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ANTIOXIDANT LEAF PIGMENTS AND VARIABILITY IN VEGETABLE AMARANTH

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Twenty-three vegetable amaranth genotypes were evaluated for variability, interrelationships among antioxidant leaf pigments and foliage yield. Five genotypes found to be a rich source of antioxidant leaf pigments and might be directly selected as antioxidant leaf pigments enriched high yielding varieties. Two genotypes had high content of antioxidant leaf pigments with low yield might be used as donor parents for antioxidant leaf pigments genes to develop transgressive segregant or pigment enriched transgenic vegetable amaranth varieties. The insignificant negative genotypic correlation was observed between total carotene versus all antioxidant leaf pigments, ascorbic acid versus all antioxidant leaf pigments and foliage yield versus rest of all traits. Improvement of vegetable amaranth regarding carotene and ascorbic acid might be possible without compromising yield loss. On the other hand, most of the interrelationships among antioxidant leaf pigments traits indicated that improving of one antioxidant leaf pigment significantly improved the other antioxidant leaf pigments.

Key words: Betalains, carotene, ascorbic acid, chlorophyll *a* and *b*, correlation

Abbreviations

CD = Critical difference, GA =Genetic advance, GAMP = Genetic advance in percent of mean, GCV = Genotypic coefficient of variation, h^2_b = Heritability in broad sense, $\sigma^2 g$

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= genotypic variance, σ^2p = phenotypic variance, PCV = phenotypic coefficient of variation, RCBD = Randomized complete block design

INTRODUCTION

Vegetable amaranth serves as an alternative source of nutrition for people in developing countries since it is a rich and inexpensive source of mineral, vitamins, protein, dietary fiber, flavonoids, polyphenols, antioxidant leaf pigments like betalains, carotene, and chlorophyll (ALI *et al*., 2009; VENSKUTONIS and KRAUJALIS, 2013).

Coloring food products have been put forward in recent years as they considerably affect the acceptability of foods and are fundamentally linked to multisensory interactions including perception of flavor and significant enjoyment of food. The growing interest of consumers in the aesthetic, nutritional and safety aspects of food has increased the demand for natural pigments such as betalains and carotene. Betalains are water-soluble compounds found in a limited number of families of the plant order Caryophyllales like *Amaranthus* have a unique source of betalains and important free radical-scavenging activity (CAI *et al*., 2003; DANTAS *et al*., 2015). B-cyanins are red to purple colored betalains (absorbance ranging from 530 to 545 nm and condensation of betalamic acid and cyclo-Dopa, considering hydroxycinnamic acid derivatives or sugars as residue) and yellow colored betalains known as β-xanthins (absorbance ranging from 475 to 485 nm and imine condensation products between betalamic acid and amines or amino acid residues) (HERBACH *et al*., 2006; STINTZING and CARLE, 2004, 2007; REPO-CARRASCO-VALENCIA *et al.,* 2010; AZERADO, 2009; PAVOKOVI and KRSNIK-RASOL, 2011). Similarly, carotene grouped into alpha-carotene, β-carotene and xanthophyll. They are hydrophilic nitrogenous secondary metabolites which replace anthocyanins in the flowers and fruits of most plants in families of Caryophyllales. β-cyanins, β-xanthins and carotene are also free radical scavengers (antioxidants) (ESATBEYOGLU *et al*., 2015; REPO-CARRASCO-VALENCIA *et al.*, 2010), which play an important role in human health. Their pharmacological activities include anticancer (ZOU *et al*., 2005; SZAEFER *et al*., 2014), antilipidemic (WROBLEWSKA *et al*., 2011) and antimicrobial (CANADANOVIC-BRUNET *et al*., 2011) activities, indicating that betalains and carotene may be a potential source for the production of functional foods. Presently, the only commercial source of betalains and carotene is the red beet root. The colorant preparations from red beet root labelled as E-162 are exempted from batch certification. E-162 is used in processed foods such as dairy products and frozen desserts (STINTZING and CARLE, 2007). Among the naturally occurring vegetable pigments, betalains are rare and limited to a few edible vegetables such as red beet and amaranth, while chlorophylls are widely distributed in plant species (SCHWARTZ and VON ELBE, 1980). The active ingredients of betalains and carotene provide anti-inflammatory property to our food and act as potential antioxidants and reduce the risk of cardiovascular disease and lung and skin cancers and is widely used as additive for food, drugs, and cosmetic products because of natural properties and absence of toxicity (KAPADIA, *et al.*, 1996; MAZZA, 2000; KANNER *et al*., 2001; BUTERA, *et al*., 2002; TESORIERE *et al*., 2003; ALI *et al*., 2009).

We are able to extract red color juice for natural drinks containing leaf color pigments betalains, and carotene from *Amaranthus*. It demands more genotypes enriched with leaf pigments. We found lots of variations in vegetable amaranth germplasm in respect to mineral, vitamins, leaf color, quality, and agronomic traits in our earlier studies (SARKER *et al*., 2014; SARKER *et al.* 2015a, 2015b, 2016, 2017, 2018a). Therefore, to fill the lacuna, an investigation

was carried out i) to estimate amount of antioxidant leaf pigments and foliage yield in 23 cultivated genotypes of vegetable amaranth, ii) to select appropriate high yielding genotypes containing high antioxidant leaf pigments and (iii) to find out possible ways for improving the antioxidant leaf pigments without compromising foliage yield.

MATERIALS AND METHODS

Seeds of 23 promising vegetable amaranth genotypes were selected in our previous studies of 122 genotypes, above selected genotypes were identified as promising due its high yield potential as well as variation in stem and leaf color.

The genotypes were sown in a Randomized Complete Block Design (RCBD) with 3 replications, during three successive years (2013-14 and 2014-15). Each accession was sown in 1 m^2 plot. The spacing was 20 cm from row-to-row and 5 cm from plant-to-plant, respectively. Total compost (10 ton/ha) was applied during final land preparation. Urea, Triple super phosphate, muriate of potash and gypsum were applied at 200, 100, 150 and 30 kg/ha, respectively. Appropriate cultural practices were also maintained. Thinning was done to maintain appropriate plant density within rows. Weeding and hoeing was done at 7 days interval. Day temperature during experimental period ranged from 25 to 38°C. Irrigation was provided in 5-7 days interval. Data were collected at 30 days after seed sowing for foliage yield and antioxidant leaf pigments.

Data collection of foliage yield

Data were collected 30 days after sowing of seeds. The data were recorded on 10 randomly selected plants in each replication for foliage yield plant^{-1} in gram.

Determination of chlorophyll and total carotenoid content

Chlorophyll *a*, chlorophyll *b* and total chlorophyll were determined from 96% ethanolic extracts of the fresh-frozen amaranth leaves following LICHTENTHALER and WELLBURN (1983) method and total carotenoid content was determined from acetone: haxen extract of the fresh-frozen amaranth leaves using spectrophotometer (Hitachi, U-1800, Tokyo, Japan) at 665, 649, and 470 nm for chlorophyll *a*, chlorophyll *b* and total carotenoid contents, respectively.

Determination of β-cyanins and β-xanthins content

β-cyanins and β-xanthins were extracted from fresh-frozen amaranth leaves using 80% methanol containing 50 mM ascorbic acid according to SARKER and OBA (2018b). B-cyanins and β-xanthins were measured spectrophotometrically at 540 and 475 nm, respectively. The quantifications were done using mean molar extinction coefficients, which were 62 x 10^6 cm^2 mol^{-1} for β-cyanins and 48 x 10^6 cm^2 mol^{-1} for β-xanthins. The results were expressed as nanograms betanin equivalent per gram fresh-frozen weight (FFW) for β-cyanins and nanograms indicaxanthin equivalent per gram FFW for β-xanthins.

Ascorbic acid

Ascorbic acid was analyzed by the method given by ROE (1954). To extract the sample, 5 g fresh leaves were grounded with 5% H_3PO_3 – 10% acetic acid (5% Meta phosphoric acid (H_3PO_3) –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 volume was made up to 1 liter 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 bubbler and the air was passed till bromine color disappeared. The bromine oxidized solution was placed in 2 matched tubes. In first tube 1 mL of 2, 4-DNP thio urea reagent (2, 4-dinitrophenyl hydrazine-thio urea reagent was prepared by dissolving 2 g 2, 4-DNP in 100 mL of 9 N H_2SO_4. Four g 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. Five mL of 85% H_2SO_4 (100 mL distilled water +900 mL concentrated H_2SO_4; sp. gr. 1.84) was added drop wise by the burette in the tube, placed in a beaker of ice water. In second tube, 1 mL of 2,4-DNP thio urea reagent was only added to prepare the blank solution. After 30 min, the absorbance reading of the sample was taken at the 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 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. 10 mL of this dehydro-ascorbic acid was pipetted in 500 mL volumetric flask and the solution was made up to 500 mL with the 85% H_2SO_4 solution. The solutions of different dilution were prepared by pipetting 5, 10, 20, 30, 40, 50 and 60 mL of the above solution into 100 mL volumetric flasks and volumes were made up to 100 mL of each by the addition of 85% H_2SO_4 solution of each flask was taken separately and further the procedure was followed as discussed above for the sample. The calibration curve was prepared by plotting absorbance values against concentration of ascorbic acid (in µg).

The amount of ascorbic acid (mg 100 g^{-1}) was calculated as follows:

ascorbic acid (µg 100 g^{-1}) = (µg from curve)/1000 × (mL of extract taken)/4 × 100/(sample weight. in g). Finally, it was converted in to mg 100 g^{-1}

Statistical analysis

The raw data of the both years (2013-14 and 2014-15) were compiled by taking the means of all the plants taken for each treatment and replication for different traits. The mean data of consecutive years were averaged and the averages of both years' means were statistically and biometrically analyzed. Analysis of variance was done according to PANSE and SUKHATME (1978) for each character. Genotypic and Phenotypic variances, genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), heritability (h^2_b) in broad sense and genetic advance in percent of mean (GAMP) were estimated according to SINGH & CHAUDHARY (1985). Correlation among the traits was analyzed following JOHNSON *et al*. (1955a).

RESULTS AND DISCUSSION

Mean performance

Mean performance, coefficient of variation (CV, %) and critical difference (CD) of leaf pigments and foliage yield for 23 vegetable amaranth genotypes are presented in Table 1. The analysis of variance revealed significant differences among the genotypes for all the 9 traits, indicating the validity of further statistical analysis (Table 1).

Leaf pigments serves as an antioxidant help to protect many diseases including cancer, cardiovascular diseases, neurodegenerative diseases and inflammation and prevent aging (VENSKUTONIS and KRAUJALIS, 2013).

Table 1. Mean performance, %CV and CD for antioxidant leaf pigments in vegetable amaranth.

Genotypes	Chlorophyll *a* (µg g^{-1})	Chlorophyll *b* (µg g^{-1})	Total chlorophyll (µg g^{-1})	β-cyanins (ng g^{-1})	β-xanthins (ng g^{-1})	Betalains (ng g^{-1})	Total carotene (mg 100 g^{-1})	Ascorbic acid (mg 100 g^{-1})	Foliage yield $plant^{-1}$ (g)
VA21	131.46	64.19	196.66	152.26	171.77	323.95	123.91	91.79	13.48
VA22	204.40	57.62	263.03	284.63	294.60	579.16	132.32	87.59	18.54
VA24	254.38	93.33	348.72	228.75	252.75	481.42	125.17	36.89	15.48
VA25	360.71	120.97	482.69	352.26	364.29	716.47	113.38	58.53	10.88
VA26	176.83	77.09	254.94	301.49	311.15	612.57	118.80	84.17	18.64
VA27	238.91	124.47	364.40	203.95	226.51	430.39	91.27	82.27	26.50
VA28	172.75	97.55	271.31	134.51	129.40	263.84	116.76	18.87	22.04
VA29	170.52	49.63	221.16	106.37	99.94	206.23	117.41	185.89	15.14
VA30	295.19	170.28	466.49	330.52	337.47	667.92	97.15	58.53	9.61
VA31	257.87	83.50	342.38	252.70	249.25	501.88	112.35	84.33	6.45
VA32	230.69	78.52	310.23	177.54	182.72	360.18	113.68	46.67	7.88
VA33	200.52	91.90	293.44	256.25	250.48	506.65	125.32	42.36	12.54
VA34	126.47	65.83	193.31	238.51	246.30	484.73	93.61	65.69	10.87
VA35	221.61	100.62	323.24	246.05	243.32	489.29	114.95	45.25	13.64
VA36	242.90	105.79	349.70	211.69	236.92	448.54	102.89	132.45	12.46
VA37	221.27	211.93	434.22	241.17	245.42	486.51	129.30	67.85	11.12
VA38	348.06	189.41	538.49	338.95	326.77	665.65	112.79	49.06	16.96
VA39	154.75	68.71	224.47	315.88	319.20	635.01	68.19	64.69	14.76
VA40	208.30	58.07	267.38	177.09	176.16	353.18	104.52	36.05	9.20
VA41	231.02	125.33	357.36	161.57	167.96	329.46	96.37	106.81	12.88
VA42	303.89	172.64	477.54	298.38	254.10	552.41	62.21	54.37	18.73
VA43	380.80	130.01	511.83	289.51	282.39	571.83	117.67	86.17	13.94
VA44	251.86	50.72	303.37	349.15	370.76	719.84	123.04	102.65	11.86
Mean	234.14	103.83	338.97	245.62	249.55	495.09	109.26	73.43	14.07
Mean	85.42	452.25	912.35	354.24	4211.26	637.67	2798.26	78.29	5432.8
SE	0.1455	0.1634	0.0666	0.0192	0.0583	0.0354	0.1526	0.2452	0.0131
CV%	2.21	3.24	2.13	1.64	2.45	2.62	1.95	2.15	3.24
CD	0.2503	0.2811	0.1146	0.0330	0.1003	0.0609	0.2625	0.4217	0.0226

* Significant at 5% level, ** Significant at 1% level

Chlorophyll a

In statistical analysis, the chlorophyll *a* content had significant pronounced variations among the genotypes. Accession VA43 had the highest chlorophyll *a* content (380.80 µg g^{-1}), followed by VA25 and VA38. The lowest amount of chlorophyll *a* was found in VA34 (126.47 µg g^{-1}). Ten genotypes showed above average mean values for chlorophyll *a* content. The mean chlorophyll *a* content was 234.14 µg g^{-1}. The estimated CV for chlorophyll a was 2.21%.

Chlorophyll b

Accession VA37 had the highest chlorophyll *b* content (211.93 µg g^{-1}), followed by VA38 and VA30. The lowest amount of chlorophyll *b* was found in VA29 (49.63 µg g^{-1}). The mean

chlorophyll *b* content was 103.83 µg g^{-1}. Nine genotypes showed above average mean values for chlorophyll *b* content. The estimated CV for chlorophyll *b* was 3.24%.

Total Chlorophyll

The total chlorophyll content showed a highly pronounced variation among all the chlorophyll traits. VA38 had the highest total chlorophyll content (538.49 µg g^{-1}), followed by VA43, VA25, VA42, and VA30. The lowest amount of total chlorophyll was found in VA34 (193.31 µg g^{-1}). The mean total chlorophyll content was 338.97 µg g^{-1}. Eleven genotypes showed above average mean values for total chlorophyll content. The estimated CV for total chlorophyll was 2.13%.

β-cyanins

There were significant variations among the genotypes in β-cyanins contents and the average β-cyanins content was 245.62. The highest β-cyanins content was observed in VA25 (352.26 ng g^{-1}), followed by VA44, VA38, VA30, VA39, and VA26, while the lowest β-cyanins content was observed in VA29 (106.37 ng g^{-1}). The CV (1.64%) was the least among all the traits analyzed. Out of 23 genotypes, 12 showed above-average values for β-cyanins content.

β-xanthins

There were significant variations among the genotypes in β-xanthins contents. The average β-xanthins content was 249.55. The highest β-xanthins content was observed in VA44 (370.76 ng g^{-1}), followed by VA25, VA30, VA38, VA39, and VA26, while the lowest β-xanthins content was observed in VA29 (99.94 ng g^{-1}). The CV was 2.45%. 12 showed above-average values for β-xanthins content.

Betalains

There were significant variations among the genotypes in betalains contents. The average betalains content was 495.09. The highest betalains content was observed in VA44 (719.84 ng g^{-1}), followed by VA25, VA30, VA38, VA39, and VA26, while the lowest betalains content was observed in VA29 (206.23 ng g^{-1}). The CV was 2.62%. Out of 23 genotypes, 10 showed above-average values for betalains content.

Total carotene

There were significant variations among the genotypes in total carotene contents. The average total carotene content was 109.26. The highest total carotene content was observed in VA22 (132.32 mg 100 g^{-1}), followed by VA24, VA33, VA37, VA21, and VA44, while the lowest total carotene content was observed in VA42 (62.21 mg 100 g^{-1}). The CV (1.95%) was the second least among all the traits analyzed. Out of 23 genotypes, 15 showed above-average values for total carotene content.

Ascorbic acid

There were significant variations among the genotypes in ascorbic acid contents. The average ascorbic acid content was 73.43 mg 100 g^{-1}. The highest ascorbic acid content was observed in VA29 (185.87 mg 100 g^{-1}), followed by VA36, VA41, and VA44, while the lowest ascorbic acid content was observed in VA28 (18.87 mg 100 g^{-1}). The CV was 2.15%. Out of 23 genotypes, 10 showed above-average values for ascorbic acid content.

Foliage yield

It had significant and the highest variations among the genotypes. The highest value was found in VA27 (26.50 g) followed by VA28, VA26, VA22, VA42, VA38, VA24, and VA29. The lowest value was observed in VA31 (6.45 g) followed by VA40, VA30, and VA25. The average was 14.07 g. The CV (3.24%) was the highest among all the traits analyzed. Nine genotypes showed above-average values.

The present investigation revealed that vegetable amaranth is rich in chlorophyll *a* (234.14 µg g^{-1}), chlorophyll *b* (193.83 µg g^{-1}), Total chlorophyll (338.97 µg g^{-1}), β-cyanins (245.62 ng g^{-1}) and β-xanthins (249.55 ng g^{-1}), betalains (495.09 ng g^{-1}), total carotene (109.26 mg 100 g^{-1}) and ascorbic acid (73.43 mg 100 g^{-1}). Five genotypes, VA27, VA26, VA24, VA42, and VA38 showed high foliage yield and also found to be a rich source of antioxidant leaf pigments and ascorbic acid. Selection of these genotypes would be economically useful for antioxidant leaf pigments and ascorbic acid and high yield aspects. The genotypes VA22 and VA29 had above average foliage yield along with low content of the antioxidant leaf pigments. These genotypes can be used as high yielding but colorant bioactive compound content varieties. The genotypes VA25 and VA43 had the highest amount of the antioxidant leaf pigments and below-average foliage yield. These genotypes can be used as a donor parent for integration of potential genes of the high antioxidant leaf pigments into other genotypes.

Variability studies

Variability plays a vital role in the selection of superior genotypes in crop improvement program. Agronomic traits are quantitative in nature, and interact with the environment under study, so partitioning the traits into genotypic, phenotypic, and environmental effects is essential to find out the additive or heritable portion of variability. The genotypic and phenotypic variance (σ^2g, σ^2p), coefficients of variation (GCV, PCV), h^2_b, GA and GAMP are presented in Table 2. The highest genotypic variance was observed for betalains (20318.65), followed by total chlorophyll, β-xanthins, β-cyanins, and chlorophyll *a* (4326.36). Chlorophyll *b*, ascorbic acid exhibited moderate genotypic variances. On the other hand, the lowest genotypic variance was observed for foliage yield.

Table 2. Genetic parameter for antioxidant leaf pigments in vegetable amaranth

Genetic parameter	Chlorophyll *a* (µg g^{-1})	Chlorophyll *b* (µg g^{-1})	Total chlorophyll (µg g^{-1})	β-cyanins (ng g^{-1})	β-xanthins (ng g^{-1})	Betalains (ng g^{-1})	Total carotene (mg 100 g^{-1})	Ascorbic acid (mg 100 g^{-1})	Foliage yield $plant^{-1}$ (g)
σ^2g	4684.08	2106.41	10522.15	5116.08	5157.75	20318.65	321.32	1311.99	21.52
σ^2p	4750.25	2215.25	10835.62	5242.63	5345.65	20762.35	355.26	1402.72	24.48
GCV	19.77	25.32	19.41	19.88	19.71	19.69	25.66	39.01	29.08
PCV	19.91	25.97	19.70	20.13	20.07	19.90	26.98	40.33	31.02
h^2_b	99.30	97.51	98.54	98.79	98.23	98.93	95.10	96.71	93.76
GA	140.99	94.55	211.31	147.35	147.94	293.64	36.93	74.62	9.56
GAMP	40.72	52.16	39.99	40.96	40.61	40.56	52.85	80.35	59.91

σ^2g = Genotypic variance, σ^2p = Phenotypic variance, GCV = Genotypic coefficient of variation, PCV = phenotypic coefficient of variation, h^2_b = Heritability in broad sense, GAMP = Genetic advance in percent of mean

The phenotypic variances for all the traits were slightly higher but close to the genotypic variances. GCV values ranged from 19.41 (total chlorophyll) to 39.01% (ascorbic

acid). The PCV values showed similar trends as GCV values and ranged from 19.70% (total chlorophyll) to 40.33% (ascorbic acid). In the present investigation, all the traits had high to moderate genotypic and phenotypic variances along with moderate GCV and PCV values, which indicate scope for improvement in these traits through selection due to predominance of additive gene action for these traits.

Variability alone is not of much help in determining the heritable portion of variation. The amount of gain expected from a selection depends on heritability and genetic advance in a trait. Heritability has been widely used to assess the degree to which a character may be transmitted from parent to offspring. Knowledge of heritability of a character is important as it indicates the possibility and extent to which improvement is possible through selection ROBINSON *et al.*, 1949). However, high heritability alone is not enough to make sufficient improvement through selection generally in advance generations unless accompanied by substantial amount of genetic advance (JOHNSON *et al*., 1955b). The expected genetic advance is a function of selection intensity, phenotypic variance and heritability and measures the differences between the mean genotypic values of the original population from which the progeny is selected. It has been emphasized that genetic gain should be considered along with heritability in coherent selection breeding program (SHUKLA *et al*., 2006). It is considered that if a trait is governed by non-additive gene action it may give high heritability but low genetic advance, which limits the scope for improvement through selection, whereas if it is governed by additive gene action, heritability and genetic advance would be high, consequently substantial gain can be achieved through selection.

The heritability estimates were high for all the traits and ranged from 93.76% (foliage yield) to 99.30% (chlorophyll *a*). The highest expected genetic advance was exhibited for betalains (293.64%) followed by total chlorophyll, β-xanthins, β-cyanins, and chlorophyll *a*. Genetic advance in percent of the mean (GAMP) ranged from 39.99 to 80.35. The highest GAMP was found in ascorbic acid (80.35), followed by foliage yield, total carotene, and chlorophyll *b*. Chlorophyll *a*, total chlorophyll, β-cyanins, β-xanthins and betalains showed moderate GAMP (around 40%). In the present study, the heritability and genetic advance values were high for all the traits except foliage yield indicated preponderance of additive gene effects.

Correlation studies

The phenotypic and genotypic correlations between the various characters are presented in Table 3. The genotypic correlation coefficients were very much close to the corresponding phenotypic values for all the traits.

In the present investigation, the genotypic correlation coefficients were very much close to the corresponding phenotypic values for all the traits indicating additive type of gene action for the expression of these traits. The chlorophyll *a* had a significant positive correlation with chlorophyll *b* in both the level. Chlorophyll *a* and chlorophyll *b* had a significant positive association with total chlorophyll in both the level. A significant positive association was observed between β-cyanins and chlorophyll *a*, as well as β-cyanins and total chlorophyll in both the level. β-xanthins exhibited significant positive interrelationship with chlorophyll *a*, total chlorophyll and β-cyanins. Betalains showed a significant positive association with chlorophyll *a*, total chlorophyll, β-cyanins, and β-xanthins. Total carotene exhibited a negligible insignificant negative association with all the traits. Similarly, ascorbic acid showed a negligible insignificant

negative interrelationship with all the traits except total carotene and foliage yield exhibited a negligible insignificant correlation with all the traits except chlorophyll *b*.

Table 3. Genotypic and phenotypic correlation co-efficient (r_g and r_p) for antioxidant leaf pigments in vegetable amaranth.

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Traits		Chlorophyll b (µg g^{-1})	Total chlorophyll (µg g^{-1})	β-cyanins (ng g^{-1})	β-xanthins (ng g^{-1})	Betalains (ng g^{-1})	Total carotene (mg 100 g^{-1})	Ascorbic acid (mg 100 g^{-1})	Foliage yield (g)
Chlorophyll *a* (µg g^{-1})	r_g	0.594**	0.933**	0.545*	0.491*	0.521**	-0.032	-0.132	-0.077
	r_p	0.592**	0.932**	0.542*	0.490*	0.520**	-0.030	-0.131	-0.076
Chlorophyll *b* (µg g^{-1})	r_g		0.844**	0.315	0.240	0.279	-0.186	-0.253	0.099
	r_p		0.842**	0.314	0.238	0.278	-0.185	-0.252	0.098
Total chlorophyll	r_g			0.504*	0.435*	0.472*	-0.105	-0.202	-0.008
	r_p			0.503*	0.433*	0.471*	-0.104	-0.201	-0.007
β-cyanins (ng g^{-1})	r_g				0.978**	0.994**	-0.132	-0.240	-0.083
	r_p				0.976**	0.992**	-0.131	-0.238	-0.082
β-xanthins (ng g^{-1})	r_g					0.995**	-0.052	-0.194	-0.095
	r_p					0.994**	-0.051	-0.193	-0.094
Betalains (ng g^{-1})	r_g						-0.093	-0.218	-0.089
	r_p						-0.092	-0.217	-0.088
Total carotene (mg 100 g^{-1})	r_g							0.063	-0.142
	r_p							0.062	-0.140
Ascorbic acid (mg 100 g^{-1})	r_g								-0.011
	r_p								-0.010

* Significant at 5% level, ** Significant at 1% level

The insignificant negative genotypic correlation was observed between total carotene versus all antioxidant leaf pigments, ascorbic acid versus all antioxidant leaf pigments and foliage yield versus rest of all traits. This indicates that selection for antioxidant leaf pigments and ascorbic acid content might be possible without compromising yield loss. On the other hand, most of the interrelationship among antioxidant leaf pigments traits was significant. A similar trend was observed by earlier work in *A. tricolor* (SHUKLA *et al*., 2006; SARKER *et al.*, 2014). The chlorophyll *a* had a significant positive correlation with chlorophyll *b*. Chlorophyll *a* and chlorophyll *b* had a significant positive association with total chlorophyll. A significant positive association was observed between β-cyanins and chlorophyll *a* as well as β-cyanins and total chlorophyll. β-xanthins exhibited significant positive interrelationship with chlorophyll *a*, total chlorophyll and β-cyanins. Betalains showed a significant positive association with chlorophyll *a*, total chlorophyll, β-cyanins and β-xanthins. It indicates that increasing of one antioxidant leaf pigments significantly increased the other antioxidant leaf pigments in vegetable amaranth. SHUKLA *et al.* (2010) observed a positive association of foliage yield with beta carotene and ascorbic acid.

CONCLUSIONS

Considering high genotypic and phenotypic variances along with GCV and PCV values, high heritability coupled with GAMP, all the traits except foliage yield could be selected for the improvement of 23 vegetable amaranth genotypes under study. However, the correlation study revealed a strong positive association among all the antioxidant leaf pigments. Selection based on antioxidant leaf pigments could economically viable to improve the antioxidant potential of vegetable amaranth genotypes. Insignificant negative genotypic correlation was observed between total carotene versus all antioxidant leaf pigments, ascorbic acid versus all antioxidant leaf pigments and foliage yield versus rest of all traits. This indicates that selection for antioxidant leaf pigments and ascorbic acid content might be possible without compromising yield loss. Based on mean performance of the genotypes, five vegetable amaranth genotypes VA27, VA26, VA24, VA42, and VA38 were identified as high yielding having substantial antioxidant leaf pigments and ascorbic acid content. These varieties might be suitable for extraction of juice for Drinks production.

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ANTIOKSIDANS PIGMENTI LISTA I VARIJABILNOST KOD AMARANTUSA

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Izvod

Dvadeset i tri genotipova amarantusa su ispitana za varijabilnost, međupovezanost antioksidans pigmenta lista i prinos. Pet genotipova su bogati izvori antioksidansih pigmenta lista i mogu direktno da se selekcionišu kao visoko rodne sorte bogate antioksidasnim pigmentima lista. Dva genotipa sa visokim sadržaj antioksidansnih pigmenta lista sa niskim prinosom mogu da se koriste kao donori za gene za antioksidansne pigmente lista za razvoj segregirajućih potomaka ili transgenih genotipova. Nedovoljna negativna genetička korelacija je dobijena između ukupnih karotena i svih antioksadativnih pigmenta lista, askorbinske kiseline i svih antioksidativnih pigmenta lista i prinosa sa ostatkom ostalih osobina. Poboljšanje karotena i askorbinske kiseline može biti moguće bez gubitka prinosa. S druge strane, većina međuodnosa između svojstva antioksidansnih pigmenta lista ukazuje da poboljšanje jednog značajno utiče na povećanje drugih svojstava.

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