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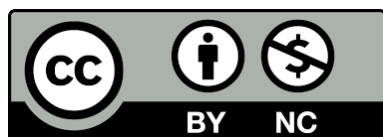
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Evaluation of chili (*Capsicum annuum* L.) genotypes for nutritional phytochemicals and mineral content

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Abstract: Chili (*Capsicum annuum* L.) is a worldwide important crop known for its nutritional and phytochemical properties. This study aimed to identify suitable candidates for breeding programs by evaluating the phytochemical and mineral content of 28 chili genotypes. The capsaicin concentration varied from 0.08% (G20) to 0.44% (G28). The maximum ascorbic acid concentration was recorded in G7 (125.56 mg/100 g), whilst the minimum was in G20 (19.45 mg/100 g). β -carotene concentration ranged from 0.29 mg/100 g FW in G12 to 0.13 mg/100 g in G13 and G26. The chlorophyll content (a and b) reached its zenith in G24 (0.30 and 0.32 mg/g FW, respectively), whereas the minimal amounts were seen in G15 and G18. The anthocyanin concentration varied from 4.18 μ g/g FW in G18 to 0.48 μ g/g FW in G11. Genotype G16 demonstrated the highest overall phenolic and antioxidant levels, whereas G25 exhibited the highest flavonoid concentration. Mineral analysis indicated that G4 and G26 contained the highest sodium (0.39%), G21 had the highest potassium (2.07%), and G10 exhibited the highest calcium (1.65%) and magnesium (0.63%). Cluster IV had high levels of ascorbic acid and anthocyanin, while cluster I had low levels, according to the heatmap analysis of the genotypes. Significant connections were discovered among ascorbic acid, anthocyanin, potassium, and calcium concentration. In breeding initiatives for nutritionally improved chili varieties, genotypes G2, G7, G12, G16, G17, G18, and G25 stand out with their excellent phytochemical and mineral profiles. This study gives important information about the genetic diversity of chili genotypes in Bangladesh.

Keywords: Pepper, total phenol, flavonoids, antioxidant activity, anthocyanin content.

Introduction

Chili (*Capsicum annuum* L.) is referred to by various names globally, including red pepper, bell pepper, pod pepper, hot pepper, paprika, cayenne pepper, and pimento. It is closely associated with tomato, eggplant, potato, and tobacco (Faustino et al., 2007). The domestication of chili initially took place in central America, predominantly in Mexico, with additional concentrations in Guatemala and Bulgaria (Salvador, 2002). The principal chili-producing nations include India, Mexico, Japan, Ethiopia, Uganda, Nigeria, Thailand, Turkey, Indonesia, China, and Pakistan. It is also cultivated to considerable degree in Italy, Spain, and the United States. India is the leading producer and consumer of chili globally (Salvador, 2002). The chili fruits are utilized to provide pungency in both their immature and mature stages. The fruit ranges in size from 1 to 20 cm in length, exhibiting forms from slender elongated to conical and robust blocky shapes. The popularity of chili stems from its diverse shapes, sizes, and sensory characteristics, including color, pungency, and piquancy, which enhance the flavor of otherwise bland staple foods like grains and vegetables. In the food and beverage industries, chili is utilized as oleoresin, facilitating enhanced distribution of color and flavor in products. Pungency results from the presence of capsaicin (Parthasarathy, 2008).

Capsaicin is an alkaloid found in the fruit and placenta that may directly neutralize various free radicals (Bhattacharya et al., 2010) and has extensive applications in the food, medical, and pharmaceutical sectors. It has been utilized as a topical analgesic for arthritis pain and inflammation (Deal et al., 1991). Capsaicin interacts with the same category of nociceptors that elicit sensations of pain from heat and acid (Julius and Basbaum, 2001), and it alleviates pain and inflammation by depleting the neurotransmitters that convey pain signals. Capsaicin exhibits anti-mutagenic properties (Ramirez-Victoria et al., 2001; Morr  and Morr , 2003) and possesses significant antioxidant activity (Lee et al., 1995; Chakrabarty and Islam, 2017). It is utilized in balm formulation, while color extracts (carotenoid pigments) serve as color additives in the food and prawn feed industries. The primary functional attributes of chili are pungency, vitamin

C, natural colors, and several minerals such as sodium, potassium, calcium, magnesium, iron, and zinc (Starykh and Nosova, 1982). Green chilies are abundant in Vitamin A and Vitamin E. Chili is extensively utilized in curry powder, curry paste, various pickles, sauces, and soups. The quality of dried chili is measured by several different parameters such as color, hotness, ascorbic acid content, and volatile compounds (Kim et al., 2006; Wang et al., 2009; Yaldiz et al., 2010). Ribes - Moya et al. (2020) characterized 14 genotypes of *Capsicum* based on phytochemicals present in pepper fruits harvested at various maturity stages. Paredes Andrade et al. (2020) characterized 198 genotypes of *Capsicum* collected from 21 different countries. They found a strong correlation between polyphenols and flavonoids, while weak correlation was observed between polyphenol and antioxidants.

The development of high-yielding cultivars necessitates an understanding of the existing genetic variation and the degree of connection between yield-contributing traits. The observed variability represents a composite assessment of genetic and environmental factors, of which only the genetic component is heritable. Nevertheless, the heritability estimate alone does not indicate the anticipated gain in the subsequent generation; it must be evaluated alongside genetic development. Correlation and path analysis will determine the degree of link between yield and its components, elucidating the relative significance of their direct and indirect impacts, so providing a thorough comprehension of their relationship with yield (Vijaya et al., 2014). This study was initiated to assess the phytochemical contents of 28 chili genotypes available at Bangabandhu Sheikh Mujibur Rahman Agricultural University.

Materials and Methods

Plant materials and field experiments

The field experiment was conducted at the experimental farm of the Department of Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur. All chemical analyses were carried out at the laboratory of the Department of Genetics and Plant Breeding and the Department of Crop Botany,

BSMRAU. Seeds of 28 genotypes of chili were sown in the pots to raise seedlings as it ensures uniform growth which facilitates better plant establishment after transplanting. The seedlings were ready to transplant in the main field 45 days after sowing (DAS).

A population of twelve plants was maintained per replication per genotype by planting a single plant per hill. Gap filling was done after one week whenever the death of a previously transplanted seedling occurred. One seedling was transplanted per hill and light irrigation was given immediately after transplanting. The spacing was maintained at 45 cm between rows and 45 cm between plants within a row for all the genotypes. One-meter distance was kept between blocks of the replication. Soil fertility was ensured by applying additional quantities of Urea, triple superphosphate (TSP), muriate of potash (MOP), Gypsum, Zinc Sulphate, and Boric Acid in amounts of 270-170-100-150-115-88 kg/ha, respectively (Anonymous, 1999). Total TSP, MP, Gypsum, zinc sulphate, and boric acid were applied during final land preparation. Cowdung was also applied @ 10/ha during final land preparation. Total urea was applied in three installments, at 15, 30, and 50 days after transplanting (DAT). Recommended inter-cultural operations were done to raise the healthy chili crop. Five plants were selected randomly from each replication and selected plants were marked by labeling for recording data. Data on the following qualitative characters were recorded after the harvest of mature green chili. The solution preparation protocols used in the analysis of different chilli's phytochemicals are presented in Supplementary Table 1.

Capsaicin content

The capsaicin concentration in fruits was assessed using the colorimetric technique outlined by Bajaj (1980). 0.5 g of dry chili powder was measured into a glass-stoppered test tube; 10 ml of dry acetone (prepared by adding 25 g of anhydrous sodium sulfate to 500 ml of acetone at least one day prior) was added into the test tube and allowed to extract overnight. The following day, samples were centrifuged at 10,000 rpm for 10 minutes to get a clear supernatant. 1 cc of the supernatant was transferred to a test tube and evaporated to dryness

using a hot water bath. The residue was subsequently dissolved in 5 ml of 0.4% NaOH solution, followed by the addition of 3 ml of 3% phosphomolybdic acid. The contents were agitated and then allowed to remain undisturbed for one hour. After one hour, the solution was promptly filtered into centrifuge tubes to eliminate any suspended material and subsequently centrifuged at 5000 rpm for 15 minutes. The transparent blue solution was directly put into the cuvette, and absorbance was measured at 650 nm alongside a reagent blank. A standard graph was constructed with 0-200 µg of pure capsaicin. Simultaneously, 0.2, 0.4, 0.6, 0.8, and 1 ml of the working standard solution were aliquoted into new test tubes. The stock standard capsaicin solution was prepared by dissolving 50 mg of capsaicin in 50 ml of 0.4% NaOH solution (1000 µg/ml). The working standard solution was prepared by diluting 10 ml of the stock standard to 50 ml with 0.4% NaOH solution (200 µg/ml) and processed as previously described. Simultaneously 0.2, 0.4, 0.6, 0.8, and 1 ml of working standard solution (stock standard capsaicin solution was prepared by dissolving 50 mg capsaicin in 50 ml of 0.4% NaOH solution (1000 µg/ml) and working standard solution prepared by diluting the 10 ml of the stock standard to 50 ml with 0.4% NaOH solution (200 µg/ml)) was taken into new test tubes and proceeded as mentioned above.

Calculation: Percent capsaicin calculated using the formula mentioned below-

$$\text{Capsaicin content (\%)} = (\mu\text{g capsaicin} \times 100 \times 100) / (1000 \times 1000 \times 1 \times 0.5)$$

Total ascorbic acid content

The ascorbic acid content was determined as per the procedure described by Pleshkov (1976). 5 ml extract solution was taken in a 50 ml conical flask. Then 5 ml of 5% KI, 2 ml of 100% glacial acetic acid, and 2 ml of 2% starch solution were added to it respectively. Total ascorbic acid was estimated by titrating that aqueous extract solution against 0.001 N of KIO₃ solution. The total ascorbic acid content was quantified by using the formula:

$$\text{Ascorbic acid (mg/100g)} = (T \times F \times V \times 100) / (v \times W)$$

Here,

$$T = \text{Titrated value of KIO}_3 \text{ ml}$$

$F = 0.088$ mg of ascorbic acid per ml of 0.001 N of KIO_3

V = total volume of the sample extracted (ml)

v = volume of the extract (ml) taken

W = weight of the sample taken (g)

Total antioxidant content

Total antioxidant content was determined by the procedure described by [Abdul-Hafeez et al. \(2014\)](#) with slight modification. The 1.0 ml plant extracts and standards (Butylated hydroxyl toluene standards) were taken in the test tubes and 1.0 ml methanol (instead of plant extract) was taken in another test tube which served as the control. Then 3 ml 0.2 mM of DPPH solution was added to each test tube and incubated the reaction mixture at 25°C for 5 minutes. After incubation, the absorbance was measured at $\lambda = 517$ nm and methanol was used as the blank. DPPH radical scavenging activity of each plant extract and standards were calculated as the percentage inhibition.

% Inhibition DPPH radical activity = $(A_{\text{control}} - A_{\text{sample}}) \times 100 / A_{\text{control}}$

Then a calibration curve from the standards was prepared and expressed the antioxidant capacity ($\mu\text{g/g}$ fresh weight) as BHT equivalent.

Total β -carotene content

Total β -carotene content was determined by the procedure described by [Nagata and Yamashita \(1992\)](#). A 1.0 g fresh sample of chili was taken into a mortar and homogenized with 10 ml acetone:hexane (4:6) solution. This sample was centrifuged and the optical density of the supernatant was measured by spectrophotometer (Model 200-20, Hitachi, Japan) at 663 nm, 645 nm, 505 nm, and 453 nm.

Calculation was done by the following formula:

β -Carotene (mg/100g) = $0.26 (\mathbf{OD}_{663}) + 0.452 (\mathbf{OD}_{453}) - \mathbf{1.22}(\mathbf{OD}_{645}) - \mathbf{304} (\mathbf{OD}_{505})$

Where the bold figure indicates optimal density.

Chlorophyll and carotenoid content

A 100 mg of fresh chili sample was taken in a glass vial. Then 5 ml of 80% (v/v) acetone was added and made the vial air tight. After that the vial was kept at 4°C in the dark for 24 hrs. 1 ml supernatant was taken in a 1 ml glass cuvette and the absorbance was read at 663 nm, 646 nm, and 470 nm, corresponding to chl a, chl b and carotenoids respectively. 80%

acetone was used as a blank ([Porra et al., 1989](#)). Quantification of chl a, chl b, and carotenoids was done as described by [Lichtenthaler and Wellburn \(1983\)](#).

Chl a ($\mu\text{g/ml}$) = $12.21 (A_{663}) - 2.81(A_{646})$

Chl b ($\mu\text{g/ml}$) = $20.13 (A_{646}) - 5.03(A_{663})$

Carotenoids ($\mu\text{g/ml}$) = $\{1000 (A_{470}) - 3.27 (\text{Chl a}) - 104 (\text{Chl b})\} / 229$

Expressed the amount as $\text{mg/g} = (\mu\text{g ml}^{-1} \times V) / (1000 \times W)$

Where,

V = Volume of acetone used (ml)

W = Weight of fruit sample (g)

Total anthocyanin content

1.0 g tissue was taken in an ice-cold glass vial. Then 5 ml of extraction solution was added and make the vial airtight. After that, the vials were kept at 4°C in the dark for 24 hrs. 2ml solution was taken from the vial in a centrifuge tube and added 2 ml distilled water. After that 2 ml chloroform was added to separate anthocyanin (insoluble in chloroform) from the chlorophylls. Then the mixture was centrifuged for 15 minutes at 5000 rpm at 4°C. After that 3 ml of the top layer (containing anthocyanin) was taken in a glass cuvette and the absorbance was read at 530 nm. The extraction solution was used as blank ([Hughes and Smith, 2007](#)). Total anthocyanin content was calculated (as cyaniding-3-glucoside equivalent) using the absorbance and a molar extinction coefficient for anthocyanin at 530 nm of $30000 \text{ L}^{-1}\text{M}^{-1}\text{cm}^{-1}$ ([Murray and Hackett, 1991](#)). Anthocyanin ($\mu\text{g/g}$ FW) = $(\text{Abs} \times \text{MW} \times V \times \text{DF} \times 1000) / (30000 \times w)$

Where,

A = absorbance at 530 nm

MW = Molecular weight of cyaniding-3-glucoside ($449.22 \text{ g mol}^{-1}$)

V = Volume of extraction solution used (ml)

DF = Dilution factor

w = Used sample weight

FW = Fresh weight

Total phenolic content

Total phenolic content was determined spectrophotometrically according to the Folin-Ciocalteu's method described by [Abdul-Hafeez et al. \(2014\)](#) with slight modification. 1.0 ml plant extracts and standards (gallic acid standards) were

taken into test tubes and 1.0 ml methanol (instead of plant extract) was taken in another test tube which served as the control. Then 0.5 ml 10% (0.2 N) Folin-Ciocalteus reagent was added to each test tube. Then the test tubes were shaken for 10 seconds, covered, and incubated the reaction mixture for 15 minutes at room temperature. After incubation, 2.5 ml 700 mM Na₂CO₃ aqueous solution was added and mixture was again shaken, covered incubated the reaction mixture for 2 hrs. The absorbance was measured at $\lambda = 765$ nm using methanol as the blank. A calibration curve from the standards was prepared and expressed the phenolic content ($\mu\text{g/g}$ fresh weight) as gallic acid equivalent.

Total flavonoid content

The total flavonoid concentration was quantified spectrophotometrically using the aluminum chloride colorimetric assay (Zhishen *et al.*, 1999) with minor modifications. 1.0 ml of plant extracts and quercetin standards were placed in test tubes, whereas 1.0 ml of methanol was used in a separate test tube as the control. Subsequently, 0.4 ml of a 5% NaNO₂ solution was included into the mixture. After 5 minutes, 0.6 ml of 10% AlCl₃·6 H₂O was introduced, and at after 6 minute, 2 ml of 1M NaOH was added to the mixture. The mixture was agitated for thorough homogenization, and the absorbance was recorded at $\lambda = 510$ nm, with methanol as the blank. A calibration curve derived from the standards was established, quantifying the flavonoid concentration ($\mu\text{g/g}$ fresh weight) as quercetin equivalent.

Total sodium, calcium, magnesium and potassium content

To calculate the total content of sodium, calcium, magnesium, and potassium in chili, ripe fruits were subjected to air-drying at ambient temperature for a duration of 3 days. The air-dried samples were subjected to oven drying at 70°C, after which 0.5 gr of the ground oven-dry samples were digested using a nitric acid (HNO₃) and perchloric acid (HClO₄) mixture in a 5:1 ratio for 2 hours. The digests were employed to ascertain the amounts of sodium, calcium, magnesium, and potassium utilizing the methodologies outlined by Piper (1947) with an atomic absorption spectrophotometer (Model 200-30, Hitachi, Japan). Statistical analysis was conducted with the software STAR (statistical

tools for agricultural research). The data underwent one-way analysis of variance for mean comparison, and significant differences were determined using Fisher's LSD test. The data were presented as the mean. Differences with a p-value of less than 0.05 were deemed statistically significant.

Results

Analysis of nutritional phytochemicals

Analysis of variance (Table 1) revealed highly significant differences among the genotypes for five quality traits such as ascorbic acid content, total phenolic content, total flavonoids content, total antioxidant content, and anthocyanin content indicating the presence of variability in the materials and considerable scope for their further improvement. Capsaicin content of fruits of 28 chili genotypes was assessed. The solution preparation protocol is provided in Supplementary Table 1, and the results are presented in Supplementary Table 2. The results showed that the capsaicin content of fruits ranged from 0.08 % to 0.44 %. The highest amount of capsaicin content was observed in genotypes G20 followed by G27 (0.39 %) and G25 (0.33 %). The lowest amount of capsaicin content was observed in the genotypes G28 followed by G2 (0.16 %) and G13 (0.17 %). Capsaicin content showed an 8.85 % coefficient of variation.

Ascorbic acid content in fruits ranged from 19.45 and 125.56 (mg/100g) (Supplementary Table 2). The highest value of ascorbic acid content was observed in the fruits of genotype G7 (125.56 mg/100g) followed by G17 (113.26 mg/100g), G3 (109.33 mg/100g), G10 (102.53 mg/100g) and G14 (101.16 mg/100g). The lowest amount of ascorbic acid content was observed in the genotypes G20 (19.45 mg/100g) followed by G19 (19.46 mg/100g) and G1 (40.83mg/100g). The ascorbic acid content of fruits showed a 12.04% coefficient of variation. β - carotene content showed low variability among the 28 genotypes of chili and it ranged from 0.13 to 0.29 (mg/100g) (Supplementary Table 2). The highest amount of β - carotene content was observed in genotype G12 (0.29 mg/100g) whereas the lowest was in genotype G9 and G26 (0.13 mg/100g). The mean value of G2, G20 and G24 were same which followed by G3, G16, G28, G4, G11, G15, G22.

Chlorophyll a content of the genotypes varied between 0.05 mg/g and 0.30 mg/g, the maximum being in G24 followed by G25 (0.28mg/g), G6 (0.24mg/g) and G9 (0.22 mg/g) in Supplementary Table 2. The minimum chlorophyll content was observed in the genotypes G15 followed by G3 (0.07 mg/g) and G8 (0.07 mg/g). The chlorophyll content showed an 11.23 % coefficient of variation. Chlorophyll b content (mg/g) ranged from 0.04 to 0.32 mg/g and maximum being in G24 followed by G9 (0.24 mg/g) and G13 (0.23 mg/g) in Supplementary Table 2. The minimum chlorophyll b content observed in the genotypes G18 followed by G3 (0.06 mg/g), G8 (0.06 mg/g), G15 (0.07 mg/g) and G19 (0.07 mg/g). The chlorophyll b content showed 13.53% coefficient of variation. Wide range of variation was observed among 28 genotypes of chili for anthocyanin content of fruit extract

(Supplementary Table 2). The highest anthocyanin content was found in the genotype G18 (4.18 $\mu\text{g/g}$ FW) followed by G23 (3.26 $\mu\text{g/g}$ FW) and G24 (2.83 $\mu\text{g/g}$ FW). The lowest anthocyanin content was observed in genotype G11 (0.48 $\mu\text{g/g}$ FW) followed by G7 (0.74 $\mu\text{g/g}$ FW), G27 (0.74 $\mu\text{g/g}$ FW) and G21 (0.84 $\mu\text{g/g}$ FW). Total carotenoid content ranged from 0.04 to 0.47 (mg/g) among 28 genotypes of chili (Supplementary Table 2). The highest amount of total carotenoid content was observed in the genotype G14 followed by G10 (0.14 mg/g), G19 (0.14 mg/g), and G6 (0.13 mg/g). The lowest amount of carotenoid content was observed in the genotype G17 followed by G21 (0.05 mg/g), G13 (0.06 mg/g), and G20 (0.06 mg/g). Variation in the total phenol content of 28 genotypes of chili is presented in Figure 1.

Table 1. Analysis of variance (ANOVA) for nutritional phytochemicals and mineral contents in chili.

Source of variation	df	Mean squares (MS)													
		CAP	AAC	BBC	TPC	TFC	TAO	Chl a	Chl b	TCC	ANC	Na	K	Ca	Mg
Replication	2	0.00	143.2	0.001	592.56	1127.55	229.18	0.003	0.002	0.04	0.01	0.02	0.00	0.00	0.00
Genotype	27	0.02 ^{ns}	2129.84 ^{**}	0.005 ^{ns}	334905.59 ^{**}	540124.88 ^{**}	2124.62 ^{**}	0.013 ^{ns}	0.012 ^{ns}	0.02 ^{ns}	1.94 [*]	0.02 ^{ns}	0.022 ^{ns}	0.15 ^{ns}	0.01 ^{ns}
CV (%)	-	8.85	12.04	8.38	13.45	11.53	14.23	11.23	13.53	11.41	4.66	6.85	2.02	7.53	3.78
Error	54	0.00	77.46	0.00	218.83	505.7	43.39	0.00	0.00	0.01	0.01	0.02	0.001	0.01	0.00

*, ** and ns indicate significance at 5% and 1% levels and non-significance, respectively, df – Degrees of freedom, CAP- Capsaicin content (%), AAC - Ascorbic acid content (mg/100g), BCC - β - carotene content (mg/100g), TPC - Total phenolic content ($\mu\text{g/g}$ FW), TFC - Total flavonoids content ($\mu\text{g/g}$ FW), TAO - Total antioxidant content ($\mu\text{g/g}$ FW), Chl a - Chlorophyll a content (mg/g), Chl b - Chlorophyll b content (mg/g), TCC - Total carotenoid content (mg/g), ANC - Anthocyanin content ($\mu\text{g/g}$ FW), Na - Sodium content (%), K - Potassium content (%), Ca - Calcium content(%), Magnesium content (%).

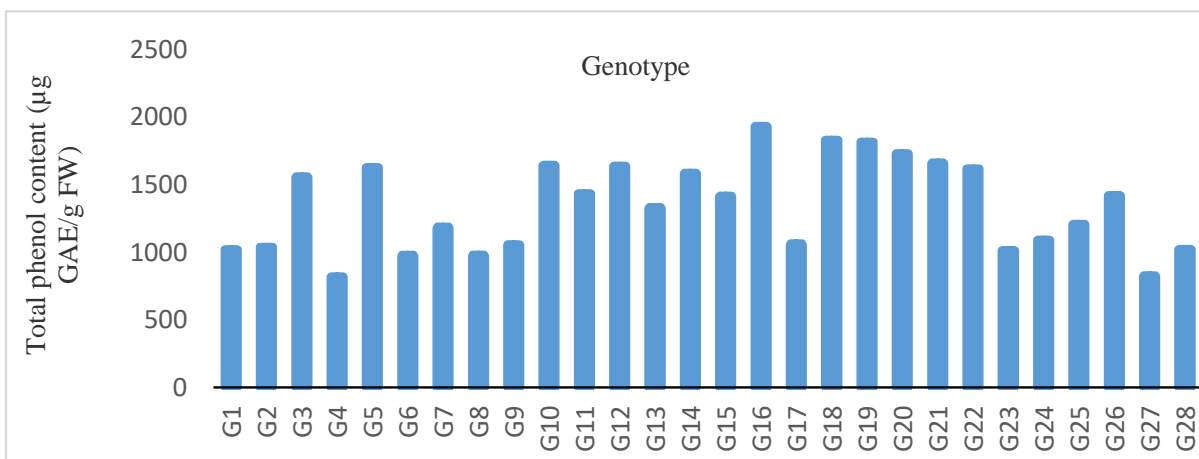


Figure 1. Variation in total phenol content in the fruit extract of 28 genotypes of chili, (GAE= Gallic Acid Equivalent).

Table 2. Means of nutritional phytochemicals and mineral contents of fruits of 28 chili genotypes arranged in four clusters. Abbreviations are as defined in Table 1.

Cluster	Counts	CAP	AAC	BBC	Chl a	Chl b	TCC	ANC	Na	K	Ca	Mg
1	2	0.37	19.46	0.22	0.14	0.09	0.10	1.74	0.10	1.94	0.85	0.56
2	10	0.23	53.99	0.18	0.17	0.18	0.09	1.53	0.18	1.93	0.99	0.53
3	10	0.21	81.56	0.22	0.12	0.10	0.09	1.45	0.12	1.89	1.06	0.53
4	6	0.25	108.60	0.21	0.12	0.13	0.16	1.83	0.14	1.93	1.15	0.53

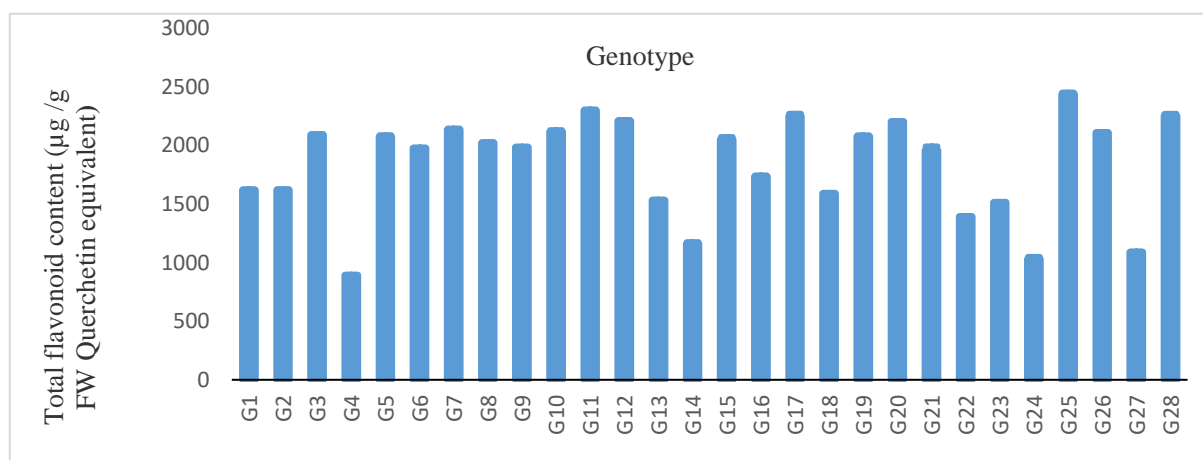


Figure 2. Variation in total flavonoid content in the fruit extract of 28 genotypes of chili.

Among the genotypes, total phenol content was high in G16 followed by G18, G19, G20, G21, G10, G12, G5, G22, G14, G3, G11, G26, G15 and G13. The lowest amount of total phenol content was observed in the genotype G4 followed by G27, G6, G8, G23, G1, G28, G2, G17, G9, G24, G7, and G25. Variation in total flavonoid content of chili fruit extract of 28 genotypes is presented in Figure 2. Total phenol content was high in G25 followed by G11, G17, G28, G12, G20, G7, G10, G26, G3, G19, G5, G15, G8, G9, G6, G21, G23, G13. The lowest amount of total phenol content was observed in the genotype G4 followed by G24, G27, G14, G22, G13, G18, G1, G2, and G16. The total antioxidant content of 28 genotypes of chili is presented in Figure 3. Among the genotypes, total antioxidant content was high in G2 and G16. The lowest amount of total antioxidant content was observed in the genotype G20 followed by G22, G5, G27, and G17.

Mineral composition of fruit extract of chili

Among the 28 genotypes of chili, sodium content (%) showed low variability and ranged from 0.06% to 0.39%. The highest amount of sodium was observed in genotypes G4 and G26 (0.39%) followed by G10 (0.27%) and G13 (0.23%). The lowest amount of sodium was observed in the genotype G8 (0.06%) followed by G5, G7, and G19 (0.08%). The mean sodium content in 28 genotypes was 0.15% and six genotypes showed above-average value (Supplementary Table 3). Fruit extract of 28 genotypes of chili was investigated for potassium content (%) and found pronounced variability which ranged from 1.71% to 2.07% (Supplementary Table 3). The highest amount of potassium content was observed in the fruit extract of the genotype G21 followed by G17 (2.01%) and G22 (2.01%).

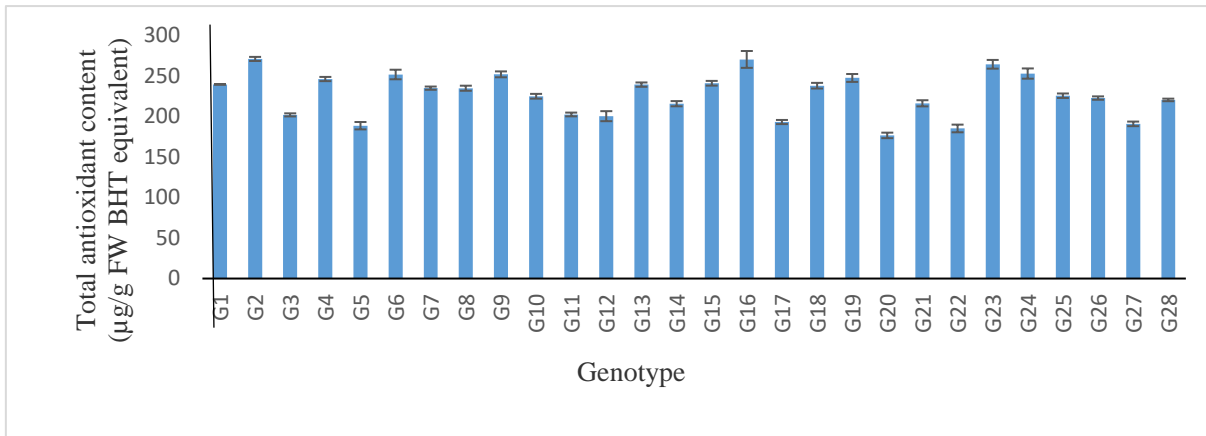


Figure 3. Variation in total antioxidant content in the fruit extract of 28 genotypes of chili, (BHT = Butylated Hydroxy Toluene).

The lowest amount of potassium content was observed in the fruit extract of the genotype G25 (1.71%) followed by G23 (1.79%) and G26 (1.8 %). The mean of potassium content in the fruit extract of 28 genotypes was 1.92% and among them 15 genotypes showed above average mean value. Calcium content (%) of the fruit extract of the chili genotypes under investigation noticed wide range of variation and was ranged between 0.72% and 1.65% (Supplementary Table 3). The highest amount of calcium content was observed in the fruit extract of the genotype G10 followed by G11 (1.48 %) and G15 (1.41 %). The lowest amount of calcium content was observed in the fruit extract of the genotypes G9 (0.72%) and G19 (0.72%) followed by G13 (0.75%) and G8 (0.83 %). The mean of calcium content in 28 genotypes was 1.04% and 12 genotypes showed above-average calcium content in the fruit extract. Magnesium content (%) showed low variability in the fruit extract of 28 genotypes of chili and ranged from 0.40% to 0.63 % (Supplementary Table 3). The highest amount of magnesium was observed in the fruit extract of G10 followed by G12 (0.62 %), G21 (0.62 %) and G27 (0.62 %). The lowest amount of magnesium was observed in the fruit extract of the genotype G13 (0.4 %) followed by G3 (0.44 %) and G14 (0.45 %). The mean of magnesium content in 28 genotypes was 0.54 %.

Heatmap and correlations among traits

By using 2-way clustering and heatmap, a dendrogram was constructed based on nutritional

phytochemicals and mineral contents of fruits of 28 chili genotypes which clustered the genotypes into 4 clusters (Figure 4). The trait variables visualized as co-cluster heatmap were classified into 3 groups. The chili genotypes grouped into row cluster showed high similarity while the genotypes in column cluster showed strong association. Among the 4 clusters, the cluster I contains minimum number of genotypes (2: G19, G20), remaining 3 clusters contains 10 (cluster II), 10 (cluster III) and 6 (cluster IV) genotypes (Figure 4). The results of cluster mean showed that genotypes of cluster I had maximum values for capsaicin content (0.37%), β -carotene content (0.22 mg/100g), potassium content (1.94 %) and magnesium content (0.56 %). Genotypes of cluster II had maximum mean values for Chl a content (0.17 mg/g), Chl b content (0.18 mg/g) and sodium content (0.18 %). Cluster mean of cluster II depicted the highest mean values only for β -carotene content (0.22 mg/100g) along with genotypes of cluster I (Supplementary Table 2, Figure 4). The genotypes of cluster IV had maximum mean values for ascorbic acid content (108.60 mg/100g), total carotenoid content (0.16 mg/g), ANC - anthocyanin content (1.83 μ g/g FW) and calcium content (1.15 %). Heatmap shows the genotypes of cluster IV was rich in ascorbic acid content (ACC) and anthocyanin content (ANC), on the other hand, the genotypes of cluster I was poor for ascorbic acid content. The other variables had poor associations with these four and among each other.

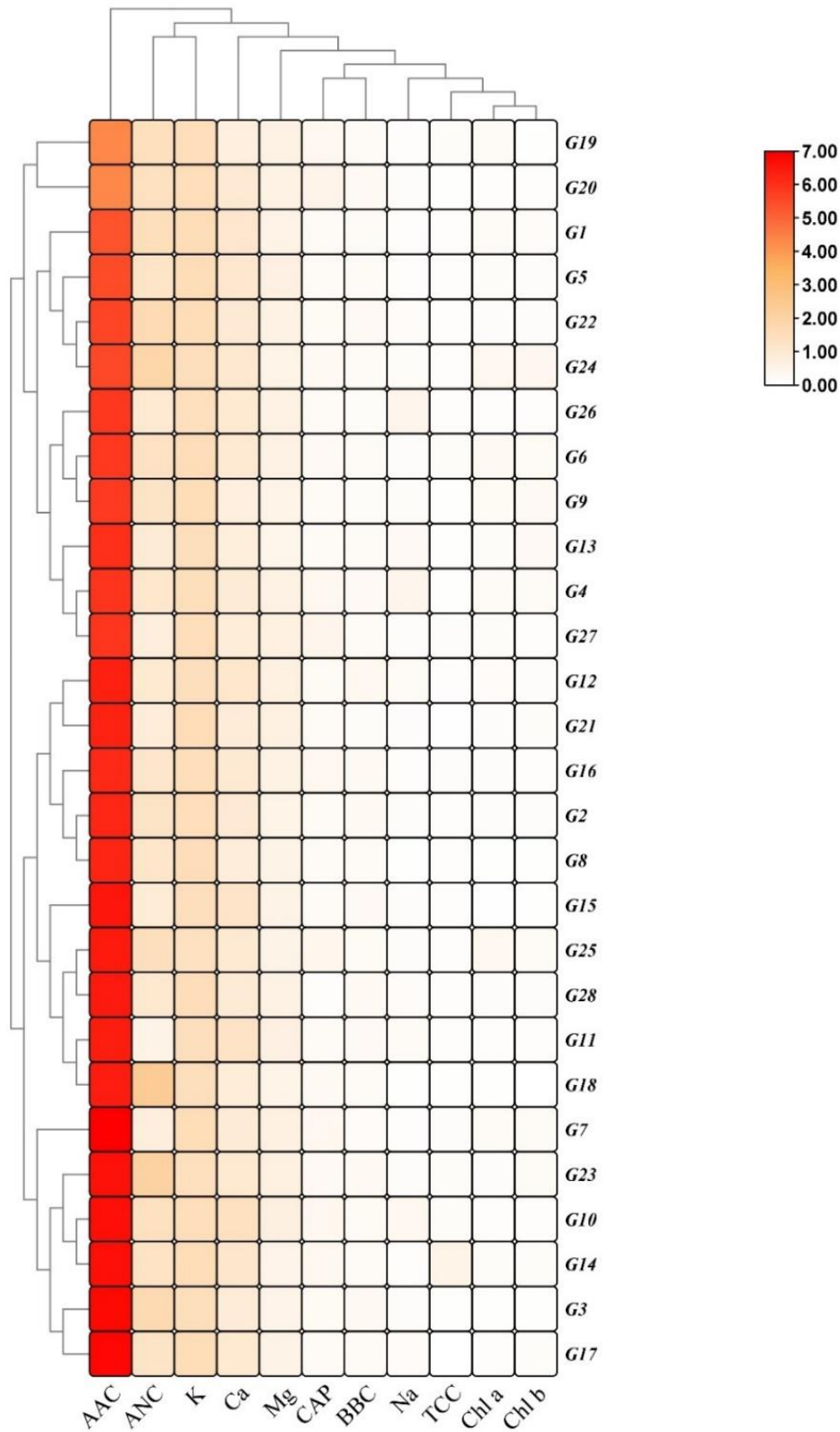


Figure 4. Cluster heatmap showing visual representation of 28 genotypes of chili in different groups based on similarity and correlations among traits. Abbreviations are as defined in Table 1.

Discussion

The evaluation of the extent of genetic variation within species has become now an essential tool in breeding program for their improvement (Dubey *et al.*, 2015). The degree of genetic diversity of genotypes and association of yield and quality traits are the important determinant for any breeding program of the crops (Shrestha, 2023). Genotype screening for yield and quality traits are the established techniques for selecting and managing genetic resources of any crops. The present research was conducted to evaluate chili genotypes for their nutritional phytochemicals and mineral content. A significant variation was observed for ascorbic acid content, total phenolic content, total flavonoids content, total antioxidant content, and anthocyanin content of fruits of 28 chili genotypes. The results of the current study are in accordance with the findings of Guzman *et al.* (2021) who evaluated genotypes of capsicum for bioactive compounds. Other traits under this study showed no variation among the genotypes for these traits and further improvement would not be possible by selection. Variations in other biochemical traits were also noted by González-López *et al.* (2021).

The greater amount of capsaicin content was observed in genotypes G20, G27 and G25 from 28 chili genotypes. The comparable genotypic differences were also reported by Bhagawati and Saikia (2015) for capsaicin content in different chili cultivar. Constantino *et al.* (2020) also confirmed the findings of previous studies. The genotypes with high capsaicin content could be utilized for the development of highly pungent variety and will serve as potential sources in the local and global markets. The capsaicin content in chili observed to be an effective antioxidant (Olatunji and Afolayan, 2018). The ascorbic acid content showed a significant variation among the 28 chili genotypes and produces the highest amount of 125.56 mg/100g and the lowest amount of 19.45 mg/100g. Similar results were reported by Pradhan *et al.* (2018). Janaki *et al.* (2015) found that the ascorbic acid content of chili fruits ranged from 43.99 to 223.22 mg/100g. The highest amount of ascorbic acid content was observed in the fruits of genotype G7 (125.56 mg/100g) and G17 (113.26 mg/100g) could

be useful in the development of nutritionally rich varieties.

β - carotene content showed low variability among the 28 genotypes of chili and the highest amount of β - carotene content was observed in genotype G12 (0.29 mg/100g) whereas the lowest was in genotype G9 and G26 (0.13 mg/100g. Sarker *et al.* (2012) reported the highest beta carotene content (0.39 mg/100g) in chili. A wide range of variation was observed for anthocyanin content in chili genotypes. The highest anthocyanin content of fruit extract was found in the genotype G18 (4.18 μ g/g FW) and the lowest in the genotype G11 (0.48 μ g/g FW). The anthocyanin content showed a 4.66 % coefficient of variation. Arnnok *et al.* (2012), and Adhikari and Pradhan (2014) reported that anthocyanin content ranged from 0.796 to 4.70 mg CGE kg⁻¹ fresh weight.

Campos *et al.* (2013) showed total carotenoid content ranged from 1.00 to 1.26 mg/100 g sample of chili. The genotypes identified based on bioactive compounds could be helpful in future breeding programs for cultivar improvement (Karim *et al.*, 2021). Chili contains a large number of phenolic compounds or flavonoids called quercetin, luteolin, and capsaicinoids (Hasler, 1998; Nahak *et al.*, 2017). The consumption of these bioactive compounds provides beneficial effects on human health due to their antioxidant properties, which protect against damage to cells and thus prevent the development of common degenerative diseases such as cancer, cardiovascular diseases, cataracts, and diabetes (Blanco-Ríos *et al.*, 2013). These chemical compounds also prevent the oxidation of essential fats within the cells of the brain that are considered necessary for its optimal functioning (Oboh and Rocha, 2007). Total phenolic content varies from cultivar to cultivar (Hamed *et al.*, 2019). Dubey *et al.* (2015) reported the existence of variations in the concentration of different polyphenols and flavonoids in chili accessions. The presence of phenolic compounds in Capsicum fruits were reported by Sukrasno and Yeoman (1993). The presence of various individual phenols and flavonoids were also reported in chili by Materska *et al.* (2003).

Fruit extract of 28 genotypes of chili was investigated for potassium, calcium and magnesium content (%) and found noticeable

variability. The highest amount of potassium content was observed in the fruit extract of the genotype G21. The highest amount of calcium and magnesium content was observed in the fruit extract of the genotype G10 (1.65% and 0.63%, respectively). Potassium, calcium and magnesium are very important nutrient elements for the body. Potassium prevents high blood pressure, plays a role in neurotransmission and helpful for synthesis of protein and amino acids (Khan *et al.*, 2019). Calcium is important for muscle movement and it helps to carry message between brain and other body parts. Magnesium plays important role in the release of parathyroid hormone which is important for kidney function, backbone activities, and also acts as catalyst for converting Vitamin D (Khan *et al.*, 2019). The amount of these minerals in the chili fruits depends on their genotypes as well as fertility status of the soil and farming practices (Emmanuel-Ikpeme *et al.*, 2014). The fruit extracts of chili genotypes are an excellent source of health-related phytochemicals and minerals such as ascorbic acid (Vitamin C), carotenoids (pro-Vitamin A), flavonoids, capsaicin, potassium, calcium, and magnesium. These elements are very much essential for preventing several chronic diseases such as cancer, asthma, sore throats, diabetes, cardiovascular disease (Shrestha, 2023).

Cluster analysis represents to define the pairwise differences between genotypes. Two-way clustering partitioned data in two directions, it clustered the genotypes together and also clustered the variables at the same time (Hageman *et al.*, 2012). The heatmap classified chilli genotypes into 4 groups and variables (phytochemicals and nutrient contents) into 3 groups. Heatmap shows the genotypes of cluster IV was rich in ascorbic acid content (ACC) and anthocyanin content (ANC), on the other hand, the genotypes of cluster I was poor for ascorbic acid content. So, genotypes from cluster IV can be considered as parent for future breeding program specially G7 and G17 which contain high amount of ascorbic acid in fruits (Supplementary Table 2). The variables ascorbic acid, anthocyanin, K, and Ca content correlated strongly. These associations can help to improve these traits together in chilli. The other variables had poor associations with these four and among each other and couldn't considered together to improve

phytochemical content in chili through any breeding program.

Conclusion

The present research through evaluation of nutritional phytochemical and mineral content provides an understanding of cultivated and available chili genotypes in Bangladesh. For capsaicin, ascorbic acid, and β -carotene content the genotypes G20, G7, and G12 were found to be superior than other genotypes. The highest amount of anthocyanin, carotenoid, total phenol, total flavonoid, and total antioxidant content was found in the genotypes G18, G14, G16, G25, and G2, respectively. The genotypes G4 and G26 contained a higher amount of Na and G21 had a higher amount of K. The highest amount of Ca and Mg were observed in the genotype G10. In case of heatmap analysis based on nutritional phytochemicals and minerals content, 28 chili genotypes were divided into 4 distinct clusters and traits into 3 clusters. Heatmap showed the genotypes of cluster IV was rich in ascorbic acid and anthocyanin content, and genotypes of cluster I was poor. The traits ascorbic acid, anthocyanin K and Ca content revealed strong association among them. Based on above findings the genotypes G2, G7, G12, G16, G17, G18 and G25 could be selected as parents for further improvement of chili through breeding program.

Supplementary Materials:

The supplementary material for this article can be found online at: https://www.jpmb-gabit.ir/article_717989.html

Supplementary Table 1. Solution preparation protocol used in the analysis of different phytochemicals in chili.

Supplementary Table 2. Mean performance of fruits of 28 chili genotypes for seven quality traits.

Supplementary Table 3. Mean performance of 28 chili genotypes mineral content of fruit extract of chili.

Author Contributions

Conceptualization, A. K. M. A. I. and P. H. C.; methodology, P. H. C., M. M. U. and M. M. H. S.; writing—original draft preparation, P. H. C.; writing—review and editing, A. K. M. A. I. and P. H. C.

C. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest Statement

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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ارزیابی ژنوتیپ‌های فلفل قرمز (*Capsicum annuum* L.) از نظر خصوصیات فیتوشیمیایی و مواد مغذی

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Chowdhury, P. H., Uddin, M. M., and Hasan Saikat, M. M. and Aminul Islam, A. K. M. (2024). Evaluation of chili (*Capsicum annuum* L.) genotypes for nutritional phytochemicals and mineral content. *J Plant Mol Breed.* 12 (1): 70-84. doi:10.22058/jpmb.2024.2030425.1300

چکیده: فلفل قرمز (*Capsicum annuum* L.) یک محصول مهم در سراسر جهان بوده که به دلیل خواص تغذیه‌ای و فیتوشیمیایی آن شناخته شده می‌باشد. این مطالعه با هدف شناسایی ژنوتیپ‌های مناسب در برنامه‌های اصلاحی، با ارزیابی محتوای فیتوشیمیایی و معدنی ۲۸ ژنوتیپ فلفل قرمز انجام شد. غلظت کپسایسین از ۰/۰۸ درصد (G20) الی ۰/۴۴ درصد (G28) متغیر بود. حداکثر غلظت اسید اسکوربیک در G7 (۱۲۵/۵۶ میلی‌گرم در ۱۰۰ گرم)، در حالی که حداقل آن در G20 (۱۹/۴۵ میلی‌گرم در ۱۰۰ گرم) تعیین شد. غلظت β -کاروتن از ۰/۲۹ میلی‌گرم در ۱۰۰ گرم FW در G12 تا ۰/۱۳ میلی‌گرم در ۱۰۰ گرم در G13 و G26 متغیر بود. حداکثر محتوای کلروفیل (a و b) در G24 (به ترتیب ۰/۳۲ و ۰/۳ میلی‌گرم بر گرم وزن تر) مشاهده شد، در حالی که حداقل مقادیر در ژنوتیپ‌های G15 و G18 ثبت گردید. غلظت آنتوسیانین از ۴/۱۸ میکروگرم بر گرم FW در G18 تا ۰/۴۸ میکروگرم بر گرم FW در G11 متغیر بود. ژنوتیپ G16 بالاترین سطح فنول کل و آنتی‌اکسیدان را نشان داد، در حالی که G25 بالاترین غلظت فلاونوئید را نشان داد. تجزیه و تحلیل مواد معدنی نشان داد که G4 و G26 حاوی بالاترین سدیم (۰/۳۹ درصد)، G21 دارای بالاترین پتاسیم (۲/۰۷ درصد)، و G10 دارای بالاترین کلسیم (۱/۶۵ درصد) و منیزیم (۰/۶۳ درصد) بودند. طبق تجزیه و تحلیل نقشه حرارتی ژنوتیپ‌ها، خوشه IV دارای سطوح بالایی از اسید اسکوربیک و آنتوسیانین بوده، در حالی که خوشه I از سطوح پایینی برخوردار بود. ارتباط قابل توجهی بین غلظت اسید اسکوربیک، آنتوسیانین، پتاسیم و کلسیم شناسایی شد. در برنامه‌های اصلاحی خصوصیات تغذیه‌ای و واریته‌های فلفل، ژنوتیپ‌های G2، G7، G12، G16، G17، G18 و G25 با پروفایل‌های فیتوشیمیایی و معدنی قابل توجه خود حایز اهمیت می‌باشند. این مطالعه اطلاعات مهمی در مورد تنوع ژنتیکی ژنوتیپ‌های فلفل قرمز در بنگلادش می‌دهد.

کلمات کلیدی: فلفل، فنول کل، فلاونوئیدها، فعالیت آنتی‌اکسیدانی و محتوای آنتوسیانین.