Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2016) 40: 526-535
© TÜBİTAK
doi:10.3906/tar-1405-83

Genetic variation and interrelationships among antioxidant, quality, and agronomic traits in vegetable amaranth

Umakanta SARKER[1,*], Md. Tofazzal ISLAM[2], Md. Golam RABBANI[3], Shinya OBA[4]

[1]Department of Genetics and Plant Breeding, Faculty of Agriculture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh

[2]Department of Biotechnology, Faculty of Agriculture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh

[3]Department of Horticulture, Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh, Bangladesh

[4]Laboratory of Field Science, Faculty of Applied Biological Science, Gifu University, Gifu, Japan

Received: 21.05.2014 • **Accepted/Published Online:** 07.03.2016 • **Final Version:** 14.06.2016

Abstract: This investigation was aimed to evaluate 22 promising vegetable amaranth genotypes for antioxidant, leaf quality, and agronomic traits for two test seasons. The experiment was conducted to study the degree of genetic parameters, associations among different traits, and their contribution towards foliage yield. The analysis of variance for twelve traits was found highly significant. High mean, high range of variability, and high genotypic variance were observed for all the traits except content of Ca, protein, and carotenoid. Close differences between genotypic and phenotypic variances and genotypic and phenotypic coefficients of variation were observed for all the traits. High to moderate genotypic coefficients of variation and heritability coupled with high to moderate genetic advance in percent of mean was observed for all the traits. Considering all genetic parameters, selection based on Fe, Zn, Mn, ascorbic acid content, fiber content, plant height, leaves plant^{-1}, diameter of stem base, and foliage yield would be effective for the improvement of vegetable amaranth. Foliage yield had a significant positive genotypic correlation with three antioxidant traits (ascorbic acid, Fe, and Mn), two leaf quality traits (protein and fiber), and three agronomic traits (plant height, leaves plant^{-1}, and stem base diameter). Fiber content, leaves plant^{-1}, plant height, and stem base diameter had high positive direct effects and Fe, Mn, and carotenoid exhibited moderate positive direct effects on foliage yield. Based on genetic interrelationships and path coefficient values, direct selection for antioxidants (Fe and Mn), fiber content, leaves plant^{-1}, plant height, and stem base diameter would significantly improve the foliage yield of vegetable amaranth. Selection based on protein content concomitantly required considering the Fe and Ca contents of the genotypes.

Key words: Ascorbic acid, beta-carotene, fiber, genetic advance, heritability, protein

1. Introduction

In the study of the U.S. National Academy of Sciences entitled "Underexploited Tropical Plants with Promising Economic Value", performed in 1975, amaranth was selected from among 36 of the world's most promising crops and identified as a crop of major potential (National Academy of Sciences, 1985). Among 60 species, vegetable amaranth (*Amaranthus tricolor*) is now a very popular vegetable in many Asian and African countries. It contains a high amount of protein with nutritionally critical amino acids, lysine and methionine, in addition to dietary fiber and high amounts of antioxidant compounds including vitamin C, carotenoids, and phenolic compounds such as flavonoids and dietary minerals (Malathy et al., 2012; Venskutonis and Kraujalis, 2013). The nutritional composition of both grain and vegetable amaranth has been investigated by many researchers (Teutonico and Knorr, 1985; Bressani, 1990; Shukla et al., 2006a, 2006b; Venskutonis and Kraujalis, 2013). *A. tricolor* has been rated equal or superior in taste to spinach and is considerably higher in carotenoids (90–200 mg kg^{-1}), protein (14%–30% on dry weight basis), and ascorbic acid (about 28 mg 100 g^{-1}) (Wu-Leung et al., 1968; Makus, 1990; Prakash and Pal, 1991; Venskutonis and Kraujalis, 2013). Antioxidants like carotenoids, ascorbic acid, and Fe, Mn, and Zn contents in vegetable amaranth are considerably higher than in many leafy vegetables (Sokkanha and Tiratanakul, 2006; Ali et al., 2009; Gupta and Prakash, 2009; Olayinka et al., 2012). Some metalloenzymes like catalase (Fe) and super oxide dismutase (Mn and Zn) require Fe, Mn, and Zn minerals for their antioxidant activity. Generation of oxygen radicals, such as super oxide radical (O_2), hydroxyl

* Correspondence: umakanta@bsmrau.edu.bd

radical (OH), and nonfree radical species such as H_2O_2 and singlet oxygen ($1O_2$), is associated with cellular and metabolic injury, accelerated aging, cancer, cardiovascular diseases, neurodegenerative diseases, and inflammation (Routray et al., 2012). Antioxidants neutralize or remove these products and help to protect against many diseases, including cancer, and prevent aging (Dasgupta and De, 2007). Amaranth has a great amount of genetic variability and phenotypic plasticity. It is also extremely adaptable to harsh environmental conditions, including high temperature and drought, and resistant against major diseases (Shukla et al., 2006a). Therefore, it can successfully be grown under varied soil and agroclimatic conditions (Katiyar et al., 2000; Shukla and Singh, 2000). Although vegetable amaranth has good nutritional quality and antioxidant properties, very little attention has been given to genetic improvement of this underutilized crop (Shukla et al. 2003, 2006a). A large number of studies are available on genetic variability and interrelationships among various traits such as growth, nutrient contents, and antioxidants in many other crops (Sukhchain and Saini, 1997; Lopez et al., 1998; Finne et al., 2000). However, reports on vegetable amaranth are rare (Shukla et al., 2010). Improvement of foliage yield of vegetable amaranth with high antioxidant, leaf quality, and agronomic traits through the knowledge of genetic parameters, associations among various antioxidant, and quality and agronomic traits along with direct and indirect influences of these component traits on yield has so far been lacking. Therefore, the objectives of the present investigation were to determine the genetic variability and genetic association among different antioxidant, quality, and agronomic traits on yield; determine the contribution of the component traits towards yield potential; and identify appropriate selection parameters for the improvement of vegetable amaranth for utilization in future breeding programs.

2. Materials and methods

2.1. Plant materials, site, and cultural practices

Germplasm accessions of vegetable amaranth (*Amaranthus tricolor*) collected from different ecogeographical regions of Bangladesh were used in this investigation. Twenty-two distinct and promising genotypes of vegetable amaranth were grown in 2011 and 2012 consecutively in a randomized block design with three replications at the experimental field of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh. Weeding and hoeing was done at 7-day intervals. Day temperature during the experimental period ranged from 25 to 38 °C. Irrigation was provided at intervals of 5–7 days. For foliage yield plants were cut at the base of the stem (base of ground level). The plot size for each treatment was 2 m^2 for foliage yield and 1 m^2 for antioxidant, quality, and agronomic traits. Spacing was maintained with row-to-row and plant-to-plant distances of 20 cm and 5 cm, respectively. Recommended fertilizer and compost doses and appropriate cultural practices were maintained.

2.2. Data collection on plant traits

Data were collected at 30 days after sowing of the seeds for both years. Data were recorded from 10 randomly selected plants from each replication for plant height (cm), leaves $plant^{-1}$, and stem base diameter (cm). Foliage yields were harvested on a whole-plot basis. Beside this, five antioxidant traits, beta-carotene (mg g^{-1}), ascorbic acid (mg 100 g^{-1}), iron (mg kg^{-1}), zinc (mg kg^{-1}), and Mn (mg kg^{-1}), and three leaf quality traits, protein (mg 100 g^{-1}), fiber (%), and Ca (g 100 g^{-1}), were estimated.

2.3. Extraction and estimation of antioxidant and quality traits

Protein was estimated following the method of Lowry et al. (1951).

2.3.1. Carotenoid

The extraction and estimation of carotenoid was done following the protocol previously described by Jensen (1978).

To carry out the extraction process, 500 mg of fresh leaf sample was ground in 10 mL of 80% acetone and centrifuged at 10,000 rpm for 3–4 min. The supernatant was taken and the volume was made up to 20 mL in a volumetric flask. The absorbance values were taken at 510 nm and 480 nm.

The carotenoid was calculated by the following formula:

Amount of carotenoid = 7.6(Abs. at 480) - 1.49(Abs. at 510) × final volume / (1000 × fresh weight of leaf taken).

2.3.2. Fiber

Fiber content was estimated using the method proposed by Watson (1994). Dried leaf samples of 500 mg were extracted by boiling for 30 min in 50 mL of 5% H_2SO_4 and 75 mL of distilled water. The sample was filtered through linen cloth after 1 h with the addition of some cold distilled water and residue was washed twice with distilled water. In the residue, 50 mL of 5% KOH was added and the volume was made up to the original volume. Furthermore, the solution was boiled for 30 min, allowed to stand for some time after adding a little cold distilled water, and filtered through linen cloth. The residue was again washed with hot distilled water followed by a mixture of dilute HCl ($HCl:H_2O$ at a ratio of 1:2) and 5 mL of ethyl alcohol. The residue was finally dried in a crucible at 80–100 °C, and dried weight was measured and represented as a percentage of initial material taken.

2.3.3. Ascorbic acid

Ascorbic acid was analyzed by the method given by Glick (1954). To extract the sample, 5 g of fresh leaves was ground with 5% H_3PO_3 and 10% acetic acid (5%

metaphosphoric acid (H_3PO_3) and 10% acetic acid was prepared by dissolving 50 g of H_3PO_3 in 800 mL of distilled water + 100 mL of glacial acetic acid and the volume was made up to 1 L with distilled water) for 1–3 min. The amount of extracting fluid was taken such that it should yield 1–10 µg of ascorbic acid/mL. In the solution, 1–2 drops of bromine were added and stirred until the solution became yellow. The excess bromine was decanted into a bubbler and air was passed until the bromine's color disappeared. The oxidized bromine solution was placed in 2 matched tubes. In the first tube, 1 mL of 2,4-DNP thio urea reagent (2,4-dinitrophenyl hydrazine-thio urea reagent) was prepared by dissolving 2 g of 2,4-DNP in 100 mL of 9 N H_2SO_4 and 4 g of thio urea was added and dissolved in this solution. The filtered solution was added and the tube was placed in water at 37 °C for 3 h, and 5 mL of 85% H_2SO_4 (100 mL of distilled water + 900 mL of conc. H_2SO_4; sp. gr. 1.84) was added dropwise by the burette in the tube, placed in a beaker of ice water. In the second tube, 1 mL of 2,4-DNP thio urea reagent was only added to prepare a blank solution. After 30 min, the absorbance reading of the sample was taken at a wavelength of 540 nm by spectrophotometer. The blank solution was used for setting the zero transmittance of the spectrophotometer. The standard solution was prepared by dissolving 100 mg of ascorbic acid of the highest purity in 100 mL of 5% H_3PO_3 + 10% acetic acid. The solution was oxidized with bromine water as above and 10 mL of this dehydrated ascorbic acid was pipetted into a volumetric flask of 500 mL, and the solution was made up to 500 mL with the 85% H_2SO_4 solution. Solutions of different dilutions were prepared by pipetting 5, 10, 20, 30, 40, 50, and 60 mL of the above solution into volumetric flasks of 100 mL and volumes were made up to 100 mL each by addition of 85% H_2SO_4. The solution of each flask was taken separately and the procedure was followed as discussed above for the sample. The calibration curve was prepared by plotting absorbance values against the concentration of ascorbic acid (in µg).

The amount of ascorbic acid (mg/100 gm) was calculated as follows:

Ascorbic acid content (mg/100 gm) = (µg from curve) / 1000 × (mL of extract taken) / 4 × 100 / (sample wt. in g).

For determination of mineral nutrients and antioxidant composition, the leaves were first oven-dried and then digested in a 1:4 mixture of $HClO_3$ and HNO_3. Calcium was determined by flame photometry and iron, zinc, and manganese were determined using an atomic absorption spectrophotometer (PerkinElmer 5100) (Vogel, 1962; AOAC, 1990).

2.4. Statistical analysis

The raw data were compiled by taking the means of all the plants taken for each treatment and replication for different traits. The mean data of both years were averaged and the average mean values were statistically and biometrically analyzed. Analysis of variance was done according to Panse and Sukhatme (1978) for each character. Genotypic and phenotypic variances, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in the broad sense (h^2_b), and expected genetic advance (GA %) were estimated according to Johnson et al. (1955a). Genotypic and phenotypic correlation coefficients were analyzed following the formula of Hayes et al. (1955). Path coefficient analysis was calculated according to the formula given by Dewey and Lu (1959).

3. Results

3.1. Variability studies

The mean values, range, genotypic variance (Vg) and phenotypic variance (V_p), GCV and PCV, h^2_b, GA %, and genetic advance in percent of mean (GAPM) for 12 antioxidant, quality, and agronomic traits of 22 vegetable amaranth genotypes are presented in Table 1.

In the present investigation, the mean and range for ascorbic acid content; Fe, Zn, and Mn content; plant height; leaves plant^{-1}, stem base diameter; fiber content; and foliage yield were observed to be pronounced. The genotypic variance was the highest for Fe content (161,495.25), followed by Zn (38,075.25), ascorbic acid (1015.25), and Mn content (725.28). Plant height, leaves plant^{-1}, stem base diameter, and foliage yield also exhibited moderate genotypic variances. On the other hand, the lowest genotypic variance was found for protein content (0.15), followed by Ca content (0.19). The phenotypic variances for all the traits were a little higher but close to the genotypic variances. Genotypic coefficient of variation values ranged from 11.23% (fiber content) to 56.69% (carotenoid content). The PCV values showed similar trends as GCV values and ranged from 11.67% (fiber content) to 58.61% (carotenoid content). The values of PCV were a little higher but close to the corresponding GCV values for all the traits. High values of coefficients of variation were observed for carotenoid, foliage yield, leaves plant^{-1}, Fe content, stem base diameter, plant height, protein, Ca content, ascorbic acid content, and Zn and Mn content. The heritability estimates were high for all the traits and ranged from 83.33% for protein content to 99.96% for Fe content. The expected GAPM varied from 22.27 for fiber content to 112.94% for carotenoid content. The highest expected genetic advance was exhibited for carotenoid (112.94%), followed by foliage yield (109.96%), leaves plant^{-1} (80.45%), Fe content (69.99%), stem base diameter (67.02%), plant height (65.52%), protein (60.69%), leaf area (61.84%), ascorbic acid content (53.98%), Ca content (53.38%), Zn content (51.34%), and Mn content (43.32%).

Table 1. Genetic parameters for antioxidant, quality, and agronomic traits in 22 vegetable amaranth genotypes.

Character	Mean	Range	V_p	V_g	PCV	GCV	h^2_b (%)	GA (5%)	% GAPM
Carotenoid (mg g^{-1})	0.95	0.65–1.50	0.31	0.29	58.61	56.69	93.55	1.07	112.94
Ascorbic acid (mg 100 g^{-1})	121.3001	71.50–172.55	1020.20	1015.25	26.33	26.27	99.51	65.48	53.98
Fe (mg kg^{-1})	1182.56	595.72–2355.45	161,555.42	161,495.25	33.99	33.98	99.96	827.69	69.99
Zn (mg kg^{-1})	782.65	460.50–1209.40	38,098.35	38,075.25	24.94	24.93	99.94	401.84	51.34
Mn (mg kg^{-1})	125.50	71.80–165.55	755.20	725.28	21.90	21.46	96.04	54.37	43.32
Protein (mg 100 g^{-1})	1.20	1.00–1.35	0.18	0.15	35.36	32.27	83.33	0.73	60.69
Fiber (%)	8.35	6.22–10.56	0.95	0.88	11.67	11.23	92.63	1.86	22.27
Ca (g 100 g^{-1})	1.60	0.65–2.50	0.21	0.19	28.64	27.24	90.48	0.85	53.38
Plant height (cm)	22.95	11.50–45.25	55.75	54.50	32.53	32.17	97.76	15.04	65.52
Leaves $plant^{-1}$	10.25	5.05–20.55	18.25	17.10	41.68	40.34	93.70	8.25	80.45
Stem base diameter (cm)	7.50	3.7–14.50	6.56	6.25	34.15	33.33	95.27	5.03	67.02
Foliage yield $plot^{-1}$ (kg)	4.50	3.65–7.15	5.81	5.79	53.56	53.47	99.66	4.95	109.96

V_p = Phenotypic variance, V_g = genotypic variance, PCV = phenotypic coefficient of variation, GCV = genotypic coefficient of variation, h^2_b = heritability in broad sense, GA = genetic advance, GAPM = genetic advance in percent of mean, Fe = iron, Zn = zinc, Mn = manganese, Ca = calcium.

3.2. Correlation studies

The phenotypic and genotypic correlations among various antioxidant, quality, and agronomic traits are presented in Table 2. The genotypic correlation coefficients were a little higher but very close to the corresponding phenotypic correlation coefficient values for all the traits. The genotypic correlation analysis presented in Table 2 showed some interesting results. The investigation revealed that foliage yield had a significant positive correlation with 3 antioxidant traits, ascorbic acid (0.338*), iron (0.318*), and manganese (0.319*). Foliage yield also exhibited a significant positive correlation with 2 leaf quality traits, protein (0.456**) and fiber (0.672**), and 3 agronomic traits, plant height (0.504**), leaves $plant^{-1}$ (0.514**), and stem base diameter (0.520**). Stem base diameter exhibited insignificant correlation with all antioxidant and quality traits except Ca (–0.555**) and a significant positive association with plant height (0.432*). Similarly, insignificant associations were found between leaves $plant^{-1}$ and all antioxidant traits except carotenoid (0.342*), while leaves $plant^{-1}$ had a significant negative association with Ca (–0.400**) and a significant positive association with plant height (0.564**). Plant height was significantly and negatively associated with 4 antioxidant and quality traits, ascorbic acid (–0.378*), Zn (–0.335*), Mn (–0.395*), and Ca (–0.327*), while its positive association with antioxidant carotenoid (0.375*) was significant. Calcium showed a significant positive association with antioxidant Zn (0.305*) and a significant negative association with protein content (–0.432*).

3.3. Path coefficient studies

Path coefficient analysis was carried out using genotypic correlation coefficients among twelve antioxidant, quality, and yield-contributing agronomic traits to estimate the direct and indirect effect on foliage yield (Table 3). The fiber content (0.621), leaves $plant^{-1}$ (0.537), plant height (0.518), and stem base diameter (0.333) had high positive direct effects and Fe (0.290), Mn (0.260), and carotenoid (0.141) exhibited moderate positive direct effects on foliage yield. Calcium (–0.300) showed a moderate negative direct effect and ascorbic acid (–0.038) had a negligible negative direct effect on foliage yield, while Zn (0.083) and protein (0.058) exhibited a negligible positive direct effect on foliage yield. Carotenoid had a moderate positive direct effect and insignificant positive correlation. Ascorbic acid had a negligible negative direct effect but a significant positive correlation. Fe exhibited a moderate positive direct effect and a significant positive correlation. Zn exerted a negligible positive direct effect and an insignificant correlation. Mn showed a moderate positive direct effect and a significant correlation on foliage yield. Protein exhibited a negligible positive direct effect but a significant positive correlation. Fiber content had a high positive direct effect and a significant correlation. Calcium exhibited a high negative direct effect with an insignificant negative association. Leaves $plant^{-1}$, plant height, and

Table 2. Genotypic and phenotypic correlation coefficient (r_g and r_p) for antioxidant, quality, and agronomic traits in 22 vegetable amaranth genotypes.

Traits		Ascorbic acid (mg 100 g^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Protein (mg 100 g^{-1})	Fiber (%)	Ca (g 100 g^{-1})	Plant height (cm)	Leaves plant^{-1}	Stem base diameter (cm)	Foliage yield plot^{-1} (kg)
Carotenoid (mg g^{-1})	r_g	0.069	0.135	0.126	0.187	−0.218	−0.057	0.121	0.375*	0.342*	0115	0.132
	r_p	0.067	0.132	0.125	0.185	−0.217	−0.055	0.121	0.372*	0.340*	0.114	0.130
Ascorbic acid (mg 100 g^{-1})	r_g		0.292	0.122	0.131	0.173	0.013	−0.139	−0.378*	−0.118	0.140	0.338*
	r_p		0.291	0.120	0.129	0.172	0.012	−0.137	−0.376*	−0.116	0.141	0.336*
Fe (mg kg^{-1})	r_g			0.177	0.112	0.112	0.018	0.152	−0.175	−0.052	−0.035	0.318*
	r_p			0.176	0.110	0.110	0.017	0.150	−0.172	−0.051	−0.035	0.317*
Zn (mg kg^{-1})	r_g				0.278	0.133	0.175	0.305*	−0.335*	−0.257	−0.199	0.096
	r_p				0.277	0.130	0.174	0.307*	−0.334*	−0.256	−0.198	0.095
Mn (mg kg^{-1})	r_g					−0.165	0.195	0.155	−0.395*	−0.128	−0.195	0.319*
	r_p					−0.164	0.194	0.154	−0.393*	−0.127	−0.194	0.318*
Protein (mg 100 g^{-1})	r_g						0.027	−0.432*	−0.275	0.181	0.122	0.456**
	r_p						0.025	−0.431*	−0.273	0.180	0.120	0.453**
Fiber (%)	r_g							−0.012	−0.119	0.158	−0.292	0.672**
	r_p							−0.012	−0.118	0.157	−0.291	0.670**
Ca (g 100 g^{-1})	r_g								−0.327*	−0.400**	−0.555**	−0.141
	r_p								−0.326*	−0.398**	−0.554**	−0.140
Plant height (cm)	r_g									0.564**	0.432*	0.504**
	r_p									0.563**	0.431*	0.502**
Leaves plant^{-1}	r_g										0.235	0.514**
	r_p										0.234	0.512**
Stem base diameter (cm)	r_g											0.520**
	r_p											0.519**

*Significant at 5% and **significant at 1%.

Table 3. Partitioning of genotypic correlation into direct (bold font) and indirect effect for antioxidant, quality, and agronomic traits in 22 vegetable amaranth genotypes.

Traits	Carotenoid (mg g^{-1})	Ascorbic acid (mg 100 g^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Protein (mg 100 g^{-1})	Fiber (%)	Ca (g 100 g^{-1})	Plant height (cm)	Leaves $plant^{-1}$	Stem base diameter (cm)	Genotypic correlation with foliage yield $plot^{-1}$ (kg)
Carotenoid (mg g^{-1})	**0.141**	–0.022	0.028	–0.003	0.021	0.001	0.026	–0.028	–0.126	0.102	–0.008	0.132
Ascorbic acid (mg 100 g^{-1})	0.008	**–0.038**	0.152	0.001	0.032	0.000	0.024	0.040	0.130	–0.006	–0.005	0.338*
Fe (mg kg^{-1})	0.021	–0.010	**0.290**	0.001	0.003	–0.007	0.009	–0.019	0.039	–0.020	0.011	0.318*
Zn (mg kg^{-1})	–0.035	–0.004	0.031	**0.083**	0.064	0.001	0.008	–0.094	0.091	–0.074	0.025	0.096
Mn (mg kg^{-1})	0.012	–0.005	0.003	0.002	**0.260**	0.004	–0.015	–0.054	0.127	–0.041	0.026	0.319*
Protein (mg 100 g^{-1})	–0.075	0.028	0.168	0.055	–0.037	**0.058**	0.002	0.168	0.079	0.008	0.002	0.456**
Fiber (%)	–0.034	0.000	0.004	0.001	0.037	0.000	**0.621**	0.004	0.029	0.016	–0.006	0.672**
Ca (g 100 g^{-1})	0.004	0.005	0.012	0.003	0.046	0.050	–0.003	**–0.300**	0.103	–0.131	0.070	–0.141
Plant height (cm)	–0.156	–0.107	0.123	0.103	0.102	–0.006	–0.137	–0.179	**0.518**	0.181	0.062	0.504**
Leaves $plant^{-1}$	0.044	0.001	–0.012	–0.002	–0.032	–0.002	0.063	0.120	–0.175	**0.537**	–0.028	0.514**
Stem base diameter (cm)	–0.070	0.102	0.016	0.002	0.050	–0.004	0.033	–0.159	0.149	0.068	**0.333**	0.520**

*Significant at 5% and **significant at 1%.

stem base diameter exerted high direct effects along with significant correlations on foliage yield. Furthermore, ascorbic acid exhibited a negligible negative direct effect, but its indirect positive direct effect via Fe and plant height made a significant positive association with foliage yield. Protein showed a negligible positive direct effect while its indirect positive effect via Fe, Ca, and plant height made the total correlation significant and positive on foliage yield.

4. Discussion

Vegetable amaranth is very popular leafy vegetable in Bangladesh. In Bangladesh and India vegetable amaranth is cultivated during hot summer months when no other green vegetables are available in the market (Singh and Whitehead, 1996). Vitamin and mineral antioxidants of vegetable amaranth remove free radicals from the body and help to fight against infections and other conditions including cancer, coronary artery diseases, muscular degeneration, and serious eye diseases (Dasgupta and De, 2007). On the other hand, they treat vitamin and mineral deficiency in the human diet. The contents of carotenoid, ascorbic acid, Fe, Zn, and Mn are the most important antioxidant traits of vegetable amaranth (Sokkanha and Tiratanakul, 2006; Ali et al., 2009; Gupta and Prakash, 2009; Olayinka et al., 2012; Routray et al., 2012; Venskutonis and Kraujalis, 2013). Calcium is required for growth of bones as well as muscular and neurological functions. In addition to antioxidant capacity, Fe is also important for hemoglobin. Fiber improves the palatability and digestibility of foliage. Amaranth's protein may be a source for vegetarians as well as poor people in the developing world.

The pronounced mean and range for ascorbic acid content, Fe, Zn, Mn, plant height, leaves plant^{-1}, stem base diameter, fiber content, and foliage yield indicated the scope of selection of these traits. Shukla et al. (2006a) also observed similar results for protein, ascorbic acid, fiber, plant height, stem diameter, leaf size, and foliage yield. The results of genotypic variances and genotypic coefficients of variation revealed that more variability was exhibited for Fe, Zn, and Mn content and ascorbic acid, plant height, leaves plant^{-1}, stem base diameter, fiber content, and foliage yield, indicating the greater scope of selection based on these traits. High genotypic variances and coefficients of variation validated that the chances of getting substantial gains under selection are possibly very high for these traits. The phenotypic variances for all the traits were a little higher but close to the genotypic variances, indicating the preponderance of additive gene effects for these traits. The GCV considers the best relative amount of genetic variation and it takes into account the mean value as well as the unit of measurement. The values of PCV were a little higher but close to the corresponding GCV values for all the traits. The small differences between PCV and GCV for all the traits indicated that the variability was predominately due to genotypic differences, i.e. environmental influences. Similar results were also reported by Shukla et al. (2006a), Rastogi et al. (1995), Revanappa and Madalageri (1998), and Bhargava et al. (2003b, 2006). Heritability is a measure of the genetic relationship between parent and progeny and it has been widely used to assess the degree to which a character may be transmitted from parent to offspring. It also indicates the relative importance of heredity and environmental influence in the expression of the traits. Knowledge of heritability is essential since it helps breeders make a decision about to what extent improvement is possible through selection (Robinson et al., 1949). The values of heritability estimates were high for all the traits and ranged from 83.33% for protein content to 99.96% for Fe content. Shukla et al. (2006a) and Revanappa and Madalageri (1998) also observed high heritability values in *Amaranthus*. The high value of heritability for all the traits suggests that all these traits are under genetic control, i.e. less environmental influence. However, it will be relevant to state here that the total genotypic variance is made up of additive genetic variance and nonadditive or nonfixable variance. High heritability alone is not enough to make sufficient improvement through selection generally in advance generations unless accompanied by substantial amounts of genetic advance (Bhargava et al., 2003a). The efficacy of heritability is increased with the estimation of genetic advance, which indicates the degree of gain in a trait obtained under a particular selection pressure. Thus, genetic advance is yet another important selection parameter that aids breeders in selection programs (Shukla et al., 2004). It has been emphasized that without genetic advance, heritability values would not be of practical importance in selection based on phenotypic appearance. Thus, genetic advance should be considered along with heritability in coherent selection breeding programs (Johnson et al., 1955b). Bayer and Becker (2005) obtained comparatively higher phenotypic variance values than genotypic variance for most of the traits in the crop *Vernonia galamensis*, but, in our study, the magnitude of genotypic variance and phenotypic variance was quite close due to low environmental effect ($V_e = V_p - V_g$) and therefore all the traits were under the control of genotypic variance (additive + nonadditive). However, in general, it is considered that if a trait is governed by nonadditive gene action, it may give high heritability but low genetic advance, whereas if the trait is governed by additive gene action, heritability and genetic advance both would be high. The traits that have high heritability along with high expected genetic advance could be substantially considered for making selections as these traits are mainly influenced by the major effects of additive gene action. The expected genetic advance as percent of mean varied from 22.27% for fiber content to 112.947% for carotenoid content. Only fiber content had a major role of nonadditive gene action in the transmission of this trait from parents to offspring. All the traits except fiber content also showed moderate to high coefficients of variation and high heritability values, which indicated a major role of additive gene action in the inheritance of these traits and their amenability for improvement in the population for foliage yield and its component traits. Earlier Shukla et al. (2006a) and Sarker et al. (unpublished data) showed high values of heritability and genetic advance for ascorbic acid, plant height, leaves plant^{-1}, stem diameter, foliage yield, and leaf size, and in another investigation Shukla and Singh (2000) also obtained high values of heritability and genetic advance for foliage yield and leaves plant^{-1}. It was concluded from the present investigation that a considerable amount of variability exists in the experimental material in terms of leaf quality (fiber content), antioxidants (Fe, Zn, and Mn content and ascorbic acid), and foliage yield as well as all contributing agronomic traits. The investigation revealed that vegetable amaranth is rich in Ca, fiber, Fe, Zn, Mn, protein, carotenoid, and ascorbic acid, which could be a good and economical source of antioxidants and nutrients in human diets, especially for vegetarian people in developing countries. However, considering all genetic parameters, selection could be made on the basis of contents of Fe, Zn, manganese, and ascorbic acid and plant height, leaves plant^{-1}, diameter of stem base, fiber content, and foliage yield plot^{-1}, which seemed to be effective for the improvement of vegetable amaranth.

The genotypic correlation coefficients were a little higher but very close to the corresponding phenotypic correlation coefficient values for all the antioxidant, quality, and agronomic traits, which indicated the existence of the additive nature of gene action for these traits. The higher magnitude of genotypic correlation than respective phenotypic correlations between various characters in amaranth were also reported earlier (Shukla and Singh, 2002; Shukla et al., 2010). The genotypic correlation analysis presented in Table 2 showed some interesting results. The investigation revealed that foliage yield had a significant positive correlation with three antioxidant traits, ascorbic acid (0.338*), Fe (0.318*), and Mn (0.319*). There exist strong associations among foliage yield and these three antioxidant traits; therefore, selection based on ascorbic acid, iron, and manganese content would significantly improve the foliage yield of vegetable amaranth. Foliage yield also exhibited significant positive correlations with two leaf quality traits, protein (0.456**) and fiber (0.672**), and three agronomic traits, plant height (0.504**), leaves plant^{-1} (0.514**), and stem base diameter (0.520**), indicating that foliage yield might be improved by selection based on high protein, high fiber, tall plants, more leaves per plant, and greater diameter of stem base. Shukla et al. (2010) observed a similar significant positive association in vegetable amaranth for foliage yield with plant height, diameter of stem base, and fiber content. Stem base diameter exhibited insignificant correlations with all antioxidant and quality traits except Ca (−0.555**) and a significant positive association with plant height (0.432*). This means that thicker plants were also taller and contained less calcium than thinner ones. Similarly, insignificant associations were found between leaves plant^{-1} and all antioxidant traits except carotenoid (0.342*), while leaves plant^{-1} had a significant negative association with Ca (−0.400**) and a significant positive association with plant height (0.564**), which indicated that tall plants have more leaves but less calcium than shorter plants. Insignificant associations both of leaves plant^{-1} and stem base diameter with all antioxidant and quality traits indicated that there was a great scope to select high antioxidant and high leaf quality genotypes without compromising the stem base thickness or reduction of leaves plant^{-1}. Plant height was significantly and negatively associated with four antioxidant and quality traits, i.e. ascorbic acid (−0.378*), Zn (−0.335*), Mn (−0.395*), and Ca (−0.327*), while its positive association with antioxidant carotenoid (0.375*) was found significant. This indicated that tall plants had less ascorbic acid, Zn, Mn, and Ca but high carotenoid compared to small plants, i.e. tender plants ensured high antioxidant and leaf quality. Calcium showed a significant positive association with antioxidant Zn (0.305*) and a significant negative association with protein content (−0.432*), indicating that with the increase of calcium content in vegetable amaranth Zn content increased sharply but protein content gradually declined. The insignificant interrelationship among the rest of the antioxidant and quality traits, i.e. carotenoid, ascorbic acid, Fe, Mn, and protein, indicated that within this group the increase of any one of the antioxidant and quality traits might be possible without compromising any of the rest of the four traits, including foliage yield. Therefore, concomitant selection based on high carotenoid, ascorbic acid, Fe, Mn, and protein significantly improves the foliage yield of vegetable amaranth. A similar trend was observed by earlier studies in *A. tricolor* (Sarker, unpublished data; Shukla et al., 2006b) and the vegetable *Chenopodium* (Bhargava et al., unpublished data). Path coefficient analysis was done by partitioning the correlation coefficient of foliage yield into direct and indirect effects of antioxidant, quality, and yield-contributing agronomic components. Path coefficient analysis was carried out using genotypic correlation coefficients among twelve antioxidant, quality, and yield-contributing agronomic traits to estimate the direct and indirect effect on foliage yield (Table 3). The fiber content (0.621), leaves plant^{-1} (0.537), plant height (0.518), and stem base diameter (0.333) had high positive direct effects and Fe (0.290), Mn (0.260), and carotenoid (0.141) exhibited moderate positive direct effects on foliage yield. Shukla et al. (2010) found high positive direct effects for carotenoid, protein, plant height, stem diameter, leaves plant^{-1}, and fiber content in vegetable amaranth. Calcium (–0.300) showed a moderate negative direct effect and ascorbic acid (–0.038) had a negligible negative direct effect on foliage yield, while Zn (0.083) and protein (0.058) exhibited negligible positive direct effects on foliage yield. Shukla et al. (2010) also found similar results for protein content in the same crop. It was interesting that path coefficient analysis results confirmed the similarity of the correlation coefficient analysis results, except for ascorbic acid and protein, and these traits showed high to moderate positive direct effects with significant positive correlations. Ascorbic acid had a negligible negative direct effect but significant positive correlation. Fe exhibited a moderate positive direct effect and significant positive correlation. Zn exerted a negligible positive direct effect and insignificant correlation. Mn showed a moderate positive direct effect and significant correlation on foliage yield. Therefore, direct selection based on two antioxidant traits (Fe and Mn) would be effective for the improvement of foliage yield of vegetable amaranth. Protein exhibited a negligible positive direct effect but significant positive correlation. Fiber content had a high positive direct effect and significant correlation. Therefore, selection of the quality trait of fiber content significantly improved the foliage yield of vegetable amaranth. Calcium exhibited a high negative direct effect with insignificant negative association. Leaves plant^{-1}, plant height, and stem base diameter exerted high direct effects along with significant correlations on foliage yield, indicating the best parameters to be selected for improving the foliage yield of vegetable amaranth. Shukla et al. (2010) observed similar findings for plant height, fiber, and carotenoid content in vegetable amaranth. On the other hand, ascorbic acid exhibited a negligible negative direct effect but its indirect positive direct effect via Fe and plant height made a significant positive association with foliage yield. Protein showed negligible positive direct effects while its indirect positive effects via Fe and Ca made the total correlation significant and positive on foliage yield. Selection based on protein content concomitantly requires considering Fe and Ca content of the genotypes. The investigated germplasms exhibited huge variability in respect of genetic parameters, genetic interrelationships, and path coefficient values. Breeders can use this variability in future breeding programs to develop high yield, high quality, and antioxidant-rich vegetable amaranth varieties. Direct selection on the basis of 2 antioxidant traits (Fe and Mn), 1 quality trait fiber content, and 3 agronomic traits (leaves plant^{-1}, plant height, and stem base diameter) would significantly improve the foliage yield of vegetable amaranth.

Acknowledgments
The authors are thankful to the National Science and Technology authority of the Ministry of Science and Technology of Bangladesh for providing partial financial support by special allocation to carry out the present investigation and the RMC authority of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh, for their partial financial support.

References

Ali MB, Khandaker L, Oba S (2009). Comparative study on functional components, antioxidant activity and color parameters of selected colored leafy vegetables as affected by photoperiods. J Food Agric Environ 7: 392-398.

AOAC (1990). Official Methods of Analysis. 14th ed. Washington, DC, USA: Association of Official Analytical Chemists.

Bayer T, Becker HC (2005). Genetic variability and interrelationship of traits in the industrial oil crop *Vernonia galamensis*. Euphytica 142: 119-129.

Bhargava A, Shukla S, Dixi BS, Bannerji R, Ohri D (2006). Variability and genotype *x* cutting interactions for different nutritional components in *C. album* L. Horticultural Sci 33: 29-38.

Bhargava A, Shukla S, Katiyar RS, Ohri D (2003a). Selection parameters for genetic improvement in *Chenopodium* grain on sodic soil. J Applied Hort 5: 45-48.

Bhargava A, Shukla S, Ohri D (2003b). Genetic variability and heritability of selected traits during different cuttings of vegetable *Chenopodium*. Ind J Genet Pl Breed 63: 359-360.

Bressani R (1990). Grain amaranth: its chemical composition and nutritive value. In: Proceedings of Fourth Amaranth Symposium. St. Paul, MN, USA: Minnesota Agricultural University, p. 22.

Dewey DR, Lu KH (1959). A correlation and path coefficient analysis of components of crested wheat grass seed production. Agron J 51: 515-518.

Dasgupta N, De B (2007). Antioxidant activity of some leafy vegetables of India: a comparative study. Food Chem 101: 471-474.

Finne MA, Rognli OA, Schjelderup I (2000). Genetic variation in a Norwegian germplasm collection of white clover (*Trifolium repens* L.). Euphytica 112: 57-68.

Glick D (1954). Methods of Biochemical Analysis. New York, NY, USA: International Science Publishers.

Gupta S, Prakash J (2009). Studies on Indian green leafy vegetables for their antioxidant activity. Pl Foods Hum Nutrition 64: 39-45.

Hayes HK, Immer FR, Smith DC (1955). Methods of Plant Breeding. 2nd ed. New York, NY, USA: McGraw-Hill.

Jensen A (1978). Chlorophylls and carotenoids. In: Hellebust JA, Craigie JS, editors. Handbook of Physiological Methods: Physiological and Biochemical Methods. Cambridge, UK: Cambridge University Press, pp. 5-70.

Johnson HW, Robinson HF, Comstock RE (1955a). Genotypic and phenotypic correlation in soybean and their implication in selection. Agron J 47: 477-485.

Johnson HW, Robinson HF, Comstock RE (1955b). Estimates of genetic and environmental variability in soybean. Agron J 47: 314-318.

Katiyar RS, Shukla S, Rai S (2000). Varietal performance of grain amaranth (*A. hypochondriacus*) on sodic soil. P Natl A Sci India B 70: 185-187.

Lopez AFS, Firpo IT, Garcia SM, Cointry EL (1998). Estimation of genetic parameters for yield traits in globe artichoke (*Cynara scolymus* L.). Euphytica 103: 61-66.

Lowry OH, Rosebrough NJ, Farr AL and Randall RJ (1951). Protein measurement with the Folin–phenol reagent. J Biol Chem 193: 265-275.

Makus DJ (1990). Composition and nutritive value of vegetable amaranth as affected by stage of growth, environment and method of preparation. In: Proceedings of Fourth Amaranth Symposium. St. Paul, MN, USA: Minnesota Agricultural University, pp. 35-46.

Malathy PD, Suraweera D, Daundasekara WAM, Nilanthi WDGP, Wahundeniya KB (2012). Yield keeping quality, antioxidant content and some nutritional aspects of selected accessions of *Amaranthus tricolor.* International Journal of Bioscience, Biochemistry and Bioinformatics 2: 324-328.

Olayinka OA, Kareem I, Ariyo SO, Oyebanj A (2012). Antioxidant contents (vitamin c) of raw and blanched different fresh vegetable samples. Food and Nutrition Science 3: 8-21.

Panse VG, Sukhatme PV (1978). Statistical Methods for Agricultural Workers. New Delhi, India: ICAR.

Prakash D, Pal M (1991). Nutritional and anti-nutritional composition of vegetable and grain amaranth leaves. J Sci Food Agric 57: 573-583.

Rastogi KB, Korla BN, Joshi AK, Thakur MC (1995). Variability studies in Chinese cabbage (*Brassica chinensis* L.). Adv Hort Forestry 4: 101-107.

Revanappa P, Madalageri BB (1998). Genetic variability studies regarding quantitative traits in *Amaranthus*. Karnataka J Agric Sci 11: 139-142.

Robinson HF, Comstock RE, Harvey PH (1949). Estimates of heritability and the degree of dominance in corn. Agron J 41: 353-359.

Routray R, Kar M, Sahu RK (2012). Evaluation of antioxidant potential in selected leafy vegetables of Odisha, India. International Journal of Pharmacy and Pharmaceutical Sciences 5: 232-235.

Shukla S, Bhargava A, Chatterjee A, Pandey AC, Rastogi A, Kumar A (2010). Genetic interrelationship among nutritional and quantitative traits in the vegetable amaranth. Crop Breed Appl Biot 10: 16-22.

Shukla S, Bhargava A, Chatterjee A, Singh SP (2004). Estimates of genetic parameters to determine variability for foliage yield and its different quantitative and qualitative traits in vegetable amaranth (*A. tricolor*). J Genet Breed 58: 169-176.

Shukla S, Bhargava A, Chatterjee A, Srivastava A, Singh SP (2006a). Genotypic variability in vegetable amaranth (*A. tricolor*) for foliage yield and its contributing traits over successive cuttings and years. Euphytica 151: 103-110.

Shukla S, Bhargava A, Chatterjee A, Srivastava J, Singh N, Singh SP (2006b). Mineral profile and variability in vegetable amaranth (*Amaranthus tricolor*). Pl Food for Hum Nutr 61: 23-28.

Shukla S, Pandey V, Pachauri G, Dixit BS, Banerji R, Singh SP (2003). Nutritional contents of different foliage cuttings of vegetable amaranth. Pl Food Hum Nutr 58: 1-8.

Shukla S, Singh SP (2000). Studies on genetic parameters in vegetable amaranth. Indian J Genet Pl Breed 54: 133-135.

Shukla S, Singh SP (2002). Varietal performance and foliage yield in vegetable amaranth. South Indian Horticulture 50: 241-244.

Singh BP, Whitehead WF (1996). Management methods for producing vegetable amaranth. In: Janick J, editor. Progress in New Crops. Arlington, VA, USA: ASHS Press, pp. 511-515.

Sokkanha S, Tiratanakul V (2006). Effect of plastic vinyl cover and shading on the antioxidants level of selected indigenous vegetables. In: 24 Regional Training Courses in Vegetable Production, Research and Extension. Bangkok, Thailand: Asian Vegetable Research and Development Centre.

Sukhchain SD, Saini GS (1997). Inter-relationships among cane yield and commercial cane sugar and their component traits in autumn plant crop of sugarcane. Euphytica 95: 109-113.

Teutonico RA, Knorr D (1985). Nondestructive method for oxalate determination of cultured *A. tricolor* cells. J Agric Food Chem 33: 60-64.

Venskutonis PR, Kraujalis P (2013). Nutritional components of amaranth seeds and vegetables: a review on composition, properties, and uses. Compr Rev Food Sci F 12: 381-412.

Vogel AT (1962). Quantitative Inorganic Analysis. London, UK: Longmans.

Watson CA (1994) Official and Standardized Methods of Analysis. 3rd ed. Cambridge, UK: Royal Society of Chemistry.

Wu-Leung F, Busson C, Jardin C (1968). Food Composition Table for Use in Africa. Rome, Italy: FAO.