

Elemental Micronutrient Content and Horticultural Performance of Various Vegetable Amaranth Genotypes

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ABSTRACT. Vegetable amaranth (*Amaranthus* sp.), a leafy vegetable crop consumed around the world, is actively promoted as a source of essential micronutrients to at-risk populations. Such promotion makes micronutrient content essential to the underlying value of this crop. However, the extent to which micronutrient content varies by effect of genotype is not clear, leaving breeders uninformed on how to prioritize micronutrient contents as the criteria for selection among other performance parameters. A total of 32 entries across seven *Amaranthus* species were field-grown and analyzed for Fe, Mg, Ca, Zn, yield, height, and canopy spread comprising 20 entries at New Jersey in 2013; 12 entries at Arusha, Tanzania, in 2014; and 20 entries at New Jersey in 2015. The genotype effect was significant in all trials for Fe, Mg, Ca, Zn, total yield, marketable yield, height, and canopy spread. The Fe content range was above and below the breeding target of 4.2 mg/100 g Fe in all environments except for New Jersey 2015, where all entries were found to accumulate in levels below the target. All entries in each of the environments contained levels of Ca and Mg above breeding targets, 300 mg/100 g Ca and 90 mg/100 g Mg. None of the entries in any environment met the Zn breeding target of 4.5 mg/100 g Zn.

Vegetable amaranth is a mostly self-pollinated, diploid eukaryote with C₄ photosynthesis known to be consumed in over 50 countries, primarily across sub-Saharan Africa, south Asia, and southeast Asia (Achigan-Dako et al., 2014; Jain et al., 1982; National Resource Council, 2006). Vegetable amaranth is commonly cited as having unrealized potential to deliver mineral and vitamin micronutrients as well as protein to at-risk populations in regions with high rates of nutritional deficiencies (Weller et al., 2015).

Previous studies have shown success in selecting increased Fe and Zn content in rice (*Oryza sativa*) without consequence to yield performance; these entries of rice were later observed to

be effective as a food source for the improvement of human nutrition (Gregorio et al., 2000; Haas et al., 2005). Sufficient variability has also been shown to exist within the wheat (*Triticum aestivum*) germplasm to allow for the selection of high-Fe and high-Zn entries (Cakmak et al., 2000). The genotype × environment interaction (GEI) effect is a potential issue in selecting stable performance in any trait for plant breeders (Crossa, 2012; Gregorio et al., 2000). Following the observation of sufficient variability to select high-mineral-content entries, studies have shown a sufficiently low GEI effect on mineral contents to facilitate a successful selection for stable performance in maize (*Zea mays*) and wheat (Feil et al., 2005; Velu et al., 2012). Observing whether sufficient variability in the germplasm exists to select vegetable amaranth or otherwise concluding that no further selection is needed for these traits is the correct activity to initially assess the viability of using this crop as a tool for improving human nutrition.

A fundamental advantage of delivering micronutrients through staple crops is that it is recognized as less expensive than supplementation programs (Masuda et al., 2012). However, micronutrients are typically not accumulated in high concentrations in either seed or root/tuber tissue, making staple crops less easily bred or selected for delivering one or more micronutrients associated with common deficiencies; whereas

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leaf tissue is often observed to contain some level of most or all micronutrients (Beyer, 2010). Leafy green vegetables may have the inherent advantage of being readily selectable for high accumulation of multiple micronutrients.

Codex Alimentarius Guidelines for Use of Nutrition and Health Claims have defined “high source” thresholds to indicate the capacity to deliver daily required amounts of these targeted micronutrients by consuming a reasonable amount of material; i.e., 30% nutrient reference value (NRV) per 100 g (Codex Alimentarius, 1997). Crops which are recognized as being a high source by this definition for a given micronutrient are the most promising to improve nutrition status in populations with a known deficiency of that micronutrient (Feed the Future, 2014). For the purpose of this study, breeding targets were set as the high source thresholds per nutrient trait by Codex Alimentarius (1997) definitions: 4.2 mg/100 g Fe, 90 mg/100 g Mg, 300 mg/100 g Ca, and 4.5 mg/100 g Zn, by fresh weight basis.

Previous studies have evaluated vegetable amaranth for nutrition content and field performance with substantial variability observed from one study to another (Achigan-Dako et al., 2014; Luoh et al., 2014; Schönfeldt and Pretorius, 2011; Shukla et al., 2006, 2010). Variability reported among these studies may be due to environmental conditions, genetics of lines evaluated, processing, and methods of nutritional analysis. It remains unclear how to characterize this crop for inclusion in nutrition improvement projects, especially without consideration of the most recently developed World Vegetable Center (WorldVeg) lines and progenitors of cultivars for distribution in sub-Saharan Africa which have been included in this study.

In this study, the effect of genotype was observed on horticultural performance and nutrition content of four elements: Fe, Mg, Zn, and Ca, recognized as among the most commonly deficient in humans (World Health Organization, 2002, 2009, 2017). Horticultural traits observed include total yield, marketable yield, marketable percentage, plant height, and canopy spread. Marketable yield is an arguable term being that this crop is often sold with the full stem, with or without root attached. In this study, marketable yield was differentiated from the total yield and consists of only the leaves and tender stems which would typically be marketed and eaten. Quantifying the marketable yield and percentage for vegetable amaranth and other underdeveloped leafy green vegetables is an important performance trait given the high proportion of stems that are not typically consumed or desirable (National Resource Council, 2006). Yield observations limited to above-ground biomass alone would fail to distinguish rank order for economic or consumable yields which may provide greater value for both producers and consumers. California standards allow 18% stem by mass for spinach (*Spinacia oleracea*) yield reporting using a similar harvest method to allow regrowth for successive harvests (Koike et al., 2011).

Evaluation of the materials in this study under tropical and temperate climatic zones provides unique opportunity to understand the extent of their adaptation. This study is intended to verify the genotype effect by repeatedly observing the effect of genotype across these environmental conditions with varying entries. Data presented in this study may be considered as a speculative basis for indicating the effect of GEI for entries observed in common across trials, but should not be considered conclusive.

The inclusion of advanced WorldVeg lines, commercial entries, and genetic resources from the U.S. Department of Agriculture (USDA) in this study makes observation of these entries particularly relevant for guiding recommendations in selections of vegetable amaranth and future screening priorities toward using vegetable amaranth for improving human nutrition. The selection for nutritional content and horticultural performance from advanced WorldVeg lines, breeding materials from the USDA, or both may facilitate using vegetable amaranth as a cost-effective delivery mechanism for essential micronutrients.

The purpose of this study was to understand the importance of screening for micronutrient content to inform breeders how to treat micronutrient content as criteria for selection, in addition to basic horticultural performance. Given sufficient variation of micronutrient content, the development of new lines through plant breeding is recognized as an economic strategy to improve the nutritional status of undernourished people (Mayer et al., 2008).

Materials and Methods

PLANT MATERIALS. Vegetable amaranth entries observed in this study were sourced from USDA, WorldVeg, private seed companies, and an accession (RUAM44) collected in New Jersey (NJ), and a Rutgers University advanced breeding line (RUAM24). These entries consist of various species of amaranth, namely, *Amaranthus caudatus*, *A. cruentus*, *A. dubius*, *A. hybridus*, *A. hypochondriacus*, *A. retroflexus*, and *A. tricolor*, and were analyzed for foliar micronutrient content and horticultural parameters across three field trials (Table 1). Twenty entries were evaluated at Pittstown, NJ, in 2013; 12 entries at Arusha, Tanzania, in 2014; and 20 entries at Pittstown, NJ, in 2015.

EXPERIMENTAL LOCATIONS. The experiment in Arusha, Tanzania, was carried out on-station at WorldVeg, eastern and southern Africa (lat. 36.8°E, long. 3.4°S, 1290 m elevation) in 2014. The site is characterized by well-drained clay loam soil with pH 6.4. Seedlings in Arusha, Tanzania, were grown in 72-cell trays with sterilized media composed of forest soil/compost, manure, sand, and rice husks in a ratio of 3:2:1:1 by mass. A 20N–4.4P–8.3K fertilizer was applied to beds in Arusha at 200 kg·ha⁻¹ before transplanting on 7 Aug. 2014. Furrow irrigation was applied as needed. Urea (46N–0P–0K) was applied 3 weeks after transplanting at 120 kg·ha⁻¹.

The experiments in NJ were conducted at Snyder Research and Extension Farm in Pittstown, NJ (lat. 40.6°N, long. 75.0°W, 116 m elevation) in 2013 and 2015. The soil at this site is characterized as a silt loam. Seedlings used for field trials in Pittstown, NJ, were grown for 4 weeks in 72-cell trays with growing mix (Fafard Grow Mix 2; Sun Gro Horticulture, Agawam, MA) under greenhouse conditions at the Rutgers University Research Greenhouses in New Brunswick, NJ, until transplanted in raised beds with 0.032-mm black plastic mulch with drip irrigation applied as needed. Granular 5N–17.5P–50.2K was applied on 29 Mar. 2013 at 746 kg·ha⁻¹, 46N–0P–0K was applied on 28 May 2013 at 224 kg·ha⁻¹, and soluble 10N–13.1P–16.6K was applied at transplanting 6 June 2013 at 2.3 g·L⁻¹ at ≈0.12 L/plant. Granular 12N–17.5P–50.2K–10S–1Zn was applied on 3 Apr. 2015 at 313 kg·ha⁻¹, 46N–0P–0K was applied on 27 Apr. 2015 at 224 kg·ha⁻¹, and soluble

Table 1. Listing of the vegetable amaranth entries evaluated for elemental micronutrient content and horticultural performance in each location. Scientific name of entries described as reported. NJ 2013 and 2015 trials conducted at Snyder Research and Extension Farm in Pittstown, NJ; Arusha 2014 at World Vegetable Center (WorldVeg) regional center of east and southern Africa, Arusha, Tanzania.

Name	Location(s) tested	Scientific name	Source
PI 608019	NJ 2013	<i>Amaranthus caudatus</i>	USDA, ARS, Ames, IA
PI 566897	NJ 2013; NJ 2015	<i>A. cruentus</i>	USDA, ARS, Ames, IA
Ames 5693	NJ 2013; NJ2015	<i>A. hybridus</i>	USDA, ARS, Ames, IA
PI 511724	NJ 2013	<i>A. hybridus</i>	USDA, ARS, Ames, IA
PI 210995	NJ 2013	<i>A. hypochondriacus</i>	USDA, ARS, Ames, IA
PI 477915	NJ 2013	<i>A. hypochondriacus</i>	USDA, ARS, Ames, IA
RUAM24	NJ 2013; Arusha 2014; NJ 2015	<i>A. tricolor</i>	Rutgers University, New Brunswick, NJ
PI 604669	NJ 2013; NJ2015	<i>A. tricolor</i>	USDA, ARS, Ames, IA
UG-AM-40	NJ 2013; NJ2015	<i>Amaranthus</i> sp.	WorldVeg, Arusha, Tanzania
AM-AC-45	NJ 2013; NJ2015	<i>A. cruentus</i>	WorldVeg, Arusha, Tanzania
Madiira 2	NJ 2013; NJ2015	<i>A. cruentus</i>	WorldVeg, Arusha, Tanzania
AC-NL	NJ 2013; Arusha 2014; NJ 2015	<i>A. cruentus</i>	WorldVeg, Arusha, Tanzania
AH-TL	NJ 2013; Arusha 2014; NJ 2015	<i>A. hypochondriacus</i>	WorldVeg, Arusha, Tanzania
Madiira 1	NJ 2013; Arusha 2014; NJ 2015	<i>A. cruentus</i>	WorldVeg, Arusha, Tanzania
Ex-Zan	NJ 2013; Arusha 2014	<i>Amaranthus</i> sp.	WorldVeg, Arusha, Tanzania
UNZA-A1	NJ 2013; NJ2015	<i>Amaranthus</i> sp.	Zambia Seed Co., Lusaka, Zambia (lot no. 822304)
EASEED	NJ2013; NJ2015	<i>Amaranthus</i> sp.	East Africa Seed Co., Nairobi, Kenya (lot no. 11-10-5446)
JOHNNY	NJ 2013; NJ2015	<i>A. tricolor</i>	Johnny's Selected Seeds, Winslow, ME (lot no. 38208)
RUAM44	NJ 2013; NJ2015	<i>A. retroflexus</i>	Rutgers University, New Brunswick NJ
SIMLAW	NJ 2013	<i>Amaranthus</i> sp.	Simlaw Seed Co., Nairobi, Kenya (lot no. 07-08-7009-A)
DAVID	NJ 2013	<i>A. tricolor</i>	David's Garden Seeds, San Antonio, TX ("Red Leaf")
Ames 5100	Arusha 2014; NJ 2015	<i>A. tricolor</i>	USDA, ARS, Ames, IA
Ames 5102	Arusha 2014; NJ 2015	<i>A. tricolor</i>	USDA, ARS, Ames, IA
Ames 5354	Arusha 2014; NJ 2015	<i>A. tricolor</i>	USDA, ARS, Ames, IA
Ames 26211	Arusha 2014; NJ 2015	<i>A. tricolor</i>	USDA, ARS, Ames, IA
Ames 26212	Arusha 2014; NJ 2015	<i>A. tricolor</i>	USDA, ARS, Ames, IA
PI 667171	Arusha 2014; NJ 2015	<i>A. tricolor</i>	USDA, ARS, Ames, IA
IP-5	Arusha 2014	<i>A. cruentus</i>	WorldVeg, Arusha, Tanzania
TZSMN102	Arusha 2014	<i>A. cruentus</i>	WorldVeg, Arusha, Tanzania
PI 664489	NJ 2015	<i>A. cruentus</i>	WorldVeg, Arusha, Tanzania

10N–22.7P–8.3K was applied at transplanting on 17 June 2015 at 4.0 g·L⁻¹ at ≈0.12 L/plant.

EXPERIMENTAL DESIGN AND LAYOUT. All field experiments were arranged in a randomized complete block design with three replications. Plants were grown in double rows spaced 30 cm between plants within rows with 14 plants/plot. Plots were 2.1 m long and 1.2 m wide, spaced 1 m between plots and 2 m between plot rows. Plants were mechanically transplanted using a water wheel in NJ trials and by hand in Arusha. The four border plants in each plot were excluded from data collection and five of the 10 interior plants were randomly selected for data collection at the time of harvest.

DATA COLLECTION. Horticultural traits were observed at the time of harvesting. Five interior plants were randomly selected at the time of harvest to obtain plant height, canopy spread, and yield data for each of the three replications in each trial. Plant height data were collected by measuring the distance from the tallest apical shoot to the soil. Canopy spread data were collected by measuring the distance from furthest laterally growing leaf tips. The first harvest occurred between 21 and 28 d after transplanting into the field by cutting shoots 10 cm from the soil line to allow grow-back. Subsequent harvests occurred about every two weeks following the initial harvest. Total yield was recorded as the mass of five plants including all leaves and stems collected from the cutback portion of the

plant. Marketable yield was observed by recording the mass of leaves and tender stems after separating from thicker central and axial stems. The percentage of marketable yield to total yield was calculated by dividing the marketable yield by the total yield and multiplying by 100. Marketable yield was only recorded in trials following NJ 2013 as this trait had not been predicted to be significantly variable until observations made during the NJ 2013 trial.

An elemental micronutrient analysis was conducted on foliar subsamples of the dried yields from each line by inductively coupled plasma mass spectrophotometry at Penn State Agricultural Analytical Services Laboratory, University Park, PA. The elemental analysis was performed on the first harvest in each trial, limited to the dried leaf blades, leaf petioles, and stems of comparable diameter to that of the petioles. The samples in NJ 2013 and NJ 2015 were dried using a walk-in tobacco dryer unit with propane-heated, forced air set to 40 °C for ≈14 d. The Arusha 2014 samples were sun-dried in mesh bags on clean plastic trays laid on a concrete surface for ≈14 d, samples were moved under an open-air structure on concrete surface during the evenings and rain events. All dried samples were contained in paper bags until ground using a shearing-action mill. The results of the elemental analysis are reported on a fresh weight basis by converting from an average moisture content of 10% for the dry samples to 90% moisture, the

approximate USDA-reported water content for raw amaranth leaves (Muggeridge, 2000; USDA, 2016). This conversion was done to conservatively estimate the micronutrient content as it would typically be purchased and used for preparing dishes. Furthermore, this conversion allows direct comparison with data reported by the USDA “Standard nutrient database” and Codex Alimentarius Guidelines for Use of Nutrition and Health Claims.

STATISTICAL ANALYSIS. Analysis of variance (Proc ANOVA) and mean separation by Tukey’s Studentized range (HSD) test was performed using SAS (version 9.4; SAS Institute, Cary, NC) for data observations from each of the environments. Data were recorded from a single observation of each replicate for a sample size of three for each entry across all trials for each trait. For the traits of height and spread, the single value per replicate was recorded from the mean of five observations within the replication. The yield traits were recorded as a single value per replicate from the observation of weighing five plants from each replication.

Results

FOLIAR MICRONUTRIENT CONTENT. All entries in each of the trials were observed to have quantities of Ca and Mg above the breeding targets, 300 mg/100 g Ca and 90 mg/100 g Mg in all environments. Little variation was observed in the mean and range of both Ca and Mg contents in each trial with respect to the breeding targets, but significant differences were observed among entries for Ca and Mg in each trial (Tables 2–4). Rank-order change was observed for Ca content among the entries observed in common across trials. Madiira 1 was among the lowest-scoring entries in 2013 and 2014 with 396 and 366 mg/100 g Ca, respectively, yet performed moderately in 2015

with 426 mg/100 g; PI 566897 performed moderately in 2013 with 447 mg/100 g, yet had the lowest amount in 2015 with 386 mg/100 g. UNZA-A1 consistently contained relatively low Mg in the two trials it was observed with 124 and 174 mg/100 g Mg (Tables 2 and 4). Rank-order change in Mg content was observed between the entries included in common across the three trials (Tables 2–4).

Differences between entries in Zn content were significant in all trials (Tables 2–4). Variation between trials for Zn content was considerable with the means across all entries per trial being 0.788, 0.467, and 0.565 mg/100 g Zn, in 2013, 2014, and 2015, respectively. Ex-Zan had relatively high performance compared with the other entries in 2013 and 2014, with 1.07 and 0.663 mg/100 g Zn, respectively. None of the entries in any trial contained enough Zn to be recognized as high source.

The Fe content between entries was significantly different within all trials. The range observed for Fe content included the highest-containing entries having twice as much Fe as the lowest-containing entries in each trial. The mean Fe content of all entries was 5.56, 4.70, and 2.68 mg/100 g Fe in NJ 2013, Arusha 2014, and NJ 2015, respectively (Tables 2–4). All entries observed during the NJ 2013 and Arusha 2014 trials contained an amount of Fe either above or within HSD values from the breeding target of 4.2 mg/100 g (Tables 2 and 3). None of the entries observed during the NJ 2015 trial surpassed the Fe breeding target; half of the entries observed in NJ 2013 contained Fe within HSD value from the target (Table 4). RUAM24 performed above the breeding target in NJ 2013 and Arusha 2014 trials with 5.1 mg/100 g Fe and 7.17 mg/100 g Fe, respectively. RUAM24 accumulated the highest Fe content in NJ 2015 with 4.00 mg/100 g Fe (Table 4). Entries RUAM44 and PI 664489 also had relatively high Fe contents in NJ 2015 with 3.90 and 3.95 mg/100 g Fe, respectively (Table 4).

Table 2. Mean micronutrient content and horticultural performance of vegetable amaranth entries at New Jersey in 2013.

Entry	Fe (mg/100 g)	Ca (mg/100 g)	Mg (mg/100 g)	Zn (mg/100 g)	TY (kg) ^z	HGT (cm) ^z	SPR (cm) ^z
PI 608019	6.37	367	132	0.667	1.05	54.5	46.5
PI 566897	5.33	447	185	0.700	0.690	45.9	47.5
Ames 5693	3.67	529	145	0.767	1.55	69.4	58.3
PI 511724	4.33	502	171	0.600	0.987	44.9	55.8
PI 210995	5.60	434	115	0.833	0.930	49.7	52.8
PI 477915	4.37	529	144	0.800	1.05	48.0	58.3
RUAM24	5.10	410	157	0.767	0.627	26.1	42.0
PI 604669	9.07	471	161	0.833	0.523	23.6	32.1
UG-AM-40	4.63	475	147	0.767	0.680	46.7	47.8
AC-45	5.67	480	173	0.900	1.68	52.6	55.7
Madiira 2	5.60	423	165	0.833	1.09	30.0	52.5
AC-NL	4.27	417	160	0.633	1.03	40.6	53.3
AH-TL	5.13	489	157	0.633	1.54	51.2	63.5
Madiira 1	5.97	396	166	0.800	1.12	54.1	53.6
Ex-Zan	4.63	499	164	1.07	1.30	47.9	60.3
UNZA-A1	5.70	488	124	0.767	1.29	48.9	52.6
JOHNNY	5.03	506	135	0.800	0.500	28.0	45.9
RUAM44	7.83	443	160	0.733	0.930	31.5	52.5
SIMLAW	4.23	536	158	1.23	1.28	41.6	54.5
DAVID	8.67	410	153	0.633	0.433	28.5	39.6
<i>P</i> value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
HSD ^y	3.35	88.0	32.2	0.378	0.770	18.9	15.2

^zTY = total yield of five plants; HGT = plant height; SPR = canopy spread.

^yEntries significantly differed within columns at $P \leq 0.05$ if difference between entry means are greater than honestly significant difference value as calculated by Tukey’s Studentized range (HSD) test.

Table 3. Mean micronutrient content and horticultural performance of vegetable amaranth entries at Arusha, Tanzania, in 2014.

Entry	Fe (mg/100 g)	Ca (mg/100 g)	Mg (mg/100 g)	Zn (mg/100 g)	TY (kg) ^z	MY (kg) ^z	MP (%) ^z	HGT (cm) ^z	SPR (cm) ^z
Ames 26212	3.60	539	145	0.463	0.256	0.127	50	29.3	22.0
Ames 26211	5.20	552	145	0.463	0.380	0.183	48	31.6	30.0
RUAM24	7.17	437	145	0.457	0.763	0.515	68	29.7	36.1
Ames 5100	7.27	396	123	0.413	1.16	0.758	66	38.0	37.2
Ex-Zan	4.17	382	121	0.663	3.61	1.77	49	59.7	46.7
AH-TL	3.40	368	130	0.433	3.45	1.17	34	70.0	38.5
AC-NL	3.13	417	150	0.407	3.65	1.34	37	68.3	49.7
Madiira 1	3.10	366	145	0.479	2.69	1.14	44	83.2	31.4
GYT 135-30	2.70	421	110	0.583	3.51	1.49	43	68.7	37.5
GYT 135-13	3.20	372	133	0.413	2.66	0.993	37	61.9	39.7
PI 667171	6.00	396	134	0.436	0.833	0.491	59	38.2	29.1
Ames 5102	7.50	429	123	0.424	0.941	0.654	70	32.7	38.3
<i>P</i> value	<0.0001	<0.0001	0.0039	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002
HSD ^y	2.06	95.2	32.1	0.139	1.18	0.436	14.5	17.9	17.2

^zTY = total yield of five plants; MY = marketable yield of five plants; MP = marketable percentage; HGT = plant height; SPR = canopy spread. ^yEntries significantly differed within columns at *P* ≤ 0.05 if difference between entry means are greater than honestly significant difference value calculated by Tukey's Studentized range (HSD) test.

Table 4. Mean micronutrient content and horticultural performance of vegetable amaranth entries at New Jersey in 2015.

Entry	Fe (mg/100 g)	Ca (mg/100 g)	Mg (mg/100 g)	Zn (mg/100 g)	TY (kg) ^z	MY (kg) ^z	MP (%) ^z	HGT (cm) ^z	SPR (cm) ^z
PI 566897	1.78	386	216	0.465	1.33	0.700	53	72.9	51.9
Ames 5693	1.65	449	196	0.685	1.70	0.833	49	80.1	52.0
RUAM24	4.00	410	188	0.615	1.02	0.783	78	31.6	58.1
PI 604669	2.83	418	209	0.584	0.867	0.700	80	28.6	42.8
AC-45	2.34	422	189	0.667	1.90	0.800	43	71.1	56.2
Madiira 2	2.37	401	194	0.604	2.10	1.27	61	53.6	64.6
AC-NL	1.88	441	234	0.402	1.37	0.733	54	66.1	54.7
AH-TL	1.99	511	226	0.441	2.25	0.917	41	70.1	54.1
Madiira 1	2.12	429	243	0.449	1.77	0.917	52	78.1	52.0
UNZA-A1	1.95	470	174	0.670	1.63	0.933	58	66.6	52.2
EASEED	2.19	444	213	0.460	1.70	1.07	64	73.7	56.6
JOHNNY	2.64	442	247	0.454	1.07	0.633	60	45.2	51.1
RUAM44	3.90	501	189	0.752	1.42	0.717	51	71.7	62.4
Ames 5100	3.37	509	213	0.539	1.05	0.600	61	60.0	49.5
Ames 5102	3.26	506	210	0.595	1.30	0.817	63	81.7	50.9
Ames 5354	2.36	472	206	0.500	1.12	0.550	53	55.0	55.1
Ames 26211	2.83	461	214	0.638	0.967	0.650	68	65.0	48.8
Ames 26212	3.06	519	223	0.706	1.07	0.517	53	51.7	48.3
PI 667171	3.60	436	213	0.611	0.933	0.583	63	58.3	50.6
PI 664489	3.95	435	179	0.543	0.133	0.117	89	11.6	12.3
<i>P</i> value	<0.0001	<0.0001	0.0008	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	<0.0001
HSD ^y	1.74	96.6	68.9	0.175	0.825	0.403	31.5	18.5	13.5

^zTY = total yield of five plants; MY = marketable yield of five plants; MP = marketable percentage; HGT = plant height; SPR = canopy spread. ^yEntries significantly differed within columns at *P* ≤ 0.05 if difference between entry means are greater than honestly significant difference value calculated by Tukey's Studentized range (HSD) test.

HORTICULTURAL PERFORMANCE. World Vegetable Center entries consistently ranked as the highest yielding in both total yield (Tables 2–4) and marketable yield (Tables 3 and 4), yet were among the lowest ranking by marketable percentage. Entries ranking highest in marketable percentage, RUAM24 and PI 604669, 78% and 80%, respectively, in NJ 2015 were observed to have marketable percentages twice as high as lower scoring entries for marketable percentage. RUAM24 and PI 604669 had equally high marketable yields as other high-performing entries in NJ 2015, only lower than ‘Madiira 2’, observed to have the highest marketable yield (Table 4).

Differences among entries in plant height and canopy spread were significant in all trials. The furthest varying entry from the mean height and spread in all trials was PI 664489, included in the NJ 2015 trial for its shorter stature and dense architecture.

Discussion

The observation of significant differences in elemental micronutrient content among entries in this study supports the hypothesis that vegetable amaranth can be selected for improved performance in elemental micronutrient content

through a breeding program. The selection efforts for each of the four micronutrients observed in this study can be informed differently given the results with respect to breeding targets as previously described.

The significant variation on Fe content by genotype observed in this study indicates that selection for stable, high Fe content should be a priority target. This is especially important when selecting entries to be promoted for cultivation and marketing as a health-improving dietary choice, which is nearly always the case with vegetable amaranth. The relatively high Fe content in RUAM44 observed in 2015 could potentially be due to the more pubescent leaves common to *A. retroflexus* collecting airborne dust; however, this would not explain the high Fe content of RUAM24 during 2015, which is not pubescent.

Despite significant differences observed among entries for Ca and Mg contents in each trial (Tables 2–4), the performance of all entries in each trial was observed to be above both 300 mg/100 g Ca and 90 mg/100 g Mg. This indicates that consumption of vegetable amaranth can improve the nutrition status of individuals deficient in these micronutrients.

However, the values reported for raw amaranth in the USDA standard nutrient database are 55 mg/100 g Mg and 215 mg/100 g Ca, below the Codex Alimentarius high source threshold, which is the primary source of designating whether a crop may be implemented as a delivery mechanism of a given micronutrient source in Feed the Future initiatives (Feed the Future, 2014; USDA, 2016). The results of this study indicate that these vegetable amaranth entries could be used for direct release or use in breeding programs as reliable sources of Ca and Mg. However, further evaluation in other target environments before use or promotion for commercial cultivation should be conducted.

Zn content above or within HSD from the breeding target of 4.5 mg/100 g Zn was not observed in any of the entries in any trial. Significant variation was observed between entries in all trials. The result of this study indicates that vegetable amaranth would not be an effective source of Zn for reducing Zn deficiency in human diet (Codex Alimentarius, 1997; Feed the Future, 2014). Evaluating more accessions would be necessary to potentially identify entries with high-Zn content.

GEI analysis was not conducted because of the low number of common entries among the three trials. The environment is likely to have substantial effect given the observed difference of mean Fe content, particularly between NJ2013 and NJ2015 as these trials mostly included entries in common to each other. The relative Fe content of RUAM24, AC-NL, AH-TL, and Madiira 1 across the three trials was not consistent. RUAM24 was observed to have relatively low variation compared with the accessions included in all trials for Fe content with respect to the breeding target, supporting that it is possible to select for vegetable amaranth entries with sufficiently minimal effect by GEI.

The results for yield data revealed a discrepancy between some entries which have lower-ranking total yields yet high ranking marketable yields, as in the case of RUAM24 and PI 604669 in NJ 2015 (Table 4). Such observations suggest that screening by total yield alone would potentially advance less valuable entries because of a higher proportion of inedible stems. Entries advanced with consideration of marketable proportion may have the economic advantage of requiring less labor for processing into bundles or improved efficiency for

post-harvest storage space when processing is not conducted before marketing.

The selection for reduced height without penalty to marketable yield has potential benefits similar to those of selection for marketable proportion in that it may facilitate efficiency in harvesting, processing, transport, and storage of this crop, with considerably less labor required for data collection. Entries RUAM24 and PI 604669 were among the lowest ranking for height, suggesting the possibility of developing a method comparing height with total yield for efficient selection for marketable yield, yet this is not confirmed by the results of this study.

Significant but nominal differences among entries were observed for plant spread. Entries which could be planted more closely within rows with no reduction in yield could potentially make the total area for cultivation more productive. However, this was not observed among entries in this study.

Amaranth contains a rich source of Ca and Mg but not Zn. Based on this study, a breeding program to improve or increase Fe content of vegetable amaranth is possible. The results of this study can be used to guide breeding programs for vegetable amaranth and may provide a basis for estimating elemental micronutrient variability in crops for which these traits have not previously been selected. This study provides foundational information on the potential contribution of regular vegetable amaranth consumption toward the improvement of human nutrition.

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