value (r = -0.331). However, Yoon et al. [29] found that lutein had a positive correlation (r = 0.439) with the ASTA value of *C. annuum* pepper in a previous study. This difference could be attributed to the amount of lutein found in the tested population, which was expected to exhibit carotenoid variation due to genetic differences.

R-squared (R²) is a statistical measure that represents the proportion of the variances for a dependent variable that is explained by an independent variable. A scatter plot was created to show how the total carotenoid content of the 226 pepper materials corresponded to ASTA values. The regression model equation was, y = 0.0926x + 8.4874 (R² = 0.9317). This finding is in agreement with that of Zaki et al. [49], who found a strong positive correlation between total carotenoids and the ASTA color unit of paprika powder. They also stated that the regression model was best fitted (y = 0.045x + 0.444) and explained 99.8% of the variance in the fitted data (R² = 0.998). A scatter plot was also generated based on the capsanthin content and ASTA value data sets. The regression model equation was, y = 0.192x + 12.581 (R² = 0.8946). This means that the ASTA value could explain 93.17% of the variance in total carotenoid content and 89.46% of the variance in capsanthin content, indicating a well-fitted model for estimation.

3.4. Principal Component Analysis (PCA)

The PCA assists in identifying the components with the greatest variance in the data set and determining any associations between the variables. PCA was used to examine the variation for the carotenoids and ASTA color value data sets of 226 pepper genetic resources. Table 5 shows the eigenvalues, individual, and cumulative contributions of variables in the first five principal components. PCA revealed that the highest two principal components (PC1 and PC2) explained 81.25% of the total variation (Figure 4A). PC1 accounted for 67.64% and was mostly comprised of total carotenoids, antheraxanthin, ASTA, capsanthin, β-carotene, and capsorubin. On the other hand, the PC2 explained 13.61% of the total variance, and the major contributors were β-cryptoxanthin, zeaxanthin, and α-carotene. The third, fourth, and fifth principal components explained 8.88%, 4.75%, and 2.46% of the total variance, respectively. The absolute value of the loadings is an indicator of the participation of the analyzed parameters in the PCs [30].
As shown in Figure 5A, the total carotenoid content and ASTA value are closely scattered on the PCA plot. Capsanthin and capsorubin are also closely related, whereas lutein is dispersed far from other variables on the negative side of the PCA plots. Individual germplasm was plotted in a biplot to demonstrate its distribution based on carotenoid and ASTA values (Figure 5B). Pepper genetic resources with a high total carotenoid content are distributed along the positive x-axis (in red), while pepper materials with a low carotenoid content are distributed along the negative x-axis. A few germplasms with high total carotenoid content (ids: 144, 17, 124, 141, 330, 52, etc.) were scattered far from the center of the PCA plot.

A cluster analysis was performed using the carotenoid and ASTA value data sets for 51 pepper germplasms with total carotenoid contents greater than 1000 mg/100 g DW to see how the pepper genetic resources were grouped and to find germplasms with a more similar set of data (Figure 6). Even though all the tested materials belong to the C. chinense species, the pepper germplasm with an approximate amount of carotenoids and ASTA value has been classified into four clusters. The dendrogram depicts four clusters (I, II, III, and IV) consisting of 6, 6, 20, and 19 germplasms, respectively. Accessions with a high total carotenoid content IT261426, IT183657, and IT261213 were clustered in group I. The total carotenoid content IT261426, IT183657, and IT261213 were clustered in group I.
carotenoids and ASTA values of the selected 51 pepper genetic resources are presented in Supplementary Table S2.

Figure 5. Principal component analysis (PCA) of carotenoids (Capsanthin, capsorubin, lutein, zeaxanthin, antheraxanthin, violaxanthin, α-carotene, β-carotene, and β-Cryptoxanthin) and ASTA color values (n = 226) (A). The individual pepper germplasm on the PCA plot (B). The lines starting from the center point of the bi-plot show the positive or negative associations of the parameters. The colors represented the total carotenoid content of pepper germplasms (red-blue). The pepper germplasms are indicated by their ID numbers (B) (Supplementary Table S1).

Figure 6. Dendogram resulting from the hierarchical cluster analysis showing the formation of clusters according to carotenoids (except Lutein) and ASTA color index using 51 selected pepper materials (selection was based on total carotenoid content > 1000 mg/100 g DW).
4. Conclusions

Capsicum spp. fruits are an abundant source of carotenoids. Carotenoid accumulation profiles change depending on the cultivar, stage of ripening, and fruit color. The evaluation of larger pepper genetic resources is critical for selecting carotenoid-rich pepper germplasm for breeding new cultivars. This study is also helpful in understanding the relationship between carotenoids and the ASTA color index, which is internationally recognized as the quality evaluation method of paprika. Therefore, in this study, the carotenoid content and ASTA value of pepper genetic resources were assessed and shown to have a strong positive correlation ($r = 0.965$). The findings of this study show that the ASTA value can assist in estimating the total carotenoid content of pepper. In addition, the ASTA value and capsanthin had a positive correlation ($r = 0.946$). Capsanthin content was reported as the major carotenoid in many research peppers among other carotenoids, and its contribution to total carotenoid was 45.27% in the present study. Following capsanthin, the contributions of antheraxanthin and capsorubin were the next highest, with 18.95% and 11.45%, respectively. Since pepper has wider nutritional, industrial, and therapeutic uses, some of the high-quality pepper accessions from this study (IT261426, IT183657, IT261213, and IT261413) can be used to breed new cultivars rich in carotenoid content. The findings of this study will help advance research into carotenoid estimation using the ASTA color value in large pepper genetic resources. Model development for carotenoid estimation based on the ASTA color index will help with the selection processes of pepper materials with high carotenoids. However, this type of modeling is dependent on the color of the pepper fruit, variety, and species. More research is needed in the future to develop the ASTA color index model for carotenoid estimation and understand the correlation between them using a large number of germplasms from different Capsicum species. As a result, a more comprehensive finding and broader application will be possible.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8060486/s1, Figure S1: The graphical scheme of the study design; Table S1: The IT number and origins of 226 pepper genetic resources; Table S2: The carotenoids content and ASTA color values of 51 selected pepper germplasms.

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