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Soluble sugar contents, total phenolic, and antioxidant capacity in a diverse set of amaranthaceae accessions

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Abstract

Thirty-Five Amaranthaceae accessions from seven species collected from the Germplasm Resources Information Network, USDA-ARS and then grown in a greenhouse in the USA were analyzed for their soluble sugars, total phenolic, and antioxidant capacity. The sugar contents were analyzed using high-performance liquid chromatography (HPLC). The sucrose, glucose, and fructose were found the pure sugars of the amaranth accessions with sucrose was the predominant. But wide ranges of variations were observed among the accessions. The sucrose contents ranged from 2.50-131.20 mg.100 g^{-1} DW (average 42.46 mg.100 g^{-1} DW). The glucose and fructose contents ranged 2.60-66.64 (average 23.47 mg.100 g^{-1} DW) and 0.60-34.60 (average 13.28 mg.100 g^{-1} DW). A significant positive correction found between total sugar contents and sucrose (r= 0.944), glucose (r= 0.797) and fructose (r= 0.792). Total phenolic (TP) contents were measured using the Folin-Ciocalteu reagent and was expressed as mg Tannic acid equivalents per gram of plant material on the dry basis (mg TAE. g^{-1}). The Trolox equivalents antioxidant capacity (TEAC) based on the DPPH free radical scavenging ability of the extract was expressed as milligram Trolox equivalents per gram of plant material on the dry basis (mg TEAC. g^{-1}). Variability among accessions was greater for TP contents (CV 44.81%); individuals ranged nearly ninefold from 1.39 to12.37 mg TAE. g^{-1} on a dry weight basis. Variation among accessions was also evident for TAEC values (CV 20.43%) and individually ranged nearly threefold from 0.17 to 0.44 mg TAEC. g^{-1} on a dry weight basis. There were significant differences (P> 0.005) for TP and TAEC while compared among the seven species. The highest TP content was observed in *C. argentea* (7.69 mg TAE.g^{-1}) followed by *A. hypochondriacus* (6.98 mg TAE.g^{-1}), and the TEAC was found highest in *A. blitum* and *A. hypochondriacus* (0.36 mg TEAC.g^{-1}) followed by *A. caudatus* (0.35 mg TEAC.g^{-1}). However, TP and TEAC values were not significantly correlated (r= 0.430). The significant difference among accessions regarding soluble sugar contents, total phenolic, and antioxidant capacity showed their potentiality to use the accessions for further breeding programs.

Introduction

Amaranthaceae, commonly called the Amaranth family has about 65 genera and 900 species (Ogundipe and Chase, 2009). Amaranthaceae contains several important genera including Amaranthus and Celosia (Wheeler, 1992). Some species in Amaranthaceae are weedy; some species are cultivated as ornamentals, particularly *Amaranthus caudatus, A. hypochondriacus, A. tricolor,* and *Celosia cristata.* There are four species of *Amaranthus* recognized as grown vegetables in eastern Asia: *A. cruentus, A. blitum, A. dubius,* and *A. tricolor* (Costea *et al*., 2001), and three species that are important to agricultural production as pseudocereals, *A. caudatus, A. hypochondriacus*, and *A. cruentus* (Marin *et al.*, 2011). In many southern countries, amaranthus is grown for green grass, hay, and silage and are good forage for livestock (Svirskis, 2009). Amaranth is a fast-growing crop, resistant to drought or hot climate and pests, and has little necessities on cultivation inputs (Hauptli, 1977; Paredes Lopez *et al*., 1989). Amaranth has attracted increasing interest over recent decades because of its nutritional, functional and agricultural characteristics (Queiroz *et al.*, 2009). Although the family Amaranthaceae is typical, the genus has few distinguishing characters among that 70 species (Juan *et al*., 2007). *Amaranthaceae*, with small seed size, have wide adaptability, highly scavenging root systems, C4 photosynthetic pathway and highly competitive nature. Some traits such as greater photosynthesis rate, condensed perception

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of adjacent neighbor, pest and disease resistance, drought tolerance, optimal nitrogen acquisition and utilization, which are desired in biomass crops, are anticipated in *Amaranthaceae*. Use of Amaranth biomass as a renewable energy source could be considered as a valuable crop with a large potential in the 21st century because of its ability to absorb heavy metals from the surrounding soil (Huska, 1992). Amaranth is one of the rare plants whose leaves are eaten as the vegetable while the seeds are used as cereals (Saunders and Becker, 1984; Brenner *et al*., 2000).

Phytochemicals, naturally occurring secondary metabolites, found in plants, have a broad range of biological effects, including anti-inflammatory, antimicrobial and antioxidant activities (Stine *et al*., 1996; Velioglu *et al*., 1998; Mazza, 2000). Recently, a keen interest has been given to naturally occurring antioxidants, which may play important roles in preventing both free radicals and oxidative chain reactions within tissues and membranes (Carini *et al*., 1990). From the viewpoint of their high antioxidant capacity, the evaluation of antioxidant activities of extracts and fractions is considered as an important step in the isolation of antioxidant phytochemicals they contain. The previous study confirmed that amaranth leaves are excellent sources of health beneficiary bioactive compounds such as Phenolic, betacyanin, and antioxidants (Khandaker *et al*., 2008). However, no or little information is available on soluble sugars, total phenolic and antioxidant capacity of young arial parts of plants from different species of Amaranthaceae family. Previous investigations have most often focused on a single or a few accessions or from seeds of few accessions (Stine *et al*., 2011). Understanding the variations in the content of these compounds is important as they have impacts on both plant fitness and the nutritional properties to use as vegetable or forage. The information of sugar components in amaranth is also very limited. Therefore, it is necessary to know the distribution of sugar compositions of amaranth. The purpose of this study was, therefore, to investigate the variations in the content of sucrose, glucose, fructose, phenolic and antioxidant capacity in 35 accessions belongs to seven species in Amaranthaceae family. The results generated from this study can be used for conventional and chemical breeding purposes for the improvement of food crops.

Materials and Methods

Plant materials

A total of 35 Amaranthaceae accessions were used for this study (Table 1). These accessions included seven species that were collected from the Germplasm Resources Information Network (GRIN), USDA-ARS. Accession number, species name and the source of origin of the accessions is presented in Table 1. All accessions were grown in a greenhouse environment with controlled temperature and relative humidity (approximately 78°C and 55%) at Southern United State during 2011 - 2013. Seeds were sown in 18-hole sheet pots for germination, after single germination seedling was transferred to 3 gallons containers contained *Canadian sphagnum* peat, Pro-Mix BX (peat-based growing medium, Premier Horticulture) and sand (4:4:1). Plants were irrigated as when necessary, and no additional fertilizer was applied. The experimental design was a completely randomized with three replications; one pot with one plant was used per replication. Aboveground portion/ Arial part of the plant was harvested after three weeks of showing, transferred to a paper bag, and then weighed.

Sample preparation and extraction

Amaranth samples were freeze-dried using model MD53 Millrock Technology freeze dryer for 72 hours. The dried samples were ground using mortar and pestle. The extractions of the samples were based on a slight modification of the previous method (Lewthwaite *et al*., 1997). Briefly, 5.0 gram of the ground samples were placed in a 150 ml beaker, and 50 ml of 80% (v / v) ethanol added. The homogenates were agitated for 18 hours using an orbital shaker. The resulting solution was centrifuged for 10 minutes at 3000 x g in a centrifuge. The supernatant was filtered using 0.45 µm filter paper.

Determination of soluble sugars by high-performance liquid chromatography (HPLC)

Sample analysis was conducted on a Hitachi Elite LaChrom High-Performance Liquid Chromatograph, equipped with a Refractive Index detector, Model (Hitachi L-2490). 20 µl of the filtered sample solutions and the standards were injected into the chromatograph using the autosampler, Hitachi L-2200. The sugars were separated on a Hitachi Lachrom NH_2, 5 µm, 4.6 x 250 mm column and guard column; Hitachi LaChrom NH_2-G, 5 µm, 4 x 23, in an isocratic mobile phase of 75% acetonitrile over 30 minutes at a flow rate of 1.0 ml per minute. The oven temperature was 40°C. Calibration standards, containing 0.5%, 1.0%, and 1.5% each of sucrose, fructose and glucose were prepared from stocks containing pure of each of the individual compounds in 80% ethanol solution. Identification

Table 1. Fresh matter (FM), dry matter (DM), total phenolic (TP) and antioxidant activity (TEAC) of the thirty-five accessions (Acc. No.) of Amaranthaceae family

Acc. No.	Species	Source of origin	FM (g)	DM (g)	TP (mg TAE. g^{-1})	TEAC (mg TEAC. g^{-1})
PI 553076	*A. australis*	Florida	102.74 ±8.03	7.75 ±1.02	5.21±1.15	0.24±0.04
PI 606282	*A. blitum subsp. oleraceus*	Bangladesh	148.15 ±8.78	20.06 ±2.00	4.68±0.19	0.36±0.09
Ames 5363	*A. caudatus*	Unknown	50.35 ±7.15	7.66 ±1.41	5.18±1.03	0.35±0.07
Ames 15150	*A. caudatus*	Peru	83.35 ±6.56	12.07 ±2.12	1.39±0.17	0.42±0.07
PI 642741	*A. caudatus*	Bolivia	152.92 ±5.66	16.22 ±1.54	2.99±0.73	0.29±0.05
Ames 2055	*A. cruentus*	Nigeria	118.74 ±8.78	15.66 ±1.47	1.67±0.12	0.21±0.01
Ames 5330	*A. cruentus*	California	184.24 ±12.00	19.85 ±1.41	1.48±0.18	0.21±0.02
Ames 5648	*A. cruentus*	Mexico	187.1 ±9.02	23.32 ±2.42	1.71±0.52	0.17±0.10
Ames 13887	*A. cruentus*	China	242.34 ±8.24	23.44 ±2.83	1.97±0.46	0.20±0.06
PI 451711	*A. cruentus*	Mexico	152.35 ±12.12	23.35 ±3.42	4.74±0.56	0.29±0.04
PI 477913	*A. cruentus*	Mexico	165.25 ±5.87	24.06 ±2.49	4.16±1.09	0.30±0.01
PI 490656	*A. cruentus*	Nepal	129.63 ±6.56	15.57 ±1.98	4.09±0.50	0.26±0.02
PI 490659	*A. cruentus*	Mexico	149.35 ±13.02	23.23 ±1.26	2.61±0.48	0.25±0.02
PI 511715	*A. cruentus*	Guatemala	121.41 ±7.01	17.18 ±1.54	4.90±0.24	0.31±0.02
PI 566897	*A. cruentus*	India	119.95 ±8.67	16.17 ±1.12	4.02±1.11	0.28±0.04
PI 606797	*A. cruentus*	Illinois	164.43 ±7.41	25.72 ±2.41	4.60±0.48	0.31±0.06
PI 606799	*A. cruentus*	United States	163.65 ±10.02	28.9 ±2.81	7.02±0.73	0.28±0.03
PI 618962	*A. cruentus*	Benin	117.36 ±8.83	17.03 ±3.54	5.18±1.30	0.27±0.06
PI 647848	*A. cruentus*	United States	173.53 ±6.78	22.99 ±2.02	3.58±0.40	0.28±0.02
PI 658731	*A. cruentus*	Iowa	146.34 ±2.83	21.74 ±0.71	4.90±0.62	0.29±0.04
Ames 5141	*A. hypochondriacus*	California	162.81 ±7.07	22.32 ±3.54	7.11±0.55	0.34±0.03
PI 540446	*A. hypochondriacus*	Pakistan	125.67 ±5.71	17.06 ±3.54	8.61±1.99	0.44±0.05
PI 558499	*A. hypochondriacus*	Nebraska	135.73 ±6.02	19.61 ±2.83	6.53±1.52	0.44±0.05
PI 604794	*A. hypochondriacus*	Mexico	238.18 ±6.71	24.76 ±0.88	7.72±1.43	0.28±0.02
PI 619247	*A. hypochondriacus*	Mexico	115.96 ±7.82	16.9 ±2.05	6.91±0.11	0.29±0.05
PI 619249	*A. hypochondriacus*	Mexico	160.75 ±7.07	23.18 ±2.53	5.00±0.51	0.36±0.03
Ames 2091	*A. tricolor*	Nepal	133.85 ±4.02	13.88 ±1.02	5.00±0.62	0.28±0.07
PI 419057	*A. tricolor*	China	102.74 ±3.52	16.05 ±1.83	4.42±1.47	0.25±0.02
PI 490764	*A. tricolor*	India	53.73 ±4.19	6.2 ±0.09	7.16±0.19	0.31±0.03
PI 604570	*A. tricolor*	Taiwan	152.97 ±6.98	16.91 ±1.54	6.65±1.17	0.29±0.02
PI 608761	*A. tricolor*	India	132.49 ±6.47	13.09 ±1.12	4.58±0.83	0.29±0.04
PI 649287	*Celosia argentea*	Nepal	90.97 ±5.54	8.22 ±2.02	5.97±0.55	0.32±0.06
Ames 14960	*C. argentea var. argentea*	Puerto Rico	149.18 ±6.02	12.24 ±3.54	5.79±1.30	0.21±0.04
Ames 2239	*C. argentea var. cristata*	Nepal	128.3 ±5.22	9.64 ±0.09	6.61±1.99	0.23±0.04
PI 293758	*C. argentea var. cristata*	Former Soviet Union	93.6 ±3.54	10.19 ±1.02	12.37 ±0.89	0.35±0.02
		Average	143.72	18.49	4.76	0.29
		Minimum	50.35	6.20	1.39	0.17
		Maximum	242.34	28.90	12.37	0.44
		SD	47.37	7.03	2.90	0.06
		CV%	32.96	38.04	44.81	20.43

and quantification of each component were based on the comparison of the retention times of the unknown with those of the standards (Lewthwaite *et al*., 1997).

Determination of total phenolic (TP)

Total phenolics in the whole aerial parts of amaranths was determined by a slight modification of the Folin-Ciocalteu method (Folin-Ciocalteu, 1927; Singleton *et al*., 1999; Islam *et al*., 2003). All samples were lyophilized before analysis. Ten mg of a sample was vigorously mixed with (1:1) aqueous ethanol solution. The mixture was subjected to ultrasonic treatment for 5 min under a hood, centrifuged at 5000 x g for 10 min, and the supernatant was collected. The residue was also mixed with five mL of (1:1) aqueous ethanol, subjected to ultrasonic treatment for 5 min to re-extract the phenolics. The extracts were combined, made up to 10 ml, and used for the measurement of total phenols. The alcohol extract was diluted to get an absorbance reading within the range of the standards (40-800 µg Tannic acid ml^{-1}). The absorbance was measured at 600 nm with a dual wavelength flying spot scanning densitometer (Shimadzu Co., Kyoto, Japan), with a microplate system. Total phenols were obtained from the calculation of the concentrations from the slope curve and reported as milligram Tannic acid equivalent per gram dry weight sample (mg TAE. g^{-1}).

Determination of antioxidant capacity (TEAC)

Fresh samples were freeze-dried and ground. The ground samples were extracted with methanol at a mass to volume ratio of 1:20 (g/ml) for 24 hours at room temperature. The extracts were evaporated on a rotary evaporator at 35°C and re-dissolved in 10% DMSO (dimethylsulfoxide). The extracts were kept at -80°C until analysis.

The radical-scavenging activity of the extracts was measured by slightly modifying the DPPH method (Brand-Williams *et al*., 1995; Burits and Bucar, 2000; Islam *et al*., 2003). A stock solution of DPPH (6 mM) was prepared by dissolving 0.0236g in 10 ml of ethanol. The stock solution was diluted to make a 60 µM working solution. A 10 mM stock solution of

Trolox was prepared for every sample tested, from which serial dilutions were made for calibration. From each dilution of the Trolox, 20 µL were added to 2.5 mL of DPPH stock solution and incubated in a dry bath at 37°C for 30 min. Absorbance was measured at 520 nm on an ASYS UVM 340 plate reader. Serial dilutions of each sample were also arranged from which 20 ul was taken, and 2.5 ml of the DPPH stock solution was added. After incubation, the absorbances were recorded. Dilutions were made for each sample tested. Dilution strength was dependent upon each extract's relative antioxidant capacity. TEAC values were measured by comparing the slope of sample plots to the slope of Trolox. Antioxidant capacity was reported as milligram Trolox equivalent per gram dry weight sample (mg TEAC. g^{-1}).

Statistical analysis

Results were stated as the mean ± the standard deviation of triplicate analysis. Differences were subjected to analysis of variance (ANOVA, $P < 0.05$) using XLSTAT for Microsoft Excel statistics package (XLSTAT, 2016). The genetic correlation between characters is presented as Pearson's correlation coefficients among accession means statistically significant at $P < 0.05$.

Results and Discussion

Significant natural variation exists in the mass of Arial parts of Amaranthaceae accessions in respect of fresh and dry matters. For example, the fresh matters of Ames 13887 (*A. cruentus*, 242.34 g) about 5-fold higher than that of lowest in Ames 5363 (*A. caudatus*. 50.35 g), and the dry matter of PI 606799 *(A. cruentus)* was 4-fold higher than that lowest in PI 490764 *(A. tricolor)* (Table 1). Biomass production may vary between the species and the species *A. cruentus* produced more biomass compared to *A. hypochondriacus* (De Troiani *et al*., 2004), which is similar to the results of this study. Although caution is necessary when comparing biomass yield from a single plant or single harvest, data from this experiment suggest some differences can still be considered to select promising accessions for biomass yield. However, a three-year study on biomass comparison of *A. hypochondriacus* and forage sorghum revealed that forage sorghum gave the highest green mass and dry matter yield at the tasselling stage in the first year but in second year amaranth, cultivar, gave a high green mass yield at the flowering, which was in the same rank as forage sorghum (Ana *et al*., 2009). We also found the average highest fresh mass and second most top dry

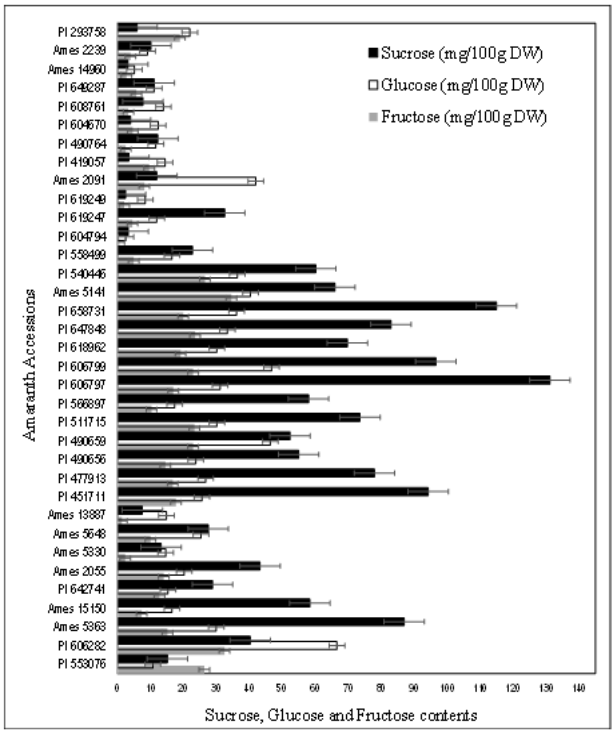

Figure 1. Distribution of sugar contents (mg 100 g^{-1} dry weight) among the thirty-five amaranth accessions studied. Data are the mean of three replication ± standard error

mass from the species, *A. hypochondriacus* among all seven species.

Soluble sugar contents

The distribution of sugar contents was presented in Figure 1. The HPLC chromatography shows the sucrose (Avg. 42.46 g. 100 g^{-1} DW), glucose (Avg. 23.47 mg. 100 g^{-1} DW) and fructose (Avg. 13.28 g. 100 g^{-1} DW) were found the pure sugar of the amaranth genotypes studied. Across all accessions, the sucrose contents were found the predominant sugars of most of the genotypes studied followed by Glucose and Fructose. But wide ranges of variations were observed among the genotypes. The sucrose contents ranged from 2.50-131.20 mg/100 g dry weight, and the glucose and fructose contents reached 2.60-66.64 (average 23.47 g. 100 g^{-1} DW) and 0.60-34.60 (average 13.28 g. 100 g^{-1} DW). The sucrose was found as the main sugar in amaranth species as reported by several authors (Becker *et al*., 1981; Venskutonis and Kraujalis, 2013). However, these results reported in different articles on numerous Amaranth species may be in a greater range (Teutonico and Knoor, 1985). Furthermore, the effects of cultural, chemical and physical treatments on amaranth species resulted in a reduction in the level of sugars (Sujak *et al.*, 2009). The correlation between the sugar contents of the thirty-five different accessions also studied (Figure 2). There was a significant positive correction were found among total sugar contents and sucrose (r=

Table 2. Values of fresh matter (FM), Dry matter (DM), total phenolic (TP) and antioxidant capacity (TEAC) of seven species of family Amaranthaceae

Species	No. of Acc.	FM (g)	DM (g)	TP (mg TAE. g^{-1})	TEAC (mg TEAC. g^{-1})
A. australis	1	102.74	7.75	5.21	0.24
A. blitum subsp. oleraceus	1	148.0	20.2	4.68	0.36
A. caudatus	3	50-153	7.7-16.2	1.39-5.18	0.29-0.42
		(95.5)	(12.0)	(3.19)	(0.35)
A. cruentus	15	119-242	15.6-25.7	1.48-7.02	0.17-0.31
		(155.7)	(21.2)	(3.77)	(0.26)
A. hypochondriacus	6	116-238	16.9-24.8	5.00-8.61	0.28-0.44
		(156.5)	(20.6)	(6.98)	(0.36)
A. tricolor	5	54-153	6.2-16.9	4.42-7.16	0.25-0.31
		(115.2)	(13.2)	(5.63)	(0.28)
Celosia argentea	4	91-149	8.2-12.2	5.79-12.37	0.21-0.35
		(115.5)	(10.1)	(7.69)	(0.28)
	P <0.5	0.00329*	0. 00490*	0.00068*	0.00412*
	CV%	33.00	38.00	31.68	29.42

Note: Values in brackets are the average; * Significant difference at P <0.5

0.944), glucose (r= 0.797) and fructose (r= 0.792). Large natural variation exists among Amaranthaceae accessions and species for their sugar components. Sucrose contents were found the predominant sugars of most of the genotypes studied followed by glucose and fructose. The results demonstrated that total sugar contents of amaranth accessions are dependent on their soluble sugars accumulation. The degree of observed genotypic variation in the sugar contents indicates Amaranthaceae is potential for plant breeding to enhance desired quality criteria that significantly impact on the future investigation(s).

Total phenol contents and antioxidant capacity

The variations of the Tannic acid equivalent (TAE) total phenolic (TP) and Trolox equivalent antioxidant capacity (TEAC) among 35 accessions are presented in Table 2. TP content ranged between 1.39 mg TAE. g^{-1} (Ames 15150; *A. caudatus*) to 12.37 mg TAE. g^{-1} (PI 293758; *C. argentea* var. cristata) with the average of 4.76 mg TAE. g^{-1}. The trolox equivalent antioxidant capacity (TEAC) ranged from 0.17 mg TAE. g^{-1} (Ames 5648; *A. cruentus*) to 0.44 mg TEAC. g^{-1} (PI 540446, PI 558499; *A. hypochondriacus*) with the average of 0.29 mg TEAC. g^{-1} (Table 2). The coefficient of variation among all 35 accessions for the TP and TEAC were 44.81% and 20.43% respectively. TP content and TEAC within individuals ranged nearly nine-fold and three-fold, respectively.

All of 35 accessions from the family Amaranthaceae belong to seven species, and the traits values were compared among the species from their average values (Table 2). ANOVA showed that there were significant differences (P> 0.005) among species for the Fresh weight, dry weight, TP, and TEAC. In general, average values of several accessions (n) showed that *A. hypochondriacus* had the highest fresh mass (156.52 g) and second largest for dry mass (20.64 g), *A. cruentus* (n=15) had second highest fresh mass (155.71 g), but highest dry mass (21.21 g), and *A. caudatus* (n=3) had lowest fresh mass (95.54 g). The Tannic acid equivalent (TAE) total phenolic (TP) content in the seven species is also presented in Table 2. The highest TP content was observed in *C. argentea* (7.69 mg TAE/g) followed by *A. hypochondriacus* (6.98 mg TAE. g^{-1}), *A. tricolor* (5.63 mg TAE. g^{-1}), *A. australis* (5.21 mg TAE. g^{-1}), A. blitum (4.68 mg TAE. g^{-1}), A. cruentus (3.77 mg TAE. g^{-1}), and *A. caudatus* (3.19 mg TAE. g^{-1}), respectively. From the results shown in Table 2, it can be seen that there is the statistical difference between the plant extracts regarding their total phenolic content (P< 0.05). There are also statistical differences among the TROLOX Equivalent antioxidant capacity (TEAC), the highest value (0.36 mg. g^{-1}) was in the species *A. blitum* and *A. hypochondriacus* followed by *A. caudatus* (0.35 mg TEAC. g^{-1}), *A. tricolor* (0.28 mg TEAC. g^{-1}), *C. argentea* (0.28 mg TEAC. g^{-1}), *A. cruentus* (0.26 mg TEAC. g^{-1}), and *A. australis* (0.24 mg TEAC. g^{-1}), respectively. In other words, the TEAC decreased in the following order: *A. blitum* and *A. hypochondriacus* > *A. caudatus* > *A. tricolor* > *C. argentea* > *A. cruentus* > *A. australis.*

The wide variety of oxidation systems and ways to measure phenolic and antioxidant capacity used in different accessions and plant's parts evaluations make it difficult to compare results from various studies. In this study, total phenolic is considerably affected by genetic factors as individual accession and plant species. Other factors like plant organ, phenological stage, and environmental factors including climatic

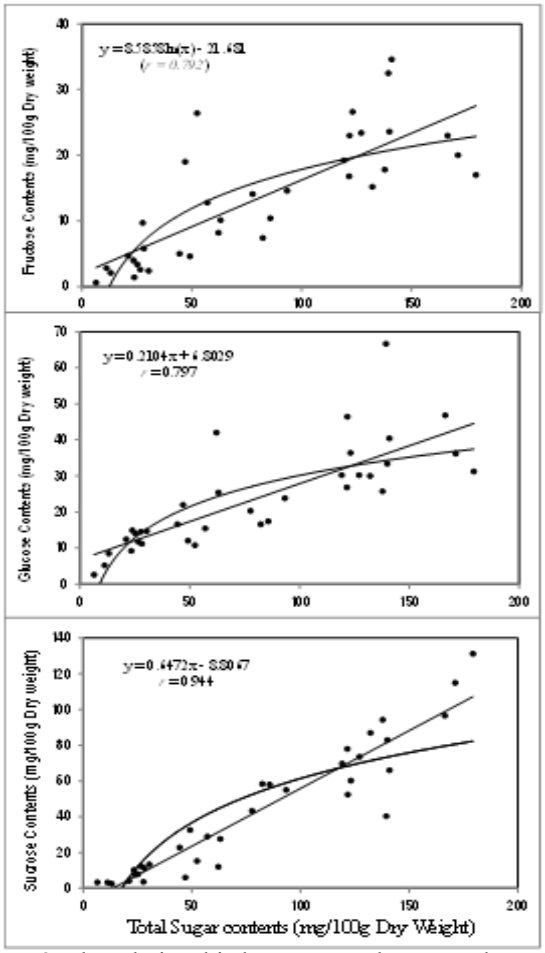

Figure 2. The relationship between total sugar and sucrose, glucose and fructose contents

conditions, biotic and /or abiotic stresses may changes the phenolic in plant (Woodhead, 1981; Conor *et al.*, 2002; Fujita *et al*., 2002; Bystricka *et al*., 2010; Stine *et al.*, 2011). However, the previous study on *A. cruentus* (Skwaryo-Bednarz and Krzepiko, 2009) found the higher amount of phenolic (1.57 mg and TAE 100 mg^{-1} of dry aerial materials) from our study. Literature data demonstrated that the variance within the varieties of *Amaranthus* accessions is, in general, highly influenced by environmental factors when the focus is on the content of Phenolic (Stine *et al.*, 2011), although phenolic in the seeds of *A. hypochondriacus* appears to be affected to a lesser degree by environmental factors (Stine *et al.*, 2011). Trolox was used as a universal standard for the calibration of all the methods used for the assessment of the total antioxidant activity because Trolox in DPPH is the most sensible one in the reaction with phenolic compounds, yielding almost 4.8-times higher values than FRAP method (Stratil *et al*., 2007, 2008). Similar to total phenolic Trolox equivalent antioxidant capacity (TEAC) shows wide variation in this study are considerably affected by genetic factors of individual accession and species. The previous study also demonstrated that the antioxidant capacity of the amaranth leaves was also found to be dependent on the amaranth variety (Skwaryo-Bednarz and Krzepiko, 2009).

Also, there was a positive but non-significant correlation ($R^2 = 0.0367$) between the total Phenolic content and antioxidant capacity of the plant samples. Numerous studies have described the relationship between phenolic content and antioxidant activity. Several authors have reported a strong correlation between phenolic content and the antioxidant activity (Velioglu *et al*., 1998; Khandaker *et al*., 2010). On the other hand, no such relationship was found by others (Kaehkoenen *et al*., 1999; Katerere *et al*., 2012) showing that the antioxidant activity of an extract cannot be predicted by its total phenolic content (Statue-Gracia *et al.*, 1997). This is probably because structure-activity relationships are governing the antioxidant activity (Nijveldt *et al*., 2001; Katerere *et al*., 2012). Therefore, there is a need to characterize phenolic compounds present within each accession's extract to allocate different antioxidant activities, to ascertain whether the phenolic structure affects antioxidant activity and also to determine whether synergism occurs among certain phenolic compounds.

Conclusion

However, the content of the soluble sugars, total phenolic and antioxidant capacity in the aerial parts of amaranth exhibit the wide variation among accessions and species. When selecting for a high and stable content of total phenolic in the amaranth studied, the accession PI 293758 of C. argentea var. cristata is the accession of highest potential. Accessions, PI 540446 and PI 558499 of *A. hypochondriacus* has a higher antioxidant capacity compare to other accessions tested. On the other hand, the accessions PI606797, PI 658731, PI 606797PI 451711 and Ames 5363 has higher soluble sugars contents. The significant difference among accessions and species regarding sugars, phenolic, and antioxidant profile also show their potential use in future breeding programs.

References

Ana, P., Milan P., Dubravko, M. and Zlatko, S. 2009. Yield and Quality of Forage Sorghum and Different Amaranth Species (*Amaranthus* spp.) Biomass. Agriculturae Conspectus Scientificus 74: 85-89.

Brand-Williams, W., Cuvelier, M.E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. Lebensm-Wiss Technology 28: 25-30.

Becker, R., Wheeler, E.L., Lorenz, K., Stafford, A.E., Grosjean, O.K., Betschart, A.A. and Saunders, R.M. 1981. A compositional study of amaranth grain.

Journal of Food Science 46: 1175-1180.

Brenner, D.M., Baltensperger, D.D., Kulakow, P. A., Lehmann, J.W., Myers, R.L., Slabbert, M.M. and Slaugh, B.B. 2000. Genetic resources and breeding of *Amaranthus*. Plant Breeding Reviews 19: 227-285.

Burns, M. and Bucar, F. 2000. Antioxidant activity of Nigella sativa essential oil. Phototherapy Research 14: 323–328.

Bystricka, U., Vollmannova, A., Margitanova, E. and Cicova, I. 2010. Dynamics of polyphenolics formation in different plant parts and different growth phases of selected buckwheat cultivars. Acta Agriculturae Slovenica 95: 225 - 229.

Carini, R., Poli, G., Diazini, M.U., Maddix, S.P., Slater, T.F. and Cheesman, K.H. 1990. Comparative evaluation of the antioxidant activity of a-tocopherol, a-tocopherol polyethylene glycol 1000 succinate and a-tocopherol succinate in isolated hepatocytes and liver microsomal suspensions. Biochemical Pharmacology 39: 1597–1601.

Conor, A.M., Luby, J.J., Tong, C.B.S., Finn, C.E. and Hancock, J.F. 2002. Genotypic and environmental variation in antioxidant activity, total phenolic content, and anthocyanin content among blueberry cultivars. Journal of the American Society for Horticultural Science 127: 89-97.

Costea, M., Waines, G. and Sanders, A. 2001. Notes on some little known Amaranthus taxa *(Amaranthaceae)* in the United States. Sida 19: 975–992.

De Troiani, R.M., Sanchez, T.M., Reinaudi, N.B. and De Ferramola, L.A. 2004. Optimal sowing dates of three species of grain- bearing Amaranth in the semi-arid Argentine Pampa. Spanish Journal of Agricultural Research 2: 385-391.

Folin, O. and Ciocalteu, V. 1927. On tyrosine and tryptophane determination in proteins. Journal of Biological Chemistry 73: 627-650.

Fujita M., Takeda K., Kohyama N., Doi Y. and Matsunaka, H. 2002. Genotypic variation in polyphenol content of barley grain. Euphytica 124: 55-58.

Huska, J. 1992. Biologization of a plant production. In Huska, J. (Ed). Proceedings of the plant production conference, p. 18. Slovakia: Slovak Agricultural University, Nitra.

Hauptli, H. 1977. Agronomic potential and breeding strategy for grain Amaranthus. In Hauptli, H. (Ed.) Proceedings of first Amaranthus Seminar, pp. 71-81. Pennsylvania: Rodale Press Inc.

Islam, M.S., Yoshimoto M. and Yamakawa, O. 2003. Distribution and physiological functions of caffeoylquinic acid derivatives in leaves of sweetpotato genotypes. Journal of Food Science 68: 111-116.

Juan, R., Pastor, J., Alaiz, M. and Vioque, J. 2007. Electrophoretic characterization of *Amaranthus* L., seed proteins and its systematic implications. Botanical Journal of Linnaean Society 155: 57-63.

Kaehkoenen, M., Hopia, A., Vuorela, H. and Rauha, J. 1999. Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. Journal of Agricultural and Food Chemistry 47: 3954-3962.

Katerere, D. R., Graziani, G., Thembo, K. M., Nyazema, N. Z. and Ritieni, A. 2012. Antioxidant activity of some African medicinal and dietary leafy African vegetables. African Journal of Biotechnology 1: 4103-4108.

Khandaker, L., Ali, M.B. and Oba, S. 2008. Total polyphenol and antioxidant activity of red amaranth (*Amaranthus tricolor* L.) as affected by different sunlight level. Journal of the Japanese Society for Horticultural Science 77: 395–401.

Khandaker, L., Akond, A., Ali, M.B. and Oba, S. 2010. Biomass yield and accumulations of bioactive compounds in red amaranth (*Amaranthus tricolor* L.) grown under different colored shade polyethylene in the spring season. Scientia Horticulturae 123: 289–294

Lewthwaite, S.L., Sutton, K.H. and Triggs, C.M. 1997. Free sugar composition of sweet potato cultivars after storage. New Zealand Journal of Crop and Horticultural Sciences 25: 33-41

Marin, D.I., Bolohan, C., Mihalache, M. and Rusu, T. 2011. Research on *Amaranthus cruentus* L. and *Amaranthus hypochondriacus* L. species grown in South-Eastern Romania, p. 297-303. Journal Series A - University of Agronomic Sciences and Veterinary Medicine (UASVM). Retrieved on May 27, 2016 from: *http://www.agro-bucuresti.ro/fisiere/file/Cercetare/Lucrari%20stiintifice%202011.pdf*

Mazza, G. 2000. Health aspects of natural colors. In Laurs, G.T. and Francis, F.J. (Eds). Natural Food and Colorants Science and Technology, p. 289-292. New York: CRC press.

Nijveldt, R.J., Van, N.E., Van, H.D.E., Boelens, P.G., Van, N.K. and Van, L.P.A. 2001. Flavonoids: a review of probable mechanisms of action and potential applications. The American Journal of Clinical Nutrition 74: 418-425.

Ogundipe, O.T. and Chase, M. 2009. Phylogenetic Analyzes of Amaranthaceae Based on matK DNA Sequence Data with Emphasis on West African Species. Turkish Journal of Botany 33: 153-161.

Paredes-López, O., Schevenin, M.L., Hernández-López, D. and Cárabez-Trejo, A. 1989. Amaranth starches isolation and partial characterization. Starch 41: 205–207.

Queiroz, Y.S., Soares, R.A.M., Capriles, V.D., Torres, E.A.F.D. and Areas, J.A.G. 2009. Effect of processing on the antioxidant activity of amaranth grain. Arch Latinoam Nutrition 59: 419–24.

Saunders, R.M. and Becker R. 1984. Amaranths: A possible food and feed resource. Advance Cereal Science and Technology 6: 357-396.

Singleton, V.L., Orthofer, R. and Lamuela-Ravento´s, R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Method of Enzymology 299: 152–178.

Skwaryo-Bednarz, B. and Krzepiko, A. 2009. Effect of various NPK fertilizer doses on the total antioxidant capacity of soil and amaranth leaves (*Amaranthus cruentus* L.). International Journal of Agrophysics 23:

61-65.

Statue-Gracia, M., Heionen, M. and Frankel, E. 1997. Antioxidant activity of anthocyanin in LDL and lecithin liposome systems. Journal of Agricultural and Food Chemistry 5: 3362-3367.

Stine, K.S., Rinnan, Å., Mortensen, A.G., Laursen, B., de Troiani, R.M., Noellemeyer, E.J., Steinmetz, K.A. and Potter J.D. 1996. Vegetables, fruits and cancer prevention. Journal of the American Dietetic Association 96: 1027-1039.

Stine, K.S., Rinnan, A., Mortensen, A.G., Laurse, B., De Troiani, R.M., Noellemeyer, E.J., Janovska, D., Dusek, K., Délano-Frier, J., Taberner, A., Christophersen, C. and Fomsgaard, I.S. 2011. Variations in the polyphenol content of seeds of field grown Amaranthus accessions. Food Chemistry 129: 131-138.

Stratil, P., Klejdus, B. and Kubaň, V. 2007. Determination of phenolic compounds and their antioxidant activity in fruits and cereals. Planta 71: 1741–1751.

Stratil, P., Kubaň, V. and Fojtova, J. 2008: Comparison of the phenolic content and total antioxidant activity in wines as determined by spectrophotometric methods. Czech Journal of Food Science 26: 242–253.

Sujak, A., Dziwulska-Hunek, A. and Kornarzynski, K. 2009. Compositional and nutritional values of amaranth seeds after pre-sowing He-Ne laser light and alternative magnetic field treatment. International Agrophysics 23: 81-86.

Svirskis, A. 2009. Prospects for Non-Traditional Plant Species Cultivated for Forage in Lithuania. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 37: 215-218.

Teutonico, R.A. and Knorr, D. 1985. Amaranth: composition, properties and applications of a rediscovered food crop. Food Technology 39: 49-61.

Velioglu, Y.S., Mazza, G., Gao, L. and Oomah, B.D. 1998. Antioxidant activity and total Phenolics in selected fruits, vegetables and grain products. Journal of Agricultural and Food Chemistry 46: 4113-4117.

Venskutonis, P.R. and Kraujalis, P. 2013. Nutritional components of amaranth seeds and vegetables: A review on composition, properties, and uses. Comprehensive Review in Food Science and Food Safety 12: 381-412.

Wheeler, J.R. 1992. Amaranthaceae. In Wheeler, J. R., Rye, B. L., Koch, B. L. and Wilson, A. J. G. (Eds.). Flora of the Kimberley Region- Department of Conservation and Land Management, p. 106–33. Como: Western Australian Herbarium.

Woodhead, S. 1981. Environmental and biotic factors affecting the phenolic content of different cultivars of *Sorghum bicolor*. Journal of Chemical Ecology 7: 1035-1047.

XLSTAT. 2016. Intuitive statistical software for PC and Mac- Integrates seamlessly into Microsoft Excel. Retrieved on May 04, 2016 from XLSTAT website: *http://www.xlstat.com/en/*