Nutrients, minerals, antioxidant pigments and phytochemicals, and antioxidant capacity of the leaves of stem amaranth

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We evaluated 17 genotypes of stem amaranth (Amaranthus lividus) in terms of dietary fiber, moisture, carbohydrates, fat, ash, gross energy, protein, minerals, phytopigments, total antioxidant capacity (TAC), vitamins, total flavonoids (TFC), total polyphenols (TPC) and their variations. Stem amaranth leaves have abundant dietary fiber, moisture, carbohydrates, and protein. We found significant amount of potassium, calcium, magnesium (9.61, 24.40, and 29.77 mg g\(^{-1}\) DW), iron, manganese, copper, zinc, (1131.98, 269.89, 25.03, and 1006.53 µg g\(^{-1}\) DW), phytopigments such as chlorophyll \(a\), chlorophyll \(b\), chlorophyll \(b_1\) (27.76, 42.06, and 14.30 mg 100 g\(^{-1}\) FW), betalain, betaxanthin, betacyanin (62.92, 31.81, 31.12 µg 100 g\(^{-1}\) FW), total carotenoids, beta-carotene (1675.38, 1289.26 µg g\(^{-1}\) FW), vitamin C (1355.46 µg g\(^{-1}\) FW), TPC, TFC (228.63 GAE and 157.42 RE µg g\(^{-1}\) DW), and TAC (DPPH, ABTS\(^{+}\)) (26.61, 51.73 TEAC µg g\(^{-1}\) DW) in the leaves of stem amaranth. Genotypes exhibited a wide range of variations. Three genotypes DS40, DS30, and DS26 could be used as an antioxidant profile enriched stem amaranth. Phenolics, phytopigments, flavonoids, and vitamins of stem amaranth leaves exhibited strong antioxidant activity. Stem amaranth could be a potential source of dietary fiber, moisture, carbohydrates, protein, minerals, phenolics, phytopigments, flavonoids, and vitamins in our daily diet for attaining nutritional and antioxidant sufficiency.

Amaranth has great variability and phenotypic plasticity with many culinary uses. In Bangladesh including south-east Asia, Africa, South America, the edible stem amaranth leaves are a very famous vegetable. Its popularity is continuously increasing in the Asian continent and elsewhere because of high nutritional value, taste, and attractive leaf color. In Bangladesh, stem amaranth is grown year-round and it could be grown in the gaps period of leafy vegetables between winter and hot summer. It is an inexpensive vegetable and has abundant dietary fiber and protein with essential amino acids such as methionine and lysine, minerals, pigments and phytochemicals like betacyanin, betaxanthin, chlorophyll, carotenoids, beta-carotene, vitamin C, phenolic compounds, and flavonoids.

In the world, food insecurity results in a continuous calorie deficit of approximately 795 million malnourished people. Deficiency of vitamins or minerals results in hidden hunger in over two billion people. Staple foods are deficient of micronutrients, mainly iron, zinc and iodine, pro-vitamin A, carotenoids, vitamin C, E, albeit these are a source of energy. Consequently, staple foods in our daily diet result in hidden hunger. We can ensure a balanced and healthy diet by consumption of fruit and vegetables as a source of vitamins and minerals accomplished with staple food. Furthermore, we protect human health and reduce the risk of cancer, cardiovascular, and other chronic diseases by feeding fruit and vegetables. Phytochemical compounds such as leaf pigments, vitamin C, phenolic and flavonoids are thought to contribute to those health benefits.

Recently, natural antioxidants of vegetables attracted consumers and researchers. Leaf pigments (betacyanin, betaxanthin, chlorophyll, and carotenoids), vitamin C, phenolics and flavonoids are available natural antioxidants in amaranths. These natural antioxidants phytochemicals defense against several diseases like cardiovascular

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diseases, cancer, cataracts, atherosclerosis, retinopathy, arthritis, emphysema, and neurodegenerative diseases.© Amaranth is also tolerant to abiotic stresses like drought and salinity.©

Stem amaranth is a very popular vegetable in Bangladesh. It is consumed both as a leafy vegetable in early stages and vegetables (stem only) in the later stage. In the younger stage, around 30 days old, the whole plant including leaves and tender succulent stems are used as leafy vegetables. The large barreled stem of this amaranth is succulent and juicy and become edible as vegetables up to initiation of flowering. It takes approximately two to three months to flower, even though some photosensitive cultivar takes 9 to 12 months to flower. Those large barreled juicy and succulent stems are a famous vegetable in Bangladesh and consumed year-round. However, the literature has shown that amaranth leaf had much higher nutrients, minerals, pigments, phytochemicals, and antioxidants in comparison to the stem of the plant.© For this reason, we evaluated the stem amaranth as leafy vegetables in terms of nutrients, minerals, antioxidant pigments and phytochemicals, and antioxidant capacity. Although it is abiotic stress tolerant and inexpensive sources of minerals, dietary fiber, protein, and antioxidant phytochemicals like leaf pigments, vitamin C, phenolics, and flavonoids, there is a scarce of information in this species. In our earlier study, we evaluated A. tricolor for morphological, proximate, minerals, antioxidant leaf pigments, antioxidant phytochemicals.© To our knowledge, it is the first report on proximate and mineral compositions, phenolics, flavonoids, leaf pigments, and vitamins in a huge number of diversified stem amaranth germplasms available in Bangladesh and elsewhere. Therefore, to fill these gaps, the present investigation was undertaken to evaluate proximate and mineral compositions, leaf pigments, vitamins, phenolics, and flavonoids content in 17 stem amaranth genotypes. To determine the variability of these traits in 17 stem amaranth genotypes.©

Results and Discussion

Proximate compositions. Table 1 represents the proximate compositions of stem amaranth. The leaf water content ranged from 82.05 to 88.43 g 100 g−1 FW. As high leaf dry matter obtained from lower moisture contents, five genotypes (17–18% dry matter) had a considerable dry matter. The maturity of the plant directly associated with the leaf moisture content of stem amaranth. The findings obtained in this study were fully agreed to the reports of amaranth and sweet potato leaves by Sarker and Oba© and Sun et al.© respectively.

The protein content of the leaf of stem amaranth exerted much pronounced variations. The protein content ranged from 5.76 to 1.47 g 100 g−1 FW. Nine genotypes had higher protein content compared to their average values. As leafy vegetables, the genotype DS36, DS34, DS26, DS30, DS25, and DS39 had high protein content. Stem amaranth is the main source of protein for poor people of the low-income countries and vegetarians. Our results showed that stem amaranth exhibited high protein content (3.46 g 100 g−1 FW) than A. tricolor (1.26%) of our previous study.©

The fat of stem amaranth ranged from 0.43, 0.42 to 0.21 g 100 g−1 FW with a grand mean value of 0.29 g 100 g−1 FW, and showing the following order: DS33 > DS32 > DS34 > DS37 > DS41. Sarker and Oba© and Sun et al.© observed similar results in A. tricolor and the leaf of sweet potato, respectively. They reported that cell function, body temperature, and the insulation of body organs were maintained through catabolism of fat. Fats are an excellent source of omega-6 and omega-3 fatty acids. Absorption, digestion, and transport of fat-soluble vitamins such as A, D, E, and K mainly depend on fats. The carbohydrates content ranged from 9.85 to 2.21 g 100 g−1 FW with a mean value of 7.24 g 100 g−1 FW. The energy ranged from 33.38 to 35.91 Kcal 100 g−1 FW with a grand mean value of 43.66 Kcal 100 g−1 FW. Ash content ranged from 5.43 to 2.09 g 100 g−1 FW with a grand mean value of 3.58 g 100 g−1 FW.

The significant variations were observed in 17 stem amaranth genotypes in terms of dietary fiber. Dietary fiber ranged from 95.72 to 62.40 µg g−1 FW with a mean value of 78.89 µg g−1 FW. Dietary fiber significantly contributed to the cure of constipation, digestibility, and palatability. Our results showed that the leaf of stem amaranth were a good source of dietary fiber, moisture, carbohydrates, and protein. The results of this study corroborated with the results of Sarker and oba©.

Composition of minerals. Table 2 represents the content of minerals of stem amaranth. In this study, the content of potassium (K) varied from 6.54 mg g−1 to 14.21 mg g−1 DW. High potassium content was obtained from eight genotypes with a grand mean value of 9.61 mg g−1 DW. The potassium content of ten genotypes was much higher than their grand mean. The range of Ca content was 16.06–31.22 mg g−1 DW. High Ca content was noted in eight genotypes which were better than the respective average value. Mg content did not exhibit pronounced variations in 17 stem amaranth genotypes (27.71 to 32.53 mg g−1 DW). The average Mg content was 29.77 mg g−1 DW. High Mg content was noted in three genotypes. In our present study, we found a significant amount of K (9.61 mg g−1), calcium (24.40 mg g−1) and magnesium (29.77 mg g−1) in the leaf of stem amaranth, albeit we determined based on the dry weight. Chakraborty et al.© in stem amaranth and Sarker and Oba© in A. tricolor also observed similar results. Jimenez-Aguilar and Grusak© reported a good amount of Mg, K, and Ca in different species of amaranth. They reported that Mg, Ca, and K content of different species of amaranth was much higher than kale, black nightshade, spider flower, and spinach.

Iron content showed the prominent variations in terms of genotypes (739.04 µg g−1 DW to 2546.25 µg g−1 DW). The grand mean value of 17 genotypes was 1131.98 µg g−1 DW. High iron content was obtained from four genotypes which were higher than the mean value. The range of manganese content varied from 174.63 µg g−1 DW to 375.33 µg g−1 DW, with a mean value of 269.89 µg g−1 DW. Six genotypes had high manganese content. The significant and notable variations in copper content were reported in the genotypes studied (17.56–42.15 µg g−1 DW). High copper was obtained from eight genotypes which were higher than the mean value. The zinc content of stem amaranth varied significantly in terms of genotypes (741.50 µg g−1 DW to 1525.92 µg g−1 DW). High zinc content was observed in five genotypes which were higher than the grand mean value (1006.53 µg g−1 DW). Stem amaranth leaves contained higher zinc and iron content than the cassava leaves© and beach pea©. Our study
showed that leaves of stem amaranth had considerable iron (1131.98 µg g⁻¹), manganese (269.89 µg g⁻¹), copper (25.03 µg g⁻¹), and zinc (1006.53 µg g⁻¹), albeit it was measured based on the dry weight. Jimenez-Aguilar and Grusak reported a good amount of iron, manganese, copper, and zinc in the different species of amaranth. They reported that iron, manganese, copper, and zinc content of different species of amaranth were much higher than kale, black nightshade, spider flower, and spinach.

### Composition of antioxidant leaf pigments.

Table 3 represents the composition of antioxidant leaf pigments of stem amaranth. Chlorophyll a content differed remarkably in stem amaranth (12.25 to 50.86 mg 100 g⁻¹). Chlorophyll a content was high in three stem amaranth genotypes. Chlorophyll a content of seven genotypes was higher than the average value. There were prominent variations in chlorophyll b content of 17 stem amaranth genotypes (5.67 to 27.38 mg 100 g⁻¹). Prominent variations were also observed in chlorophyll ab (18.86 to 74.37 mg 100 g⁻¹). Four genotypes exhibited high chlorophyll ab content, Nine genotypes had higher chlorophyll ab than the mean value. Our study revealed that stem amaranth genotypes had a considerable amount of chlorophyll ab (42.06 mg 100 g⁻¹), chlorophyll a (27.76 mg 100 g⁻¹), and chlorophyll b (14.30 mg 100 g⁻¹), whereas, chlorophylls content of A. tricolor reported by Khanam and Oba were relatively lower.

Betacyanin ranged from 15.42 to 53.36 µg 100 g⁻¹ with a mean value of 31.12 µg 100 g⁻¹. Betaxanthin content showed the significant and notable differences in 17 stem amaranth genotypes (17.27 to 55.24 µg 100 g⁻¹). High betaxanthin content was observed in four genotypes. Eight genotypes had higher betaxanthin content than the mean value. Betalain ranged from 32.70 to 108.60 µg 100 g⁻¹. High betalain content was observed in five genotypes. Eight genotypes had higher betalain content than average value. The range of total carotenoids content was 469.29 µg g⁻¹ to 1675.38 µg g⁻¹. Three genotypes showed the highest total carotenoids content. Similarly, high total carotenoids were found in four genotypes. Ten genotypes had higher total carotenoids than average value. In this study, we found a significant amount of betacyanin (31.12 µg 100 g⁻¹), betaxanthin (31.81 µg 100 g⁻¹), betalain (62.92 µg 100 g⁻¹) and total carotenoids (1675.38 µg g⁻¹) in the stem amaranth. Khanam et al. reported corroboration results for betacyanin, betaxanthin, betalain and total carotenoids content of A. tricolor.

### Antioxidant phytochemicals.

Table 4 represents TAC, vitamins, TPC, and TFC of stem amaranth. The range of beta-carotene content was 355.35 µg g⁻¹ to 1289.26 µg g⁻¹. Four genotypes showed high beta-carotene. Ten genotypes had higher beta-carotene than average beta-carotene. The range of vitamin C content was 431.14 to 431.22 µg g⁻¹ with a mean value of 746.58 µg g⁻¹. Seven genotypes had higher vitamin C than average vitamin C. Vitamin C content was high in four genotypes. The range of total polyphenol content (TPC) was 78.22 GAE µg g⁻¹ DW to 228.66 GAE µg g⁻¹ DW with a mean value of 156.25 GAE µg g⁻¹ DW. Five genotypes showed high polyphenol content. Ten genotypes showed higher polyphenol than average polyphenol content. Prominent variations were noted in the TPC content of stem amaranth genotypes, with a range of 65.89 RE µg g⁻¹ DW to 157.42 RE µg g⁻¹ DW. The mean value of TFC was 105.84 RE µg g⁻¹ DW. TFC showed the following order: DS30 > DS26 > DS40 > DS35 > DS34. Eight genotypes showed higher TFC value than average TFC. The range of TAC (DPPH) was 8.94 TEAC µg g⁻¹ DW to 26.61 TEAC µg g⁻¹ DW. Five genotypes had high TAC (DPPH). Seven genotypes exhibited higher TAC (DPPH) than average value. The range of TAC (ABTS⁺) was 16.71 TEAC µg g⁻¹.
Table 2. Mineral compositions (Macrolelements mg g\(^{-1}\) DW and microelements µg g\(^{-1}\) DW elements) of 17 stem amaranth genotypes. CV, Coefficient of variation; K, Potassium; Ca, Calcium, Mg, Magnesium; Fe, Iron; Mn, Manganese; Cu, Copper; Zn, Zinc; n = 6; **Significant at 1% level, Different letters in each column are differed significantly by Tukey’s HSD test.

| Genotypes | K (mg g\(^{-1}\) DW) | Ca (mg g\(^{-1}\) DW) | Mg (mg g\(^{-1}\) DW) | Fe (µg g\(^{-1}\) DW) | Mn (µg g\(^{-1}\) DW) | Zn (µg g\(^{-1}\) DW) |
|-----------|----------------|----------------|----------------|----------------|----------------|----------------|
| DS25      | 7.34 ± 0.02f  | 16.24 ± 0.05  | 29.97 ± 0.07c | 1047.74 ± 0.86g | 228.28 ± 0.27c | 26.32 ± 0.04d | 852.24 ± 0.74o |
| DS26      | 14.43 ± 0.06a | 17.94 ± 0.05  | 31.88 ± 0.12a | 1732.94 ± 0.56b | 345.34 ± 0.46b | 23.56 ± 0.06g | 1534.56 ± 0.51a |
| DS27      | 9.85 ± 0.07d  | 25.67 ± 0.04e | 39.32 ± 0.14f | 989.67 ± 0.87i | 198.72 ± 0.39k | 20.68 ± 0.04i | 914.88 ± 0.46l |
| DS28      | 7.52 ± 0.04f  | 25.66 ± 0.05e | 29.86 ± 0.16d | 986.69 ± 0.76j | 188.76 ± 0.28l | 20.73 ± 0.04i | 941.74 ± 0.64k |
| DS29      | 11.55 ± 0.05c | 24.23 ± 0.06  | 29.55 ± 0.14e | 1033.56 ± 0.48k | 227.27 ± 0.57m | 28.17 ± 0.07e | 944.42 ± 0.51j |
| DS30      | 10.34 ± 0.05d | 31.32 ± 0.08a | 30.23 ± 0.18d | 1116.91 ± 0.34c | 321.83 ± 0.37c | 27.95 ± 0.07c | 1432.27 ± 0.41b |
| DS31      | 9.98 ± 0.04d  | 29.65 ± 0.06c | 29.22 ± 0.17f | 1384.65 ± 0.62c | 381.26 ± 0.64a | 18.14 ± 0.04f | 1241.35 ± 0.37c |
| DS32      | 8.36 ± 0.06e  | 30.46 ± 0.06b | 30.84 ± 0.14b | 2572.22 ± 0.46a | 310.87 ± 0.68a | 25.34 ± 0.04c | 1023.28 ± 0.46e |
| DS33      | 11.37 ± 0.07c | 28.25 ± 0.05d | 30.24 ± 0.16d | 968.42 ± 0.61k | 312.65 ± 0.53d | 29.33 ± 0.03b | 988.33 ± 0.34g  |
| DS34      | 12.41 ± 0.06b | 19.34 ± 0.07h | 29.89 ± 0.15d | 752.23 ± 0.42m | 176.84 ± 0.45m | 44.42 ± 0.04a | 748.47 ± 0.46g  |
| DS35      | 6.62 ± 0.06f  | 24.21 ± 0.05f | 29.32 ± 0.09g | 985.65 ± 0.82k | 246.72 ± 0.81h | 28.46 ± 0.06c | 957.18 ± 0.29m |
| DS36      | 10.06 ± 0.07d | 28.78 ± 0.04d | 29.82 ± 0.14d | 1128.56 ± 0.48g | 271.55 ± 0.68b | 24.78 ± 0.04f | 1052.33 ± 0.48d |
| DS37      | 12.16 ± 0.08b | 19.28 ± 0.05  | 28.68 ± 0.15g | 743.12 ± 0.15c | 296.76 ± 0.66e | 24.87 ± 0.02f | 1005.32 ± 0.68h |
| DS38      | 6.63 ± 0.04g  | 24.13 ± 0.07f | 29.56 ± 0.17c | 788.43 ± 0.54m | 239.54 ± 0.38j | 27.85 ± 0.06c | 889.38 ± 0.57m |
| DS39      | 7.37 ± 0.06f  | 22.79 ± 0.05g | 27.76 ± 0.12h | 1135.29 ± 0.62d | 251.31 ± 0.61g | 23.54 ± 0.07g | 976.87 ± 0.45j |
| DS40      | 11.54 ± 0.04c | 24.86 ± 0.07e | 30.58 ± 0.16c | 1062.84 ± 0.52f | 276.67 ± 0.85g | 22.32 ± 0.03h | 878.46 ± 0.51l |
| DS41      | 7.64 ± 0.05f  | 23.26 ± 0.07g | 28.71 ± 0.15g | 932.25 ± 0.38i | 337.21 ± 0.53b | 25.36 ± 0.03e | 901.38 ± 0.27l |
| Mean      | 9.72          | 24.47          | 29.73          | 1138.89        | 273.92          | 25.99          | 1016.62         |
| CV%       | 2.87%         | 1.352%         | 1.754%         | 0.528%         | 0.645%          | 0.543%         | 0.462%          |

**Correlation studies.** Correlations of phytochemicals, antioxidant pigments, and antioxidant potential of stem amaranth are shown in Table 5. The correlation coefficients shown in Table 5 had encouraging findings. We observed a significant positive correlation among TAC (DPPH), chlorophyll \(ab\) and \(b\), betacyanin, chlorophyll \(a\), betaxanthin, betalain, TAC (ABTS\(^{+}\)) and TFC. Shukla et al.\(^{31}\) also reported positive associations in their earlier work in *A. tricolor*. Similarly, betacyanin, betaxanthin, and betalain showed positive and significant interrelationship among each of them and with TAC (ABTS\(^{+}\)), chlorophylls, TPC, TAC (DPPH), and TPC which was corroborated with the results of our earlier studies in amaranth\(^{9,20-24}\) indicating increase in any pigment was directly related to increment of another pigment. The positive and significant interrelationship of TAC (DPPH), TPC, TAC and TAC (ABTS\(^{+}\)) indicated that pigments, TPC, and TAC exhibited strong antioxidant potential. The significant negative association was observed between pigments vs. total carotenoids and pigments vs. beta-carotene, while total carotenoids and beta-carotene exhibited a significant positive association with TAC (ABTS\(^{+}\)), TAC (DPPH), TPC, and TFC which was corroborated with the results of our earlier studies in amaranth\(^{20-24}\). It indicated that the increment of any leaf pigment had a direct decrement of total carotenoids and beta-carotene. Beta-carotene and total carotenoids exhibited strong antioxidant potential as these traits had significantly and positively associated with TAC (ABTS\(^{+}\)), TAC (DPPH), TPC, and TFC. There were positive associations between beta-carotene and total carotenoids. In contrast, the negligible insignificant association was observed between vitamin C and all the leaf pigments. Jimenez-Aguilar and Grusk\(a\)\(^{39}\) reported negligible insignificant association for ascorbic acid in amaranth. Whereas, vitamin C was positively and significantly correlated with TAC (ABTS\(^{+}\)), TAC (DPPH), TPC, and TFC indicating the strong contribution of vitamin C of stem amaranth to antioxidant activity. TAC (ABTS\(^{+}\)), TAC (DPPH), TPC, and TFC associated significantly and positively among each other, as well as vitamins and pigments, indicated that vitamins, flavonoids, pigments, phenolics strongly contributed to the antioxidant activity of amaranth. In the present investigation, it revealed
that leaf pigments, vitamins, phenolics, flavonoids played a significant contribution to the antioxidant capacity of stem amaranth.

In conclusion, stem amaranth leaves were good sources of potassium, calcium, magnesium, iron, manganese, copper, zinc, chlorophylls, vitamin C, betacyanin, betaxanthin, TAC, betalain, carotenoids, betacarotene, protein, dietary fiber, TPC, carbohydrates, and TFC. It could be used as a leafy vegetable for potential sources of antioxidant leaf pigments, betacarotene, vitamin C, phenolics, minerals and proximate, flavonoids in the human diet for attaining nutritional and antioxidant sufficiency.

Methods

Experiment materials, layout, design, and cultural practices. Seventeen stem amaranth genotypes selected from 156 genotypes were sown in Bagangbandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, in a randomized complete block design (RCBD) with three replications. It is consumed as a leafy vegetable in the early stage (30 days old). In the later stage (up to 4 months) only stems were eaten as vegetables in different curries which tend to be less nutritious. The experimental unit was 1 m × 1 m. Stem amaranth genotype was grown maintaining the distance of 20 cm between rows and 5 cm between plants. The experimental site was located in the center of the Madhupur Tract (AEZ 28), about 24°23'N 90°08'E, with a mean elevation of 8.4 msl. The site falls under the subtropical zone and has mean temperatures of 29 °C (summer) and 18 °C (winter). There was no precipitation during the cropping season. The experimental field was a high land having silty clay soil. The soil was slightly acidic (pH 6.4) and low in organic matter (0.87%), total N (0.09%) and exchangeable K (0.13 cmol/kg). The soil S content was at par with a critical level, while P and Zn contents were above the critical level (Critical levels of P, S, and Zn were 14, 14 and 0.2 mg kg⁻¹, respectively and that of K was 0.2 cmol kg⁻¹). During land preparation total compost (10 ton/ha) was applied. We applied recommended fertilizer doses, such as Urea, triple super phosphate, murate of potash and gypsum at 200, 100, 150, and 30 kg/ha, respectively. Thinning was done to maintain appropriate spacing between plants of a row. As a necessity, weeding and hoeing were done at 7 days interval to control the weeds. Proper irrigations were provided to maintain the normal growth of the crop. Leaf samples were collected 30 days after the sowing of seed.

Chemicals. Solvent: methanol, ethanol, and acetone. Reagents: dithiothreitol (DTT). HNO₃, standard compounds of pure Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid), H₂O₂, potassium persulfate, ascorbic acid, folin-ciocalteu reagent, gallic acid, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS⁺, rutin, 2, 2-dipyridyl, sodium carbonate, aluminum chloride hexahydrate, and potassium acetate. We bought all solvents and reagents from Kanto Chemical Co. Inc. (Tokyo, Japan) and Merck (Germany).

Measurement of the composition of proximate. Ash, crude fat, moisture, crude protein contents, fiber, and gross energy were determined through AOAC method 15,36. Crude protein was estimated through the Micro-Kjeldahl method multiplying nitrogen by 6.25 (AOAC method 976.05). To estimate carbohydrate (g 100g⁻¹ FW), the sum of the percentage of crude protein, ash, crude fat, and moisture was subtracted from 100.
After removing the supernatant in a volumetric flask, the extract was centrifuged at 10,000 g for 3–4 min. The absorbance was taken at 510 nm and 480 nm using a spectrophotometer (Hitachi, U-1800, Tokyo, Japan). Data were expressed as mg chlorophyll per 100 g and µg total carotenoids per g fresh weight.

**Estimation of composition of minerals.** Stem amaranth leaves were dried at 70 °C for 24 hours in an oven. We ground the dried leaves finely in a mill. The method described by Jimenez-Aguilar and Grusak36,38 was used to estimate minerals. Concentrated HNO₃ was used to digest the samples (250 mg) overnight (room temperature). Then it was set for 2.5 h at 125 °C, followed by 30% H₂O₂ for 2 h at 125 °C. The temperature was then increased to 200 °C, and the samples were heated until they were completely dry. After cooling, the samples were resuspended in 15 mL 2% HNO₃. The following wavelengths (nm): K (404.721), Ca (219.77), Mg (294.20), Fe (262.82), Mn (257.6), Cu (327.39), and (Zn 206.19) were used to determine the concentrations through an inductively coupled plasma optical emission spectroscopy (ICP-OES, Ciros ICP-FCE12, Kleve, Germany). Certified mineral standard was followed to calibrate the ICP-OES daily. Results are expressed in mg and µg per gram of sample dry weight (DW).

**Estimation of carotenoids and chlorophylls.** Method of Sarker and Oba36,37 was followed to estimate chlorophyll ab, chlorophyll b, total carotenoids, and chlorophyll a through extracting the fresh leaves of stem amaranth in 80% aceton. The absorbance was read at 663 nm for chlorophyll a, 646 nm for chlorophyll b, and 470 nm for total carotenoids, respectively through a spectrophotometer (Hitachi, U-1800, Tokyo, Japan). Data were expressed as mg chlorophyll per 100 g and µg total carotenoids per g fresh weight.

**Estimation of betaxanthin and betacyanin content.** Method of Sarker and Oba36,38 was followed to estimate betacyanin and betaxanthin through extracting the leaves of stem amaranth in 80% methyl alcohol having 50 mM ascorbate. Betacyanin and betaxanthin were estimated using a spectrophotometer (Hitachi, U-1800, Tokyo, Japan) at 540 nm for betacyanin and 475 nm for betaxanthin, respectively. The results were expressed as microgram betanin equivalent per 100 gram fresh weight (FW) for betacyanin and micrograms indicaxanthin equivalent per 100 gram FW for betaxanthin.

**Determination of beta-carotene.** Beta-carotene content was extracted following the method of Sarker and Oba36. 500 mg of fresh leaf sample was ground thoroughly in a mortar and pestle with 10 ml of 80% acetone. After removing the supernatant in a volumetric flask, the extract was centrifuged at 10,000 × g for 3–4 min. The final volume was brought up to 20 ml. The absorbance was taken at 510 nm and 480 nm using a spectrophotometer (Hitachi, U-1800, Tokyo, Japan). Data were expressed as µg beta-carotene per g fresh weight.

The following formula was used to measure the beta-carotene content:

\[ \text{Beta-carotene} = 7.6 \times (\text{Abs. at 480}) - 1.49 \times (\text{Abs. at 510}) \times \text{Final volume/(1000 × fresh weight of leaf taken)} \]

**Determination of vitamin C.** A spectrophotometer was used to measure ascorbate (AsA) and dehydroascorbic acid (DHA) acid from the fresh stem amaranth leaves. Dithiothreitol (DTT) was used for the pre-incubation of the sample and reduction of DHA into AsA. AsA reduced Fe³⁺ to Fe²⁺. AsA was estimated...
Table 5. The correlation coefficient for antioxidant leaf pigments, beta-carotene, vitamin C, TPC, TFC, TAC (DPPH) and TAC (ABTS⁺) in 17 stem amaranth genotypes. Chi a, Chlorophyll a; Chi ab, Chlorophyll ab; TAC, Total antioxidant capacity; TPC, Total polyphenol content; TFC, Total flavonoid content; **Significant at 1% level.

through measuring Fe⁺ complexes with 2, 2-dipyridyl. Finally, the absorbance of the sample solution was read at 525 nm using a spectrophotometer (Hitachi, U-1800, Tokyo, Japan) and data were expressed as µg vitamin C per g fresh weight.

Extraction of sample for TAC, TFC, and TPC. The leaves were dried in the air in a shade for chemical analysis. 1 g of grounded dried leaves was extracted in 40 ml of 90% aqueous methanol in a tightly capped bottle (100 ml). The bottle was then placed in a shaking water bath (Thomastant T-N22S, Thomas Kagaku Co. Ltd., Japan) for 1 h. The extract was filtered for estimation of total antioxidant capacity, flavonoids, and polyphenols.

Polyphenols estimation. Method of Sarker and Oba was followed to estimate the total phenolic content of stem amaranth using the folin– ciocalteu reagent with gallic acid as a standard phenolic compound. Folin–ciocalteu reagent was previously diluted 1:4, reagent: distilled water. In a test tube, 1 ml of diluted folin-ciocalteu was added to 50 µl extract solution and then mixed thoroughly for 3 min. 1 ml of Na₂CO₃ (10%) was added to the tube and stand for 1 h in the dark. A Hitachi U1800 spectrophotometer (Hitachi, Tokyo, Japan) was used to read the absorbance at 760 nm. A standard gallic acid graph was made to determine the concentration of phenolics in the extracts. The results are expressed as µg gallic acid equivalent (GAE) g⁻¹ DW.

Flavonoids estimation. The AlCl₃ colorimetric method was used to estimate the total flavonoid content of stem amaranth extract. In a test tube, 1.5 ml of methanol was added to 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, 2.8 ml of distilled water and 500 µl of leaf extract for 30 min at room temperature. A Hitachi U1800 spectrophotometer (Hitachi, Tokyo, Japan) was used to take the absorbance at 517 nm. Method of Sarker and Oba was followed for ABTS⁺ assay. 7.4 mM ABTS⁺ solution and 2.6 mM potassium persulfate were used in the stock solutions. The two stock solutions were mixed in equal quantities and allowing them to react for 12 h at room temperature in the dark for preparation of the working solution. Exactly 2850 µl of ABTS⁺ solution (1 ml ABTS⁺ solution mixed with 60 ml methanol) was mixed with 150 µl sample of leaf extract and allowed to react for 2 h in the dark. A Hitachi U1800 spectrophotometer (Hitachi, Tokyo, Japan) was used to read the absorbance
against methanol at 734 nm. The percent of inhibition of DPPH and ABTS$^+$ relative to the control were used to determine antioxidant activity using the following equation:

$$\text{Antioxidant activity}(\%) = \left( \frac{\text{Abs. blank} - \text{Abs. sample/Abs. blank}}{\text{Abs. blank}} \right) \times 100$$

where, Abs. blank is the absorbance of the control reaction [10 µl methanol for TAC (DPPH), 150 µl methanol for TAC (ABTS$^+$)] instead of leaf extract] and Abs. sample is the absorbance of the test compound. Trolox was used as the reference standard, and the results were expressed as µg Trolox equivalent g$^{-1}$ DW.

**Statistical analysis.** Mineral, chlorophylls, carotenoids, beta-carotene, vitamin C, polyphenols, flavonoids, and antioxidant activity (DPPH & ABTS$^+$) analysis were evaluated in three independent samples per replication (each sample was prepared from a combined sample of leaves from multiple plants) and nine samples per genotype. Results were expressed as mean value ± standard deviation per genotype. Every mean represents the average of all measurements for the same genotype (Tables 1–4). ANOVA was performed using Statistix 8 software and the means were compared by Tukey’s HSD test at 1% and level of probability.

**Ethical statement.** The lab and field experiment in this study was carried out following guidelines and recommendations of “Biosafety Guidelines of Bangladesh” published by the Ministry of Environment and Forest, Government of the People's Republic of Bangladesh (2005).

**Data availability**

Data used in this manuscript will be available to the public.

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Author contributions
U.S. initiated the research work and conceived the study; U.S. performed the experiments; biochemical analysis and statistical analysis; U.S. M.A.D. drafted, edited, interpreted data and prepared the manuscript; S.O. edited the manuscript, provided valuable suggestions during the experiment and also provided valuable support and guidance preparing the manuscript.

Competing interests
The authors declare no competing interests.

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