IMPROVEMENT OF AFRICAN INDIGENOUS VEGETABLES FOR STABLE DELIVERY OF MICRONUTRIENTS

By

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ABSTRACT OF THE DISSERTATION

Improving African indigenous vegetables for delivery of stable micronutrient content

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Amaranth (*Amaranthus spp.*) is cultivated in over 80 countries as a preferred leafy green vegetable crop and is often cited as having unrealized potential to reduce multiple micronutrient deficiencies occurring at high rates in the countries where it is cultivated. Despite widespread cultivation and a wealth of genetic resources held by USDA GRIN and international germplasms, amaranth remains designated as an orphan crop due to a lack of development; producers are not often provided with options of cultivars as a leafy green vegetable. The goal of this dissertation research was to narrow the information gap preventing an effective cultivar development platform which serves goals of both farmers and organizations which promote Amaranthus among other indigenous vegetables to reduce prevalence of micronutrient deficiencies. Specific objectives were to: 1) establish breeding priorities to develop breeding lines and cultivars which can meet the needs of farmers and goals of international development goals focusing on orphan crops, 2) screen genetic diversity for traits of interest in entries which can either be utilized as breeding lines or fast-tracked for cultivar development 3) confirm consistency of genotype effect for prioritized traits and observe whether effect of genotype by environment interaction is sufficiently low to successfully select for these traits. Genotype effect on accumulation of Fe content was found to be substantial and consistently significant, with one entry identified

which accumulated high-source quantities of Fe across multiple environments including environments in which the mean Fe content across entries to fall well below high-source thresholds. A screening method to verify successful outcrossing events using SSRs was developed to facilitate breeding efforts with this crop which historically has relied on visible markers given the high rate of self-pollination, small, and highly numerous flowers on inflorescences of Amaranthus. The culmination of this study presents a case study of the first characterization of a crop to reliably provide high-source levels of three essential micronutrients which is aligned with the cultural preferences of populations often deficient in those micronutrients where it is cultivated, providing a platform for development of similar commodities toward alleviation of hidden hunger and wide-spread micronutrient deficiencies.

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DEDICATION

For my Mom, Dad, and brothers.

To my niece Ava, for affirming the power of dissent through asking honest questions.

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1.1 Introduction

Green leafy vegetables (GLV) are highlighted by the FAO and WHO among preferred foods to be consumed for optimal nutrition especially with respect to consuming adequate micronutrients (FAO, 2002; World Health Organization, 2004). Country-specific, food-based dietary guidelines invariably recommend consuming plenty of vegetables (Miller & Welch, 2013; USDA, 2015b).

Multiple micronutrient deficiencies may often occur simultaneously due to an inadequate diet (Best et al., 2011). Iron deficiency anemia has been estimated to affect 53% of school-age children worldwide (Rosso & Marek, 1996). Malnutrition, including vitamin A and zinc deficiency, has been found to be underlying 53% of the 10.6 million deaths of children under 5 years of age in sub-Sahara Africa (WHO, 2002).

Special feeding programs for women, infants, and children in the USA was found to be one of the most cost-effective methods to preventing premature death (Tengs et al., 1995). The UN sustainable development goal (SDG) 2, focused on food security and improved nutrition (UN, 2016), and SDG4, quality education are being simultaneously addressed by the home grown school meal program, which is now implemented in over 46 countries (World Food Programme, 2018). Students in many elementary schools across the USA are provided free produce through the United States Department of Agriculture (USDA) and Department of Defense (DoD) Fresh Fruit and Vegetable Program, promoting fresh market produce as “good low-calorie, low-fat sources of vitamins, minerals, and fiber,” (USDA, 2010).

While fortification of foods has been shown to reduce micronutrient deficiencies and anemia (Best et al., 2011), decision makers addressing at-risk populations with intervention strategies relying solely on locally produced, unfortified foods should have a basis for inclusion of commodities to meet specific health needs. A review such as this one is needed to consolidate and demonstrate current knowledge of GLVs within the broader class of fresh market produce for implicitly and often explicitly being promoted for content and efficacy of essential micronutrients to support or improve human health.

The treatment of micronutrient deficiencies including supplementation of multiple micronutrients has been found to have a greater effect on health outcomes than targeted, single-micronutrient supplementation alone (Best et al., 2011). Fresh produce and often GLVs especially are prioritized to address diet inadequacy, though without a reference to characterize which commodities may be most useful for the delivery of particular micronutrients, a well-diversified diet consisting of multiple commodities within this class with adequate portions may not result in dietary adequacy.

The procedure used by this review for inclusion of GLVs, data to characterize them by, and thresholds for distinction of micronutrient content follows the same procedure used by Feed the Future (FTF) (2016; 2014) for researchers implementing nutrition-sensitive indicators. Both the FAO and FTF refer to an expounded list provided by the WHO (2010) to guide researchers for including specific commodities in survey tools for making health inferences (Feed the Future, 2016; Food and Agriculture Organization, 2007). The USDA standard reference database is defined as the primary reference which researchers can use to characterize commodities by *Codex Alimentarius*-defined thresholds for being a "high source" of one or more micronutrients (Feed the Future, 2016). Commodities which are not listed in the USDA standard reference nutrient database should be characterized by the West African Food Composition Table published by the FAO (Feed the Future, 2016). Foods

which are not characterized by the USDA standard reference nutrient database nor the West African Food Composition Table may be characterized by country-specific resources (Feed the Future, 2016). The WHO list includes several cruciferous vegetables i.e. from the mustard family (*Brassicaceae*), which departs from some other characterizations of GLVs (Steinmetz & Potter, 1996), but is likely to be more aligned with consumers perception of what a GLV is for including crops like kale, Chinese cabbage (bok choy), and other leafy crucifers. The GLVs characterized by this review are listed in Table 1.1 with scientific name to prevent ambiguity often created by common names, and specifies the database supplying the information in subsequent tables.

The purpose of this review is to establish how to characterize GLVs with respect to addressing specific micronutrients deficiencies is needed to support registered dieticians and other health professionals to make dietary recommendations. This review is also intended to support initiatives intending to reduce micronutrient deficiencies through the promotion of GLVs.

A review of relevant data included the following: 1) an examination of regulations for qualifying and identifying commodities as nutrient-rich; 2) an evaluation of the nutritional composition of GLVs as presently reported by standard references; and 3) conclusions on the potential impact of consuming GLVs for making inferences on health outcomes with respect to micronutrient deficiencies.

1.2 Nutrition labeling and nutrient content claims of green leafy vegetables

Nutrient content claims are statements found on food products to distinguish foods as a source of one or more nutrients in agreement with the nutrition label (Marinangeli et al., 2017). Nutrient content claims have been shown to increase consumers' perception of healthfulness, perceived presence of healthful nutrients, and intentions to consume food

products (Iles, Nan, & Verrill, 2017). Nutritional labeling of fresh market produce in the United States is voluntary at the discretion of the retailer (US Government Publishing Office, 2002). This creates an information gap at the point of purchase for this commodity class which is widely recommended to supplement micronutrients.

Country-specific and international labeling guidelines for nutritional content claims of vitamins and minerals follow a similar pattern across jurisdictions, providing low and high thresholds for characterizing foods for the expected contents of given micronutrient with respect to jurisdiction-specific daily recommended consumption levels (Table 1.2 (Australian Government, 2016a; Canadian Food Inspection Agency, 2016; Codex Alimentarius, 1997; European Union, 2006, 2011; Food and Drug Administration, 2013)). Nutritional content claim criteria across jurisdictions differ slightly in threshold requirements, though *Codex Alimentarius* and European requirements are the most conservative with the exception of vitamin C requirements in Australia (Australian Government, 2016b).

Foods which can make nutrient content claims have a greater role in public health beyond customer influence. For studies applying FTF nutrition-sensitive indicators, quantitative improvements of health status can be inferred by observed consumption of a commodity which meets *Codex Alimentarius* high source thresholds for one or more micronutrients identified by FTF as problem micronutrients (Feed the Future, 2016) that has been documented as deficient in that region. The Codex Alimentarius Commission (CAC) was established by the FAO and WHO Food Standards Programme. Feed the Future methods for inferring health status improvement through observation of food consumption using dietary surveys relies on thresholds described in *Codex Alimentarius* Guidelines for Use of Nutrition and Health Claims.

The use of the most conservative international thresholds in this review should position the considerations that follow to apply across demographics and professional applications interested in the consumption- or consumption of GLV for health outcomes associated with micronutrient adequacy. *Codex* thresholds, being inclusive of minimum standards required to meet major regional and national requirements, may serve as a basis for comparison of commodities.

1.3 Characterizations of green leafy vegetables for micronutrient thresholds across selected commodities

The USDA Standard Nutrient Reference Database is the primary resource researchers are directed to for determining whether a commodity meets this criterion (Feed the Future, 2016); in this review it is the first reference cited to characterize each of the commodities listed on the non-comprehensive list of GLV provided by the WHO. Feed the Future further instructs researchers to refer to the West African Food Composition Table assembled by the FAO as needed, and then to any in-country ministry information that may be available (Feed the Future, 2016).

The GLVs listed in the tables in this review are not comprehensive across the GLV commodity class. Tables 1.2, 1.3, and 1.4 can be used for specifically characterizing commodities which are included, and to a more-limited extent, for consideration as a representative selection to consider how a randomly selected GLV might be characterized for a given micronutrient. Micronutrients included do not include vitamin D or vitamin B_{12} as they are not known to occur in plants and are not assigned values other than in the case of included "0" in databases.

"Dark green leafy vegetables" is an aggregate food category in both FAO and FTF dietary diversity assessment survey tools (Feed the Future, 2016; Food and Agriculture

Organization, 2007). Green leafy vegetables as an aggregate class may be difficult to characterize nutritionally due to including a diverse selection of commodities as well as an effect of variety and the environment on the genetic expression relative to the accumulation of minerals and vitamins within the edible plant part of commerce within specific commodities (Byrnes, Dinssa, Weller, & Simon, 2017; Feed the Future, 2016). Different cultures may often prefer different GLV commodities, making disaggregation essential.

Composition tables are known to vary widely. For analytical consistency, this review refers primarily to USDA standard nutrient reference database and then to the FAO-produced West African Food Composition Table to address foods from the WHO list which are not included in the USDA database as per FTF protocols. Color-coded demonstration (Tables 1.3 and 1.4) of "source" (light green) and "high source" (green) thresholds follows *Codex Alimentarius* guidelines which can serve to inform for adherence to any of the highest country or region thresholds, other than Vitamin C as per Australian regulations (Table 1.2), being that it is at least as conservative as country guidelines and abides by international organization guidelines for making health inferences (Codex Alimentarius, 1997; Feed the Future, 2016). The following summary provides a short description of each vitamin and mineral considered of importance for healthier diet and balanced nutrition.

1.3.1 Vitamin A

Among the many carotenoids observed to occur in fruits and vegetables, only those which have been found to have vitamin A activity are included for conversion to a vitamin A quantity for food labeling purposes; these carotenoids are referred to as pro-vitamin A carotenoids (Rose and Vasanthakaalam 2011). Pro-vitamin A carotenoids are converted in digestion to retinol, the active form of vitamin A in humans; while conversion rates and interactions have been observed to vary, conventions have been established for food labeling purposes (National Institutes of Health, 2013; Tang, 2010). While plants do not

provide vitamin A directly, the converted quantities of pro-vitamin A carotenoids as μg Vitamin A/100g will be discussed for consideration with international labeling conventions. From the 31 GLV commodities with data on vitamin A, 16 commodities could be considered high source, seven could be considered a source, and 8 did not contain enough to be considered a source (Table 1.3).

The WHO instructs researchers who are assessing infant and young child feeding practices to only include GLVs which can be characterized as sources of vitamin A by the same guidelines defined for this review (World Health Organization, 2010). The FAO explicitly describes GLVs as being rich in vitamin A, the WHO implicitly states as such (Food and Agriculture Organization, 2007; World Health Organization, 2010), and in a review paper with a less-inclusive definition of GLVs, all were categorized as adequately containing carotenoids (Van Duyn & Pivonka, 2000). In partial agreement with those references, other than for vitamin C and K, the GLVs included in this review do not meet or exceed source and high source thresholds as frequently for any other micronutrient. However, from the list of commodities provided by the WHO which the USDA or FAO had data to characterize for vitamin A, only slightly greater than 50% were found to be known to contain enough pro-vitamin A carotenoids to reasonably be expected to maintain or improve the health status of an individual or region at risk or suffering from vitamin A deficiency i.e. exceeded the high source threshold. Slightly under 25% were found to qualify for recognition as a source of vitamin A at all, slightly under 25% do not meet or exceed the threshold to be recognized as a source of vitamin A.

1.3.2 Thiamin

From the 32 commodities with data on thiamin, one could be considered high source, seven could be considered a source, and 23 did not contain enough to be considered a source.

1.3.3 Riboflavin

Out of the 32 commodities with data on riboflavin, seven could be considered high source, 5 could be considered a source, and 19 did not contain enough to be considered a source.

1.3.4 Niacin

Fiddlehead fern is the only standout commodity from those included in this review to exceed the high source threshold for niacin. Bean greens (leaves of a bean plant) is the only commodity that exceeded source threshold but was below the high source threshold for niacin. The remaining 30 commodities with data for niacin had been found to contain an insufficient quantity for source claims.

1.3.5 Pantothenic acid

Otherwise known as Vitamin B_5 and occasionally as "pantothenate", none of the commodities in this review exceeded high source thresholds for pantothenic acid. Chicory greens have been found to exceed the source threshold among the majority of commodities well below this threshold. Eight commodities did not have data on pantothenic acid including all which were sourced from the FAO database.

1.3.6 Folate

The GLV commodity class is often considered to be invariably rich in folate or folic acid specifically (Van Duyn & Pivonka, 2000). Of the 31 commodities, seven were found to exceed the high source threshold for folate, 13 were found to exceed the source threshold but not the high source threshold, 10 contained an insufficient quantity to exceed the source threshold. One commodity did not have data reported.

1.3.7 Vitamin C

All of the commodities included in this review were found to either exceed source, or high source thresholds. Four commodities contained only enough to be considered sources, while the remaining 27 contained enough to be considered high sources. Vitamin C is the least stable vitamin, it degrades, before and during cooking as storage, processing, exposure to oxygen and exposure to light are known to degrade this vitamin so the values in Table 1.3 may not accurately indicate the amount delivered (deMan, 2000a).

1.3.8 Vitamin K

All of the commodities included in this review that had data reported for vitamin K contained amounts substantially in excess above the high source threshold for vitamin K. Many commodities had not been analyzed as this nutrient has become a priority in recent years compared to many of the other micronutrients.

1.3.9 Calcium

The majority of GLVs were found not to exceed source thresholds for Ca content. Eleven were found to exceed the source threshold for Ca and two, baobab greens and lambs quarters have data reported which indicate these commodities to be the only two to exceed the high source threshold.

1.3.10 Iron

The majority of GLVs contained an insufficient amount of Fe to be categorized as either source or high source for this micronutrient. Eleven exceeded the source threshold, but not the high source threshold. Only one commodity, cassava greens, was found to exceed the high source threshold for Fe.

1.3.11 Magnesium

The majority of GLV commodities included in this review did not exceed the source threshold, and none of the commodities exceeded the high source threshold. Thirteen commodities were found to contain sufficient levels of Mg to be characterized as a source.

1.3.12 Zinc

None of the commodities included in this study were found to contain enough Zn to be characterized as a source. One commodity did not have data reported. Bean greens had the highest reported quantity of Zn, with 1.28 mg/100g, narrowly exceeding half the quantity designated as the source threshold.

1.4 Discussion

Presently, there is disagreement between the expected and the reported micronutrient content of GLV across commodities. Despite institutionally described associations of GLVs with vitamins and minerals, most GLVs should not be expected to readily deliver high source levels of any micronutrient, though the frequency of high-source content in GLVs varies across micronutrients (Figure 1.1).

Plant traits are generally subject to genetic by environmental interactions, to this end application of this data should be approached with skepticism without confirming performance of a specific variety of a commodity across multiple environments, preferably within each environment multiple times. Standard reference data and composition tables are limited in this respect, limiting results and conclusions drawn by this review.

Green leafy vegetables are aggregated as a discrete category in survey methods used to make health inferences on the regional, household, and individual level, indicating that those, whether cultivated or locally harvested should include “vitamin-A rich” leaves, although this is not explicitly defined (Feed the Future, 2016; Food and Agriculture

Organization, 2007; World Health Organization, 2010). The GLV commodity class is described by FAO Dietary Diversity Questionnaire (2007) as wild and locally available, "vitamin-A rich leaves such as amaranth, cassava leaves, kale, spinach, etc.". Several GLVs considered in this review would not be characterized as a source of Vitamin A, and nearly half of the GLVs would not be considered a high source of Vitamin A. Disaggregation of commodities in the GLV class should be considered for accurately assessing intake of Vitamin A.

Green leafy vegetables are commonly associated with calcium content. The dietary guidelines report from USDA highlights "mustard spinach" (*Brassica rapa var. perviridis*) as a source of calcium (USDA, 2015a). This commodity was not reported in Table 1.4 as it is not the same crop as mustard greens (*Sinapsis alba*) listed by the WHO (World Health Organization, 2010). If Ca sources were judged by mg Ca/calorie, mustard spinach as highlighted by the USDA, or other GLVs would lead this list otherwise populated largely by dairy sources (USDA, 2015a).

1.5 Conclusions

The findings of this review indicate that substantial differences exist between GLVs. and that these should often not be characterized as the same depending on the commodity and micronutrient. This creates an issue when attempting to address dietary deficiencies if incorrectly assuming a commodity has high source content of one or more micronutrients. This may have the unintended consequence of precluding the timely development of effective initiatives to improve health status in people with micronutrient deficiencies.

Greater consideration of the actual micronutrient content delivered is essential for the successful implementation of programs addressing micronutrient deficiencies by promoting the consumption of fruits and vegetables. Recognition of specific cultivars

selected for consistent delivery of high source levels of one or more micronutrients could lend credibility to the current systems described in this review.

Table 1.1. Modified list of green leafy vegetables from World Vegetable Organization (WHO) (2010)

Common name	Binomial or genus	Family	Database[z]	Identifier
Alfalfa leaves	*Medicago sativa*	Fabaceae	-	-
Amaranth greens (mchicha, xian cai)	*Amaranthus*	Amaranthaceae	USDA	11003
Arugula	*Eruca sativa*	Brassicaceae	USDA	11959
Balsam-pear (bitter gourd)	*Momordica charantia*	Cucurbitaceae	USDA	11022
Baobab greens	*Adansonia*	Malvaceae	FAO	04_001
Bean greens	*Phaseolus*	Fabaceae	USDA	11597
Beet greens (swiss chard)	*Beta vulgaris*	Amaranthaceae	USDA	11086
Bitter leaf (ewuro, ndole, onugbu)	*Vernonia calvoana*	Asteraceae	FAO	04_022
Broccoli	*Brassica oleracea*	Brassicaceae	USDA	11090
Broccoli rabe (broccoli raab)	*Brassica rapa*	Brassicaceae	USDA	11096
Carrot greens	*Daucus carota*	Umbelliferae	-	-
Cassava greens	*Manihot esculenta*	Euphotbiaceae	FAO	04_008
Chicory greens	*Cichorium intybus*	Asteraceae	USDA	11152

Chili greens	*Capsicum frutescens*	Solanaceae	-	-
Chinese cabbage (bok choy, pak choy)	*Brassica rapa*	Brassicaceae	USDA	11116
Chinese kale (chinese broccoli, kai-lai)	*Brassica oleracea*	Brassicaceae	USDA	11994
Collard greens (spring greens)	*Brassica oleracea*	Brassicaceae	USDA	11161
Cow pea greens	*Vigna unguiculata*	Papilionaceae	USDA	11201
Dandelion greens	*Taravacum*	Asteraceae	USDA	11207
Drumstick greens (moringa)	*Moringa oleifera*	Moringaceae	USDA	11222
Fenugreek greens (methi)	*Trigonella foenum*	Fabaceae	-	-
Fiddlehead fern (dod)	*Pteridium aquilinum*	Dennstaedtiaceae	USDA	11995
Garden cress (pepper grass)	*Lepidium sativum*	Brassicaceae	USDA	11203
Kale	*Brassica oleracea*	Brassicaceae	USDA	11233
Lamb's quarters (bathua)	*Chenopodium album*	Amaranthaceae	USDA	11244
Lettuce (bib, romaine)	*Lactuca sativa*	Asteraceae	USDA	11251

Malva greens (mallow)	*Malva vertcillata*	Malvaceae	-	-
Mustard greens[y]	*Sinapsis alba*	Brassicaceae	-	-
Okra (lady's finger, gumbo)	*Abelmoschus esculentus*	Malvaceae	FAO	04_004
Pumpkin greens	*Cucurbeta spp.*	Cucurbitaceae	USDA	11418
Purslane	*Portulaca olracea*	Portlacaceae	USDA	11427
Quinoa greens	*Chenopodium quinoa*	Amaranthaceae	-	-
Seaweed	*Caulerpa prolifera*	Caulerpaceae	-	-
Spinach	*Spinacia oleracea*	Amaranthaceae	USDA	11457
Sweet potato leaves	*Ipomoea batatas*	Concolvulaceae	USDA	11505
Tannia greens[x]	*Xanthosoma*	Araceae	-	-
Taro greens	*Colocasia esculenta*	Araceae	USDA	11520
Turnip greens	*Brassica rapa*	Brassicaceae	USDA	11568
Water cress	*Nasturtium officinale*	Brassicaceae	USDA	11591

Water spinach (swamp cabbage, kangkung)	*Ipomoea aquatica*	Convolvulaceae	USDA	11503
Yau choy	*Brassica napus*	Brassicaceae	-	

[z]Commodities which are not found in either the USDA, nor the FAO database have been excluded from this study, though they remain listed here without specification of database and identifier.

[y]USDA lists mustard spinach standard reference (SR) number 11274 as *Brassica rapa (*perviridis group), this crop is also known as Japanese mustard spinach or Komatsuna, and is not the same crop as *Sinopsis alba*, as listed by the WHO.

[x]WHO lists Tannia as *Xanthosoma spp.,* and FAO West African Food Composition Table provides data for *Xanthosoma* under identifier number 04_009; one of the data sources reported by FAO was USDA standard reference data on for Taro leaves SR no. 11520, which the USDA lists as *Colocasia esculenta.*

Table 1.2. Claim thresholds and requirements for nutrient content claims of vitamin and mineral micronutrients.

	Claim Threshold	Requirement
Australia[z]	General claim	10% of RDI or ESADDI
	"Good source"	25% of RDI or ESADDI
Canada[y]	"Contains," or "Source of"	5% of RDI
	"Good source of," "high in"	15% of RDI (30% for vitamin C)
	"Excellent source of," "very high in," "rich in," a valuable source of"	25% of RDI (50% for vitamin C)
Europe[x]	"Significant amount," or "source of," or "contains"	15% NRV
	"High source"	30% NRV
	"Good source," "contains," or	10%-19% of the DV per RACC.

USA[w]	"provides"	
	"High," "rich in," or "excellent source of"	20% or more of the DV per RACC
	"More," "fortified," "enriched," "added," extra," or "plus"	10% or more of the DV per RACC than an appropriate reference food.
Codex Alimentarius[v]	"Source"	15% NRV
	"High"	30% NRV

[z](Australian Government, 2016a)

[y](Canadian Food Inspection Agency, 2016)

[x](European Union, 2006, 2011)

[w](Food and Drug Administration, 2013)

[v](Codex Alimentarius, 1997)

Table 1.3. Vitamin content of green leafy vegetables listed in WHO (2010) according to USDA standard reference nutrient database and FAO West African food composition table.

Commodity[z]	Micronutrient[y]								
	Vitamin A (µg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Pantothenic acid[x] (mg)	Vitamin B6 (mg)	Folate (µg)	Vitamin C (mg)	Vitamin K (µg)
Amaranth greens	146	0.027	0.158	0.66	.	0.192	85	43.3	1140
Arugula	119	0.044	0.086	0.31	.	0.073	97	15	108.6
Balsam-pear	87	0.181	0.362	1.11	.	0.803	128	88	.
Baobab greens[w]	197	0.03	0.04	1.9	.	0.3	118	47	.
Bean greens[v]	405	0.833	0.602	3.47	0.136	0.232	16	45	.
Beet greens	316[u]	0.1	0.22	0.4	0.250	0.106	15	30	400
Bitter leaf	241	0.03	0.03	0.6	.	0.06	113	27	.

Broccoli	31[t]	0.071 (0.010)[s]	0.117 (0.20)[s]	0.639 (0.064)[s]	0.573 (0.046)[s]	0.175 (0.031)[s]	63 (12.748)[s]	89.2[r]	101.6[s]
Broccoli rabe	131[q]	0.162 (0.040)[q]	0.129 (0.014)[q]	1.221 (0.174)[q]	0.322 (0.083)[q]	0.171 (0.036)[q]	83 (7.514)[q]	20.2 (5.300)[q]	224.0 (23.561)[q]
Cassava greens[p]	286	0.25	0.46	1.6	.	0.3	118	39	.
Chicory greens	286	0.06	0.1	0.5	1.159	0.105	110	24	297.6
Chinese cabbage	223	0.04	0.07	0.5	0.088	0.194	66	45	45.5
Chinese kale	86	0.1	0.153	0.46	.	0.074	104	29.6	89.1
Collard greens	251[o]	0.054 (0.020)	0.130 (0.062)	0.742 (0.151)	0.267 (0.092)	0.165 (0.040)	129 (6.089)[n]	35.3 (5.022)	437.1[n]
Cow pea greens	36	0.354	0.175	1.12	0.06	0.177	101	36	.
Dandelion greens	508	0.19	0.26	0.81	0.084	0.251	27	35	778.4

Drumstick greens	378	0.257	0.66	2.22	0.125	1.2	40	51.7	.
Fiddlehead fern	181[m]	0.020 (0.001)	0.210 (0.013)	4.980 (0.085)	.	.	.	26.6 (2.415)	.
Garden cress	346	0.08	0.26	1	0.242	0.247	80	69	541.9
Kale	500[l]	0.11	0.13	1	0.091	0.271	141[n]	120	704.8[n]
Lambs quarters	580	0.16	0.44	1.2	0.092	0.274	30	80	.
Lettuce	436[k]	0.072 (0.002)[k]	0.067 (0.003)[k]	0.313 (0.006)[k]	0.142 (0.008)[k]	0.074 (0.003)[k]	136 (32.743)	4.0 (0.401)[k]	102.5 (7.222)[k]
Okra greens[j]	56	0.16	0.41	0.2	.	0.3	118	36	.
Pumpkin greens	97	0.094	0.128	0.92	0.042	0.207	36	11	.
Purslane	.	0.047	0.112	0.48	0.036	0.073	12	21	.
Spinach	469[l]	0.078 (0.008)	0.189 (0.008)	0.724 (0.032)	0.065 (0.008)	0.195 (0.008)	194 (35.597)	28.1 (4.129)	482.9

Sweet potato leaves	189[n]	0.156	0.345	1.13	0.225	0.19	1[n]	11	302.2[n]
Taro greens	241	0.209	0.456	1.51	0.084	0.146	126	52	108.6
Turnip greens	579	0.07	0.1	0.6	0.38	0.263	194 (4.220)	60	251
Water cress	160	0.09	0.12	0.2	0.31	0.129	9	43	250
Water spinach	315	0.3	0.1	0.9	0.141	0.096	57	55	.
High source thresholds	240	0.36	0.36	4.5	1.5	0.39	120	18	18

[z] Common name as listed by WHO (2010).

[y] Nutrients included in FTF problem nutrient list

[x] FTF lists "pantothenate", yet this vitamin is often referred to as pantothenic acid in foods (deMan, 2000b).

[w] Micronutrient data of commodities not listed in USDA standard reference nutrient database are listing data provided by the West African Food Composition Table. Citations are provided by FAO for each crop without specifying which reference is informative for each micronutrient (Achigan-Dako et al., 2009; Eyeson & Ankrah, 1975; FAO and USDA, 1968; Gning, Ndong, Wade, Dossou, & Guiro, 2007; Icard-Vernière et al., 2010; Prynne & Paul, 2011).

[v] (Achigan-Dako et al., 2009; Ejoh, Nkonga, Inocent, & Moses, 2007; Icard-Vernière et al., 2010)

[u] (Bureau & Bushway, 1986; Sweeney & Marsh, 1971).

[t] (ARS, 2001e, 2001b; Bureau & Bushway, 1986; R. J. Bushway, 1986; R. J. Bushway & Wilson, 1982; R. J. Bushway, Yang, & Yamani, 1986; Khachik, Beecher, & Whittaker, 1986; Sweeney & Marsh, 1971; Wu, Perry, & Klein, 1992).

[s] (ARS, 2001e, 2001b).

[r] (ARS, 2009).

[q] (ARS, 2001e, 2001d).

[p] (Endrias, 2006; Eyeson & Ankrah, 1975; Gning et al., 2007; Icard-Vernière et al., 2010; Perisse, Le Berre, Bergeret, & Masseyeff, 1957; West, Pepping, & Temalilwa, 1988).

[o] (ARS, 2006; Sweeney & Marsh, 1971).

[n] (ARS, 2006).

[m] (A. A. Bushway et al., 1985; R. J. Bushway & Wilson, 1982).

[l] (ARS, 2006; Quackenbush, 1987; Sweeney & Marsh, 1971).

[k] (ARS, 2001c, 2001a).

[j] (Icard-Vernière et al., 2010; West et al., 1988).

[l] (Bureau & Bushway, 1986; R. J. Bushway, 1986; Khachik et al., 1992, 1986; Sweeney & Marsh, 1971).

Table 1.4. Elemental micronutrient content of green leafy vegetables listed in WHO (2010) according to USDA standard reference nutrient database and FAO West African food composition table.

Commodity[z]	Micronutrient[y]			
	Ca (mg·100g^{-1} (SE))	Fe (mg·100g^{-1} (SE))	Mg (mg·100g^{-1} (SE))	Zn (mg·100g^{-1} (SE))
Amaranth greens	215	2.32	55	0.9
Arugula	160	1.46	47	0.47
Balsam-pear	84	2.04	85	0.3
Baobab greens[x]	313	3.9	52	0.9
Bean greens	224	4	8	1.28
Beet greens	117	2.57	70	0.38
Bitter leaf[w]	162	2.8	58	1.01
Broccoli	47 (5.130)[v]	0.73 (0.095)[v]	21 (2.104)[v]	0.41 (0.022)[v]

Broccoli rabe	108 (10.878)[u]	2.14 (0.361)[u]	22 (3.368)[u]	0.211 (0.46)[u]
Cassava greens[t]	276	5.5	58	0.69
Chicory greens	100	0.9	30	0.42
Chinese cabbage	105	0.8	19	0.19
Chinese kale	105	0.59	19	0.41
Collard greens	232 (10.092)[s]	0.47 (0.062)[s]	27 (2.703)[s]	0.21 (0.062)[s]
Cow pea greens	63	1.92	43	0.29
Dandelion greens	187	3.1	36	0.41
Drumstick greens	185	4	42	0.6
Fiddlehead fern	32	1.31	34	0.83
Garden cress	81	1.3	38	0.23
Kale	150[s]	1.47[s]	47[s]	0.56[s]

Lamb's quarters	309	1.2	34	0.44
Lettuce	33 (0.733)[r]	0.97 (0.079)[r]	14 (0.301)[r]	0.23 (0.013)[r]
Okra greens[q]	297	0.6	38	0.88
Pumpkin greens	39	2.22	38	0.2
Purslane	65	1.99	68	0.17
Spinach	99 (4.996)	2.71 (0.522)	79 (4.794)	0.53 (0.039)
Sweet potato leaves	78[r]	0.97[r]	70[s]	.
Taro greens	107	2.25	45	0.41
Turnip greens	190	1.1	31	0.19
Water cress	120	0.2	21	0.11
Water spinach (swamp cabbage, kangkung)	77	1.67	71	0.18
High source thresholds	300	4.2	90	4.5

[z] Common name as listed by WHO (2010).

[y] Nutrients included in FTF problem nutrient list

[x] (Achigan-Dako et al., 2009; Eyeson & Ankrah, 1975; FAO and USDA, 1968; Gning et al., 2007; Icard-Vernière et al., 2010; Prynne & Paul, 2011).

[w] (Achigan-Dako et al., 2009; Ejoh et al., 2007; Icard-Vernière et al., 2010).

[v] (ARS, 2001e, 2001b).

[u] (ARS, 2001e, 2001d).

[t] (Endrias, 2006; Eyeson & Ankrah, 1975; Gning et al., 2007; Icard-Vernière et al., 2010; Perisse et al., 1957; West et al., 1988).

[s] (ARS, 2006).

[r] (ARS, 2001c, 2001a).

[q] (Icard-Vernière et al., 2010; West et al., 1988).

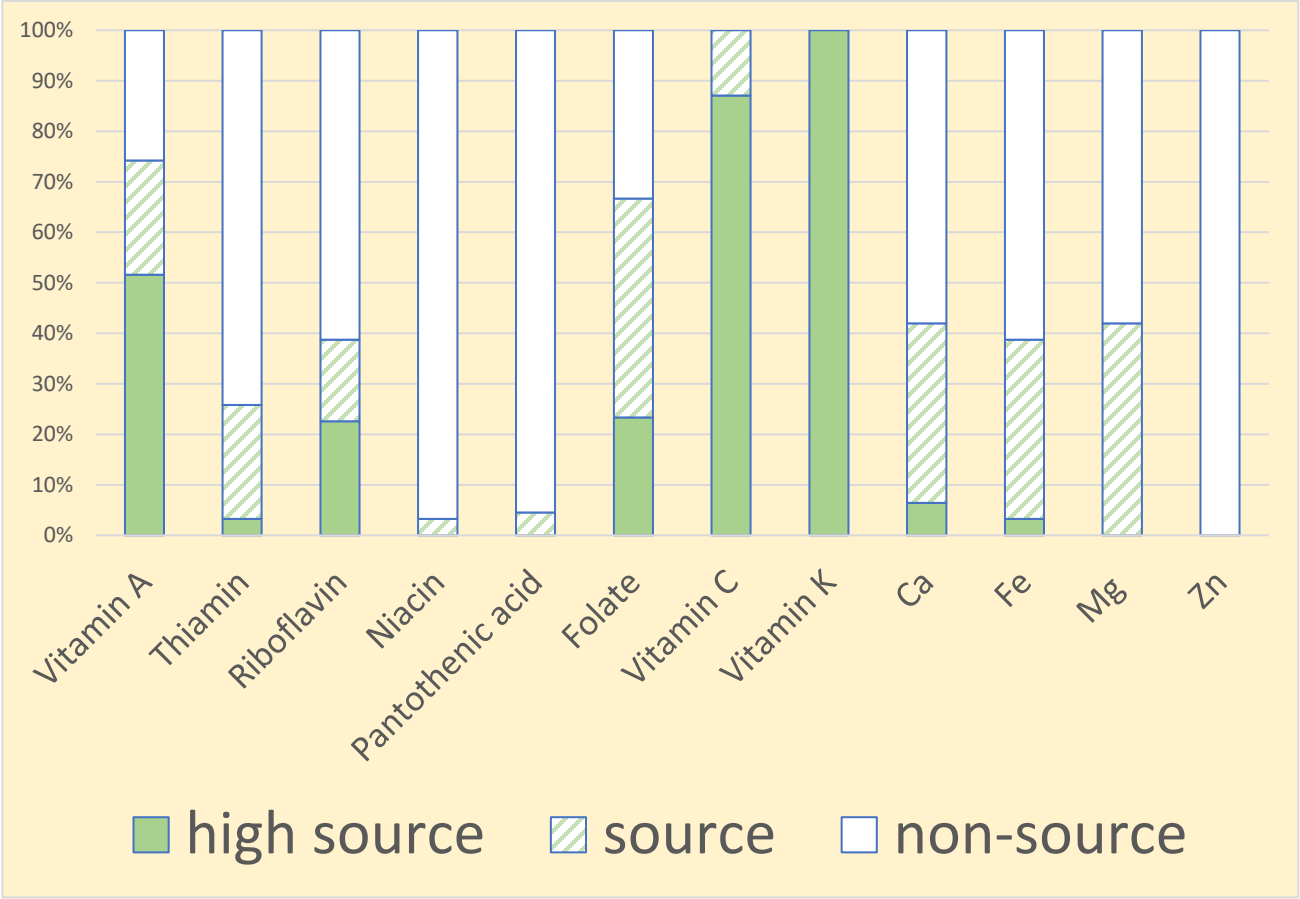

Figure 1.1. Percent of WHO-listed dark green leafy vegetables with "high source", "source", and below-"source" levels for each FTF-identified problem micronutrient.

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Chapter 2 Elemental Micronutrient Content and Horticultural Performance of Various Vegetable Amaranth Genotypes†

2.1 Introduction

Vegetable amaranth (*Amaranthus* sp.) 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 for 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 selection of high-Fe and high-Zn entries (Cakmak et al., 2000). The genotype x environment interaction (GEI) effect is a potential issue in selecting for stable performance in any trait for plant breeders (Crossa, 2012; Gregorio et al., 2000). Following the observation of sufficient variability to select for high-mineral-content entries, studies have shown sufficiently low GEI effect on mineral content to facilitate 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.

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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 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 high source by this definition for a given micronutrient are 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* 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-Sahara 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 roots 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 climactic zones provides unique opportunity to understand the extent of their adaptation. This study is intended to verify 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 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 utilizing 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).

2.2 Materials and Methods

2.2.1 Plant Materials

Vegetable amaranth entries observed in this study were sourced from USDA, WorldVeg, private seed companies, 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.

2.2.2 Experimental Locations

The experiment in Arusha, Tanzania was carried out on-station at WorldVeg, eastern and southern Africa (lat. 36.8ºE, 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. 20N–4.4P–8.3K fertilizer was applied to beds in Arusha at 200 kg·ha^{-1} prior to transplanting on 7 Aug. 2014. Furrow irrigation was applied as needed. Urea (46N-0P-0K) was applied 3 weeks after transplanting at 120kg·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 29 Mar. 2013 at 746 kg·ha^{-1}, 46N–0P–0K was applied 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 approximately 0.12 L per plant. Granular 12N–17.5P–50.2K–10S–1Zn was applied 3 Apr. 2015 at 313 kg·ha^{-1}, 46N–0P–0K was applied 27 Apr. 2015 at 224 kg·ha^{-1}, and soluble 10N–22.7P–8.3K was applied at transplanting 17 June 2015 at 4.0 g·L^{-1} at ≈ 0.12 L/plant.

2.2.3 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 time of harvest.

2.2.4 Data Collection

Horticultural traits were observed at the time of harvesting. Five interior plants were randomly selected at 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 was 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 entry by inductively coupled plasma (ICP) mass spectrophotometry at Penn State Agricultural Analytical Services Lab, 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. Samples in NJ 2013 and NJ 2015 were dried using a walk-in tobacco dryer unit using 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 a 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; U.S. Department of Agriculture, 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 to data reported by USDA "Standard nutrient database" and Codex Alimentarius Guidelines for Use of Nutrition and Health Claims.

2.2.5 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.

2.3 Results

2.3.1 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 to 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 were 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).

2.3.2 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, 078% 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.

2.4 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 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 *Amaranthus 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; U.S. Department of Agriculture, 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. Further evaluation in other target environments prior to 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 to 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 due to 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 prior to 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 to 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 contained high source levels of Ca and Mg but not Zn in this study. Based on the results of 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.

Table 2.1. Listing of the vegetable amaranth (*Amaranthus* sp.) 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	*A. caudatus*	USDA, ARS. Ames, Iowa
PI 566897	NJ 2013; NJ 2015	*A. cruentus*	USDA, ARS. Ames, Iowa
Ames 5693	NJ 2013; NJ2015	*A. hybridus*	USDA, ARS. Ames, Iowa
PI 511724	NJ 2013	*A. hybridus*	USDA, ARS. Ames, Iowa
PI 210995	NJ 2013	*A. hypochondriacus*	USDA, ARS. Ames, Iowa
PI 477915	NJ 2013	*A. hypochondriacus*	USDA, ARS. Ames, Iowa
RUAM24	NJ 2013; Arusha 2014; NJ 2015	*A. tricolor*	Rutgers University, New Brunswick, NJ
PI 604669	NJ 2013; NJ2015	*A. tricolor*	USDA, ARS. Ames, Iowa

UG-AM-40	NJ 2013; NJ2015	*Amaranthus* sp.	WorldVeg, Arusha, Tanzania
AM-AC-45	NJ 2013; NJ2015	*A. cruentus*	WorldVeg, Arusha, Tanzania
Madiira 2	NJ 2013; NJ2015	*A. cruentus*	WorldVeg, Arusha, Tanzania
AC-NL	NJ 2013; Arusha 2014; NJ 2015	*A. cruentus*	WorldVeg, Arusha, Tanzania
AH-TL	NJ 2013; Arusha 2014; NJ 2015	*A. hypochondriacus*	WorldVeg, Arusha, Tanzania
Madiira 1	NJ 2013; Arusha 2014; NJ 2015	*A. cruentus*	WorldVeg, Arusha, Tanzania
Ex-Zan	NJ 2013; Arusha 2014	*Amaranthus* sp.	WorldVeg, Arusha, Tanzania
UNZA-A1	NJ 2013; NJ2015	*Amaranthus* sp.	Zambia Seed Co. Lusaka, Zambia. Lot no. 822304
EASEED	NJ2013; NJ2015	*Amaranthus* sp.	East Africa Seed Co. Nairobi, Kenya. Lot no. 11-10-5446.
JOHNNY	NJ 2013; NJ2015	*A. tricolor*	Johnny's Selected Seeds. Winslow, ME. Lot no. 38208
RUAM44	NJ 2013; NJ2015	*A. retroflexus*	Rutgers University, New Brunswick NJ
SIMLAW	NJ 2013	*Amaranthus* sp.	Simlaw Seed Co. Nairobi, Kenya. Lot no. 07-08-7009-A
DAVID	NJ 2013	*A. tricolor*	David's Garden Seeds. San Antonio, TX. “Red Leaf”

Ames 5100	Arusha 2014; NJ 2015	*A. tricolor*	USDA, ARS. Ames, Iowa
Ames 5102	Arusha 2014; NJ 2015	*A. tricolor*	USDA, ARS. Ames, Iowa
Ames 5354	Arusha 2014; NJ 2015	*A. tricolor*	USDA, ARS. Ames, Iowa
Ames 26211	Arusha 2014; NJ 2015	*A. tricolor*	USDA, ARS. Ames, Iowa
Ames 26212	Arusha 2014; NJ 2015	*A. tricolor*	USDA, ARS. Ames, Iowa
PI 667171	Arusha 2014; NJ 2015	*A. tricolor*	USDA, ARS. Ames, Iowa
IP-5	Arusha 2014	*A. cruentus*	WorldVeg, Arusha, Tanzania
TZSMN102	Arusha 2014	*A. cruentus*	WorldVeg, Arusha, Tanzania
PI 664489	NJ 2015	*A. cruentus*	WorldVeg, Arusha, Tanzania

Table 2.2 Means of micronutrient content and horticultural performance of vegetable amaranth entries at New Jersey in 2013. Fe, Ca, Mg, and Zn reported as (mg·100 g^{-1}); TY, total yield of five plants; MY, marketable yield of five plants; HGT, plant height; SPR, canopy spread.

Entry	Fe (mg·100 g^{-1})	Ca (mg·100 g^{-1})	Mg (mg·100 g^{-1})	Zn (mg·100 g^{-1})	TY (kg)	HGT (cm)	SPR (cm)
PI 608019	6.37	367	132	0.667	1.053	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
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
HSD[z]	3.35	88.0	32.2	0.378	0.770	18.9	15.2

[z]Entries significantly different 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 2.3. Means of micronutrient content and horticultural performance of vegetable amaranth entries at Arusha, Tanzania in 2014. Fe, Ca, Mg, and Zn reported as (mg·100 g^{-1}); TY, total yield of five plants; MY, marketable yield of five plants; MP, marketable percentage; HGT, plant height; SPR, canopy spread.

Entry	Fe (mg·100 g^{-1})	Ca (mg·100 g^{-1})	Mg (mg·100 g^{-1})	Zn (mg·100 g^{-1})	TY (kg)	MY (kg)	MP (%)	HGT (cm)	SPR (cm)
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
P value	<0.0001	<0.0001	0.0039	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002
HSD[z]	2.06	95.2	32.1	0.139	1.18	0.436	14.5	17.9	17.2

[z]Entries significantly different within columns at $P \leq 0.05$ if difference between entry means are greater than honestly significant difference value calculated by Tukey's Studentized Range (HSD) Test.

Table 2.4. Means of micronutrient content and horticultural performance of vegetable amaranth entries at New Jersey in 2015. Fe, Ca, Mg, and Zn mg·100 g^{-1}; TY, total yield of five plants; MY, marketable yield of five plants; MP, marketable percentage; HGT, plant height; SPR, canopy spread.

Entry	Fe (mg·100 g^{-1})	Ca (mg·100 g^{-1})	Mg (mg·100 g^{-1})	Zn (mg·100 g^{-1})	TY (kg)	MY (kg)	MP (%)	HGT (cm)	SPR (cm)
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
P value	<0.0001	<0.0001	0.0008	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	<0.0001
HSD[z]	1.74	96.6	68.9	0.175	0.825	0.403	31.5	18.5	13.5

[z]Entries significantly different within columns at $P \leq 0.05$ if difference between entry means are greater than honestly significant difference value calculated by Tukey's Studentized Range (HSD) Test.

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Chapter 3 Genotype x environment interaction for elemental micronutrient content of vegetable amaranth, Ethiopian mustard, edible nightshade and spiderplant

3.1 Introduction

Vegetable amaranth (*Amaranthus sp.*), Ethiopian mustard (*Brassica carinata*), edible nightshade (*Solanum scabrum*), and spiderplant (*Cleome gynandra*), are leafy green vegetables consumed in at least 50 countries combined across sub-Saharan Africa, South Asia, Southeast Asia, and the Caribbean (Achigan-Dako, Sogbohossou, & Maundu, 2014; National Resource Council, 2006). High rates of micronutrient deficiencies in these regions have attracted attention to these culturally preferred horticulture crops to provide micronutrients as a public health benefit that comes with self-sufficient economic growth of smallholder farmers (National Resource Council, 2006; Weller, Van Wyk, & Simon, 2015).

Micronutrients for human consumption are typically categorized as either vitamins or elemental minerals. Elemental micronutrients are not metabolic products of plants and must be obtained from the environment, supporting the hypothesis that plants are limited from accumulating meaningful concentrations of these minerals in severely deficient soils (Ortiz-Monasterio et al., 2007). However, consistent observation of significant genotype effect, from both selection and genetic modification in various staple and horticultural crops supports that meaningful gains in elemental micronutrient content can be realized toward being utilized for reduction of nutritional deficiencies in the environments (Byrnes, Dinssa, Weller, & Simon, 2017; Kodkany et al., 2013; Masuda et al., 2012).

The concentration of Fe in solution has long been understood to decrease exponentially as pH increases, becoming less readily available for uptake by plants as Fe^{2+} is converted to the less water-soluble Fe^{3+}; this phenomenon has long made Fe availability

considered to be the most problematic among essential mineral nutrients for plants (Oertli & Jacobson, 1969). The capacity for plants to tolerate high pH soils with respect to Fe sufficiency has been demonstrated to be controlled genetically with either of two different mechanisms. In grasses, a strong chelator of Fe^{3+} is released and a specific uptake system transports without reducing to Fe^{2+} (Römheld & Marschner, 1986). In non-grasses, entries capable of overcoming Fe unavailability in high pH soils have been demonstrated to reduce F^{3+} to Fe^{2+} within the roots prior to transport (Brown, 1977). The genes that have been identified for being involved in these processes have consistently fit these models (Kobayashi & Nishizawa, 2012).

The bulk of studies in the literature on identifying genetically improved elemental mineral use in plants are primarily interested in tolerance to adverse environments and efficiency. This may result in inapplicable findings relative to high accumulation if the mechanism of tolerance is based on functioning with less rather than increased uptake. Iron-efficiency as observed previously does not appear to raise this concern as those with genetic adaptation found to be efficient for Fe usage have been shown to have much higher Fe in the leaves than those which were Fe deficient under the same conditions (Brown, Chaney, & Ambler, 1971). Genotypic resistance to calcium stress expressed as blossom end rot in tomato (*Lycopersicon esculentum*) however, has been demonstrated to be expressed as a function of both having a lower requirement as well as greater uptake (Greenleaf & Adams, 1969).

Heavy metal toxicity is known to cause Fe deficiency symptoms in plants, though some Fe-efficient varieties have been demonstrated to be capable of tolerating elevated quantities of heavy metals in the soil (Brown & Jones, 1975). This could present an unintended problem, for allowing full production of crops that can contain minerals which are deleterious to

human health from contaminated soils which would otherwise be unable to produce crops successfully.

The accumulation of Ca in plants is associated with soil conditions; plants grown on calcareous soils are expected to have high quantities of soluble Ca^{2+}, while those grown in acidic and low-calcium soil will accumulate very little Ca^{2+} (Läuchli, 1976). However, genotype effect on Ca-efficiency has been reported in several plants including tomatoes, snap beans, and broccoli (Caines & Shennan, 1999; Farnham, Grusak, & Wang, 2000; Quintana, Harrison, Nienhuis, Palta, & Grusak, 1996).

The environmental availability of Mg in soil may be induced by low soil pH, though this is limited if Mg is in abundance as in the case of certain soil parent material and proximity to major bodies of saltwater (Marschner, 1991). Efficiency of Mg has been observed in plants as an effect of genotype (Farnham et al., 2000).

Differential Zn efficiency has been observed in grasses as an effect of genotype (Cakmak et al., 1996; Graham, Ascher, & Hynes, 1992). The mechanism of Zn efficiency has been supported to be associated with the release of phytosiderophores i.e. Fe-chelating compounds, from the roots allowing greater uptake of Zn (Cakmak et al., 1996). Across Zn-efficient lines, however, an unspecified interaction has been observed weakening the correlation of phytosiderophore release with Zn-efficiency; this has been speculated to be due to transport mechanisms following uptake from soil (Erenoglu et al., 1996).

The occurrence of both Ca and Mg stress in plants is associated with soil acidity, yet the efficient use of both of these minerals is not always correlated (Farnham et al., 2000). Anion transport is facilitated by a contrast of low H^+ concentration in the soil and a higher concentration in the plasma membrane; conversely, cation transport is inhibited by a lower

soil pH, particularly Mg, which may partially demonstrate how efficiency for use of both minerals would not necessarily be correlated (Marschner, 1991).

Vegetable amaranth has previously been characterized for micronutrient content in a number of studies and is listed on the USDA National Nutrient Database for Standard Reference, yet this data includes a wide range of variability between studies and often lacks description of methodology used to quantify micronutrients (Achigan-Dako et al., 2014; Luoh, Begg, Symonds, Ledesma, & Yang, 2014; Schönfeldt & Pretorius, 2011; Shukla et al., 2006; Shukla, Bhargava, Chatterjee, Pandey, & Mishra, 2010).

A consistently significant effect by genotype on Fe, Ca, Mg, and Zn content was observed in vegetable amaranth by Byrnes et al. (2017). Observing the effect of genotype by environment interaction (GEI) is necessary to determine how to proceed with selection of entries for nutrition delivery as a complement to horticultural performance. Genetic by environment interaction, or phenotypic plasticity effect, is the phenomenon of differential trait expression between genotypes in response to environmental differences between trials (Kang, 2004). Observation of GEI is essential to variety development to reliably predict performance stability in the expression of traits of interest. GEI analysis should be conducted with genotypes of interest observed in target environments to observe performance relative to where the genotypes are intended to be promoted for cultivation.

The two basic expressions of GEI are with rank change, also known as crossover, or without rank change across environments. Sufficiently moderate GEI facilitates selection as this indicates stability in performance, while substantial GEI limits accurate prediction of genotype performance. GEI with rank order change may indicate genotypes should be selected for promotion specific to environment (Crossa, 2012; Gregorio, Senadhira, Htut, & Graham, 2000). The effect of GEI has been reported in several crops, including grain

amaranth (Bednarz, Bridges, and Brown 2000; Mekbib 2003; Riday and Brummer 2006; Fan et al. 2007; Mulema et al. 2008; De Vita et al. 2010; Guillen-Portal, F.R., Baltensperger, D.D., Nelson, L.A., D'Croz-Mason 1999) (Dia et al., 2016; Edwards, 2016; Tumwegamire et al., 2016).

There are several methods which have been introduced to observe GEI and stability through previous decades to the present. The Additive Main effects and Multiplicative Interaction (AMMI) method, previously known as FANOVA, was introduced for situations where both main effects and interaction are important (Gollob, 1968; Mandel, 1971; Zobel, Wright, & Gauch, 1988). The application of this multivariate approach updated the various univariate approaches and was found to have the effect of improved precision on yield estimates equivalent to increasing replications by a factor of 2.59 (Crossa, 1990). Genotype by genotype x environment interaction (GGE) analysis (Yan, Hunt, Sheng, & Szlavnics, 2000) is a similar method to AMMI with considerations for optimal applications of each method often discussed (Dia et al., 2016; Gauch, Piepho, & Annicchiarico, 2008).

An underlying issue of GEI models is that the observed genotype effect is affected by the environments included and vice-versa (Marcos Malosetti, Ribaut, & van Eeuwijk, 2013). Environments are generally expected to be the majority source of variance. To ensure significance of variation by genotype, genotypes likely to be commercially irrelevant are incentivized for inclusion. While this may be argued to be logical as an experimental control method, this study prioritizes advanced lines and cultivars.

In this study, the AMMI model is used indicate performance interactions with genotypes, environments, and the interactions thereof where pertinent (see discussion). A mixed model was applied to partition variance by genotype, environment, and GEI as has become commonly performed across statistical software platforms for the analysis of multi-

environment trial data for various traits and crops with multiple genotypes being compared (Gilbert et al., 2015; Jat et al., 2018; M. Malosetti, Voltas, Romagosa, Ullrich, & Eeuwijk, 2004; Mathews et al., 2008; van der Voet, Perry, Amzal, & Paoletti, 2011). The GEI variance was partitioned by genotype within the mixed model analysis where potential for selection of a genotype with relative stability appeared feasible, as also performed in (Gilbert et al., 2015).

The USDA National Nutrient Database for Standard Reference assigns a value to vegetables and vegetable products a value for each micronutrient from a variable sample size without differentiation by variety or genotype. This is the primary resource researchers can use to verify whether a food can be considered high source by FAO definitions (Feed the Future, 2016). A food which is recognized as meeting or exceeding high source thresholds for one or more micronutrients may be used to quantitatively evaluate health status improvement by observing consumption of the food by individuals deficient in those micronutrients. The target quantities of micronutrients in this study are assigned as defined in *Codex Alimentarius* Guidelines for Use of Nutrition and Health Claims. "High source" thresholds are defined as being the content necessary to provide at least 30% of the Nutrient Reference Value (NRV) per micronutrient if 100g is consumed: 4.2 mg/100g Fe, 90 mg/100g Mg, 300 mg/100g Ca, and 4.5 mg/100g Zn, by fresh weight basis (Codex Alimentarius, 1997). "Source" thresholds are defined as half the value of respective high source thresholds (Codex Alimentarius, 1997).

A GEI analysis of micronutrient content is needed for AIVs to understand whether a crop, or a genotype within a crop, can be reliably used to improve the health status of people with one or more micronutrient deficiencies.

3.2 Materials and Methods

3.2.1 Germplasm

The genotypes included for each crop are detailed in Table 3.1 below. The genotypes include advanced lines and cultivated varieties developed by the World Vegetable Center and other breeding groups such as RUAM24, which was previously found to have high marketable proportion, competitive marketable yield, and high Fe content (Byrnes et al., 2017).

3.2.2 Experimental design and field sites

All field experiments were arranged in a randomized complete block design (RCBD) with three replications for amaranth and four replications each for Ethiopian mustard, edible nightshade, and spiderplant due to having fewer entries. Plants were grown in double rows spaced 30 cm between plants within rows with 14 plants per 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 New Jersey (NJ) field trials and by hand in both Kenya and Tanzania. The four border plants in each plot were excluded from data collection and the interior plants were observed for data collection at time of harvest.

3.2.2.1 USA site

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 four 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 29 March 2013 at 746 kg·ha-1, 46N–0P–0K was applied 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 approximately 0.12 L per plant. Granular 12N–17.5P–50.2K–10S–1Zn was applied 3 April 2015 at 313 kg·ha-1, 46N–0P–0K was applied 27 April 2015 at 224 kg·ha-1, and soluble 10N–22.7P–8.3K was applied at transplanting 17 June 2015 at 4.0 g·L-1 at approximately 0.12 L per plant.

3.2.2.2 Tanzania site

The experiments in Arusha, Tanzania were carried out on-station at WorldVeg, eastern and southern Africa (lat. 36.8°S, long. 3.4°E, 1290 m elevation) in 2016 and 2017. 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. Furrow irrigation was applied as needed. 20N–4.4P–8.3K fertilizer was applied to beds in Arusha at 200 kg·ha^{-1} prior to transplanting on 7 August 2014. Urea (46N-0P-0K) was applied 3 weeks after transplanting at 120 kg·ha^{-1}. The first field trial at the Tanzanian site was conducted between December 2015 and March 2016, and the second trial between May and September 2017. Plants were lyophilized during the first trial and oven-dried at 40°C for the second trial.

3.2.2.3 Kenya site

The experiments in the Turbo region outside of Eldoret, Kenya (lat. 0.37°N, long. 35.1°E, 1789 m elevation) were carried out during three distinct cultivation periods in 2017. The soil at this site was found to have an average pH of 5.7, CEC of 14.2 meq/100g, and 4.34% OM. Drip irrigation was applied as needed. A “chimney solar dryer” was used to dry plant materials for shipment to Rutgers University (Deltsidis et al., 2018).

The first season of cultivation at the Kenyan site at Turbo was conducted during the hottest and driest period Kenya experiences annually. Seeds were sown November 17, 2016, transplanted January 12, 2017, and harvested February 15, 2017. Five grams of granular 17:17:17 fertilizer was applied to each plant at time of transplanting.

The second field season at the Kenyan site was conducted during highest annual precipitation period with more moderate temperatures. Seeds were sown April 20, 2017, transplanted June 19, 2017, and harvested July 11, 2017. Five grams of granular 17:17:17 fertilizer was applied at time of transplanting.

The third field season at the Kenyan site was conducted following the cooler period, when the average temperature increases to a moderate level with moderate precipitation from 50-100mm of rain per month on average. Seeds were sown September 9, 2017, transplanted to the field October 18, 2017, and harvested November 24, 2017.

3.2.3 Elemental analysis.

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 marketable yield of the first harvest in each trial. The marketable yield is defined in this study as inclusive of leaves and stems with diameters comparable to the petiole of leaves which would commonly be consumed.

In our mineral analysis studies, several original samples received from our African partners appeared to be unusually high in iron. The concentration of Fe found in plants for biologically relevant quantities is notably different than the amount of iron that would appear in tissue analyses due to contamination e.g. by small amounts of soil or rust. We observed several

sets of tissues appeared to have been exposed to such sources of iron and as such were not used in data analyses. Several trials were removed from the data set when unusually high levels of iron came back in the analyses. Analysis was not included from the Tanzania 2016 trial for all crops as it appears plant samples were contaminated prior to lyophilizing on-site. For amaranth, the next Tanzania trial was also excluded as it did not feature the full germplasm; the second and third Kenyan trials were also removed as it appeared several entries included in these trials may have been contaminated with soil. For nightshade, each Kenyan trial contained many entries which appeared to have been contaminated with soil, these have been excluded from analysis. Ethiopian mustard appeared to have several entries contaminated with soil from the second Kenyan trial. These removals were done in order not to bias the statistical analyses due to factors beyond normal plant accumulation of minerals, including iron from the soil.

3.2.4 Statistical analysis

Each field trial was treated as a unique environment for each location, season, and year. Significance of each of the model terms was done using an approximated F-test with the Kenward Roger (Kenward & Roger, 1997) correction for the degrees of freedom. The specific genotypes were compared by slicing the GEI variance by genotype effect and LSMeans were estimated. The model was fitted using PROC MIXED in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). AMMI analysis was conducted using R Studio.

3.3 Results and Discussion

3.3.1 Iron Content

The mean Fe content of all crops inclusive of all genotypes and environments was below the high source threshold (4.2 mg/100g) and above the source threshold (2.1

mg/100g) (Figure 3.1). Genotype effect on Fe content was only found to be significant in amaranth (Table 3.2).

Two amaranth genotypes were found to have mean Fe content above the high source threshold, though between them, Madiira2 has much greater variation than RUAM24 (Figure 3.2). Partitioning of GEI effect across genotypes indicates that RUAM24 is the only genotype which was found not to have significance of variance across environments (Table 3.3).

Ethiopian mustard had a higher mean Fe content, with relatively more data points above the high source and source threshold than edible nightshade and spiderplant. However, genotype effect on Fe in Ethiopian was not observed to be significant and neither was GEI (Table 3.2). None of the Ethiopian mustard genotypes were found to be consistently above the high source threshold for Fe content.

Edible nightshade and spiderplant had more data points below the source threshold than amaranth and Ethiopian mustard. Neither edible nightshade nor spiderplant were observed to have a significant genotype, nor GEI effect (Table 3.2).

3.3.2 Calcium Content

The mean Ca content observed in each crop was found to be within the "source" range (Figure 3.3). Genotype effect was found to be significant within amaranth, Ethiopian mustard, and edible nightshade (Table 3.2).

Amaranth was observed to have a significant genotype effect on Ca content and was found to have more data points above the high source threshold compared to the other crops (Figure 3.3). Genotypes AC45, ACNL, AHTL, and UGAM40 had means above the high

source threshold (Figure 3.4), yet none of the amaranth genotypes were observed to have significantly less variance by GEI effect (Table 3.3).

Ethiopian mustard was found to have a significant genotype effect on Ca content and an insignificant GEI effect, though none of the genotypes were found to have mean Ca content substantially greater than the other Ethiopian mustard entries (Figure 3.5). None of the Ethiopian mustard genotype means were observed to be above the high source threshold, or below the source threshold. While partitioning of the GEI effect across Ethiopian mustard genotypes would indicate that some genotypes have greater stability (Table 3.3), non-significance of effect was more likely for having included fewer trials analyzed compared to the other crops.

Edible nightshade was found to have a lower mean Ca content compared to the other crops (Figure 3.3). A significant genotype effect was observed (Table 3.2). The mean and median of entry SS042 were the highest, observed to be above the source threshold (Figure 3.6).

3.3.3 Magnesium Content

Amaranth was the only crop not found to have a significant genotype effect on Mg content (Table 3.2); it was also the only crop with a mean above the high source threshold (Figure 3.7). Ethiopian mustard, spiderplant and edible nightshade each had a significant effect by genotype (Table 3.2). Ethiopian mustard was found to have a mean Mg content below the source threshold; edible nightshade and spiderplant each had means above the source threshold (Figure 3.7).

The findings of this study would support amaranth being a high source food for Mg irrespective of genotype as a reliable delivery source of Mg. It is reasonable to speculate

that Mg content of amaranth may fall below the high source threshold as some data points had; AC45, RUAM24, and UGAM40 however, did not fall below the high source threshold for Mg (Figure 3.8). These varieties may be considered for having greater potential stability, though all genotypes were found to have significant variation across environments (Table 3.3). When observing these results using the AMMI model, the three genotypes mentioned to not have been observed to have Mg contents below the high source threshold have separation from the other entries from having less of a negative interaction from the Kenyan field trial which was the only environment with a mean Mg content below the high source threshold across amaranth genotypes (Figure 3.9).

Despite a significant genotype effect observed in each Ethiopian mustard, spiderplant, and edible nightshade, within each crop no genotype was found to have substantially preferable stability or content (Figures 10, 11, 12). Results of the mixed model analysis partitioning GEI by genotype did not indicate that any genotype within each of these crops to had insignificant GEI effect (not shown).

Ethiopian mustard was found to be a relatively poor source of Mg. 'Arumeru' and 'Chinasaki' were found to have slightly higher means, though likely not enough to warrant use as breeding lines for this purpose and if intending to supplement the diet for Mg, the results of this study find that amaranth, regardless of genotype, would be preferred if available.

3.3.4 Zinc

The zinc content of each crop across environments was found to be below the source threshold (Figure 13). The results of this study support that the included crops grown in the environments tested would not be suitable as a source of Zinc for the human diet.

3.4 Conclusions

The results of this study demonstrate a differential capacity of AIVs to deliver Fe, Ca, and Mg. Each of the samples analyzed contained quantities of Zn below the "source" threshold, which follows data on leafy green vegetables in the USDA standard reference database as discussed in Chapter 1.

The observation of samples with Fe content above the "high source" threshold complemented by Ca and Mg above "high source" thresholds would potentially have low incidence given Fe becomes less available to plants as the pH increases, while Ca and Mg becomes less available to plants as pH decreases (Läuchli, 1976; Marschner, 1991; Oertli & Jacobson, 1969). However, if a plant is genetically adapted to uptake one of more of these minerals in otherwise limiting conditions, it is reasonable to predict that quantities of these micronutrients can be accumulated simultaneously at "high source" levels given the observation that the effect of genotype can overcome environmental limitations (Brown, 1977; Caines & Shennan, 1999; Farnham et al., 2000; Kobayashi & Nishizawa, 2012; Quintana et al., 1996). The consistent observation of high Fe content in RUAM24, including observations when Fe was lower in comparison with other entries and when Ca and Mg were also high indicates the potential for this entry to be characterized as "high source" for Fe.

The objective description of a variety for application of a Plant Variety Patent (PVP) would normally be observed for three location/years (environments) in the region and season of best adaptability (U.S. Department of Agriculture, 2015a). In these studies, the unique nature of RUAM24 being stable and high in iron is a notable character that could show its distinction among comparative amaranth leafy green lines in micronutrient content. With most PVP applications, little focus has been applied to micronutrient content,

though in some crops there is consideration for tolerance of limiting conditions as in the case of soybean (*Glycine max*), which is graded for iron-chlorosis on calcareous soil (U.S. Department of Agriculture, 2015b).

The observation of micronutrients in crops over multiple environments provides needed guidance to characterize those as foods for reduction of micronutrient deficiencies and general maintenance of health for people who chose foods with an expectation of micronutrient content. The current study demonstrates that substantial differences in elemental micronutrient content exist between crops and within crops with respect to *Codex Alimentarius* thresholds for micronutrient content.

Table 3.1 Genetic entries of amaranth, spiderplant, edible nightshade, and Ethiopian mustard included in this study with species, accession as identified by source, and source of material.

Crop	Species	Accession	Source
Amaranth	*Amaranthus sp.*	UG-AM-40	WorldVeg
	A. sp.	AC-45	WorldVeg
	A. cruentus	Madiira 2	WorldVeg
	A. cruentus	AC-NL	WorldVeg
	A. hypochondriacus	AH-TL	WorldVeg
	A. cruentus	Madiira 1	WorldVeg
	A. tricolor	RUAM24	Rutgers
Spiderplant	*Cleome gynandra*	UG-SF-23	WorldVeg
	C. gynandra	ML-SF-17	WorldVeg
	C. gynandra	PS	WorldVeg
	C. gynandra	UG-SF-15	WorldVeg
	C. gynandra	ML-SF-29	WorldVeg
Edible nightshade	*Solanum scabrum*	SS 52	WorldVeg
	S. scabrum	Ex-Hai	WorldVeg
	S. scabrum	SS 49	WorldVeg
	S. scabrum	SS 04.2	WorldVeg
	S. scabrum	BG 16	WorldVeg
	S. scabrum	BG-29	WorldVeg
Ethiopian mustard	*Brassica carinata*	Arumeru	WorldVeg
	B. carinata	Rungwe	WorldVeg
	B. carinata	MBEYA GREEN	WorldVeg
	B. carinata	MBEYA PURPLE	WorldVeg
	B. carinata	RW-B-1 EM1	WorldVeg
	B. carinata	MUSTARD I	WorldVeg
	B. carinata	CHINASAKI	WorldVeg

Table 3.2 P values of Genetic (G), Environment (E), and genetic by environmental interaction (G x E) effect across crops for each micronutrient observed.

Crop	Effect	Fe	Ca	Mg	Zn
Amaranth	G	<.0001	<.0001	0.2393	0.881
	E	0.0005	<.0001	<.0001	<.0001
	G x E	<.0001	<.0001	<.0001	0.5378
Ethiopian Mustard	G	0.5016	0.0498	0.0036	0.1708
	E	0.0454	<.0001	<.0001	<.0001
	G x E	0.843	0.5298	0.0686	0.5232
Spider Plant	G	0.5737	0.8267	0.0063	0.1854
	E	0.0085	<.0001	<.0001	<.0001
	G x E	0.5521	0.0059	0.0087	0.6028
Edible nightshade	G	0.0525	0.0417	0.0242	0.0229
	E	0.0348	<.0001	<.0001	<.0001
	G x E	0.8149	0.0299	<.0001	0.0099

Table 3.3. Tests of effect slices genetic by environment for each genetic entry across each crop and micronutrient observed.

Crop	Entry	Fe	Ca	Mg	Zn
Amaranth	AC45	<.0001	<.0001	<.0001	0.0004
	ACNL	<.0001	<.0001	<.0001	<.0001
	AHTL	0.0039	<.0001	--	--
	Madiira1	0.0002	<.0001	<.0001	<.0001
	Madiira2	<.0001	<.0001	<.0001	<.0001
	RUAM24	0.2208	<.0001	--	--
	UGAM40	0.0016	<.0001	<.0001	<.0001
Ethiopian Mustard	Arumeru	--	0.0447	0.0005	<.0001
	Chinasaki	--	0.0051	0.0062	<.0001
	MbeyaGrn	--	0.1410	<.0002	<.0001
	MbeyaPrp	--	0.4921	0.0003	<.0001
	Must1	--	0.7032	<.0001	<.0001
	RWB1	--	0.0129	<.0001	<.0001
	Rungwe	--	0.1516	<.0001	<.0001
Edible nightshade	BG16	0.2352	<.0001	<.0001	<.0001
	BG29	0.0907	<.0001	<.0001	<.0001
	ExHai	0.6752	<.0001	<.0001	<.0001

	SS042	0.0246	<.0001	<.0001	<.0001
	SS49	0.1945	<.0001	<.0001	<.0001
	SS52	0.2214	<.0001	<.0001	<.0001
Spiderplant	MLSF17	0.0158	<.0001	<.0001	0.0018
	MLSF29	0.3653	<.0001	<.0001	0.0258
	PS	0.9473	<.0001	<.0001	0.0079
	UGSF15	0.3944	<.0001	<.0001	0.0041
	UGSF23	0.1137	<.0001	<.0001	0.0197

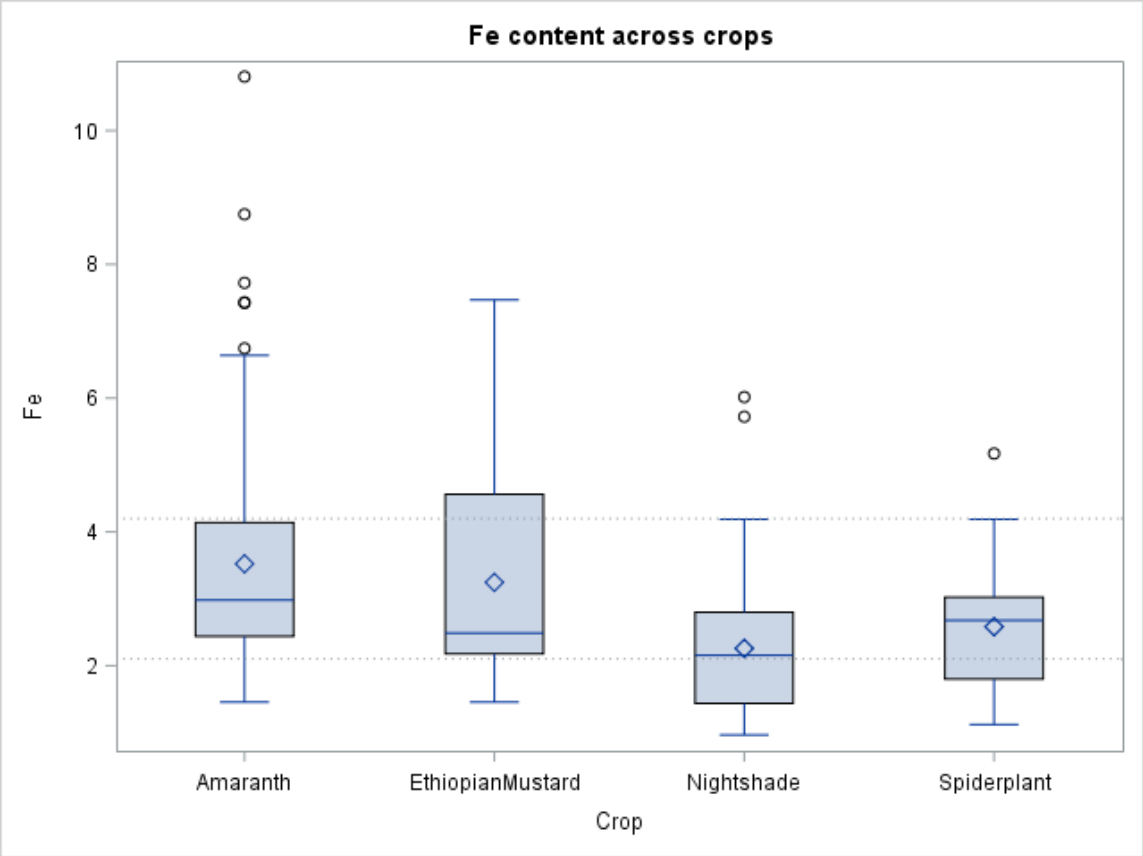

Figure 3.1. Mean Fe content of amaranth, Ethiopian mustard, edible nightshade, and spiderplant with all genetic entries aggregated, across multiple environments.

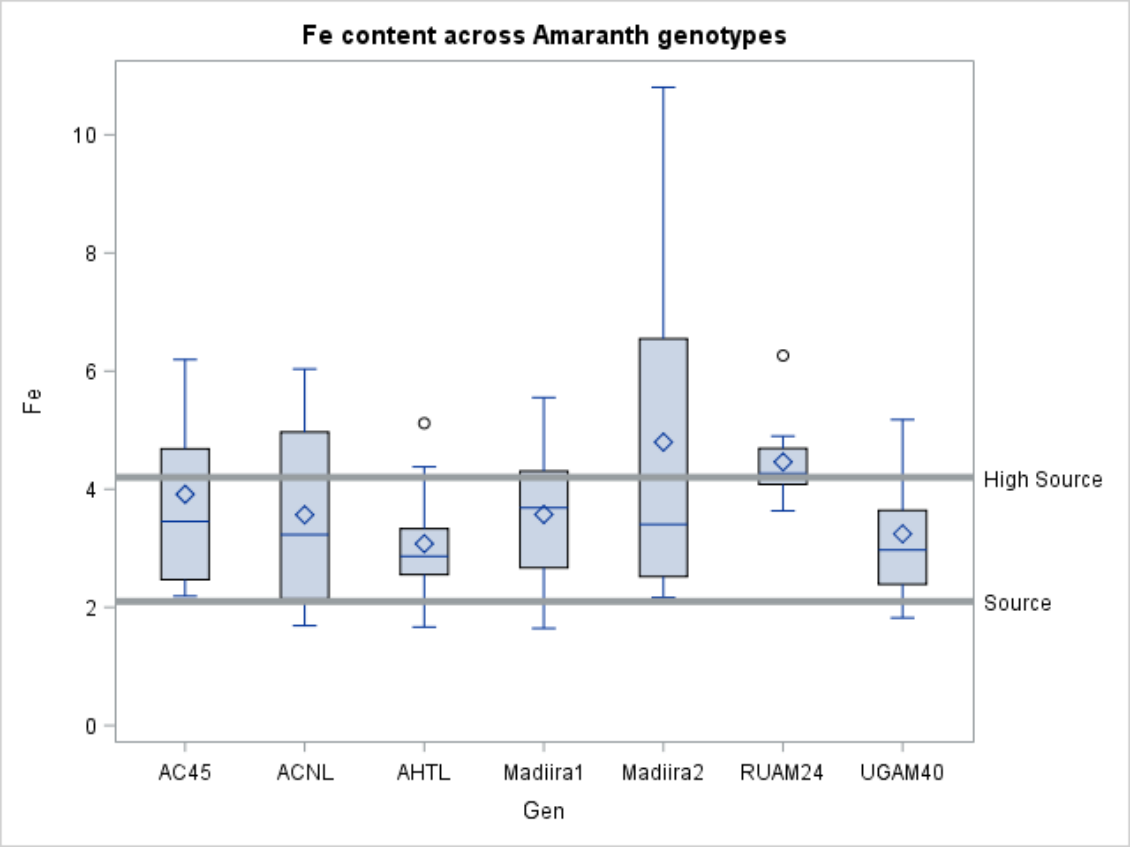

Figure 3.2. Mean Fe content of genetic entries within amaranth across multiple environments.

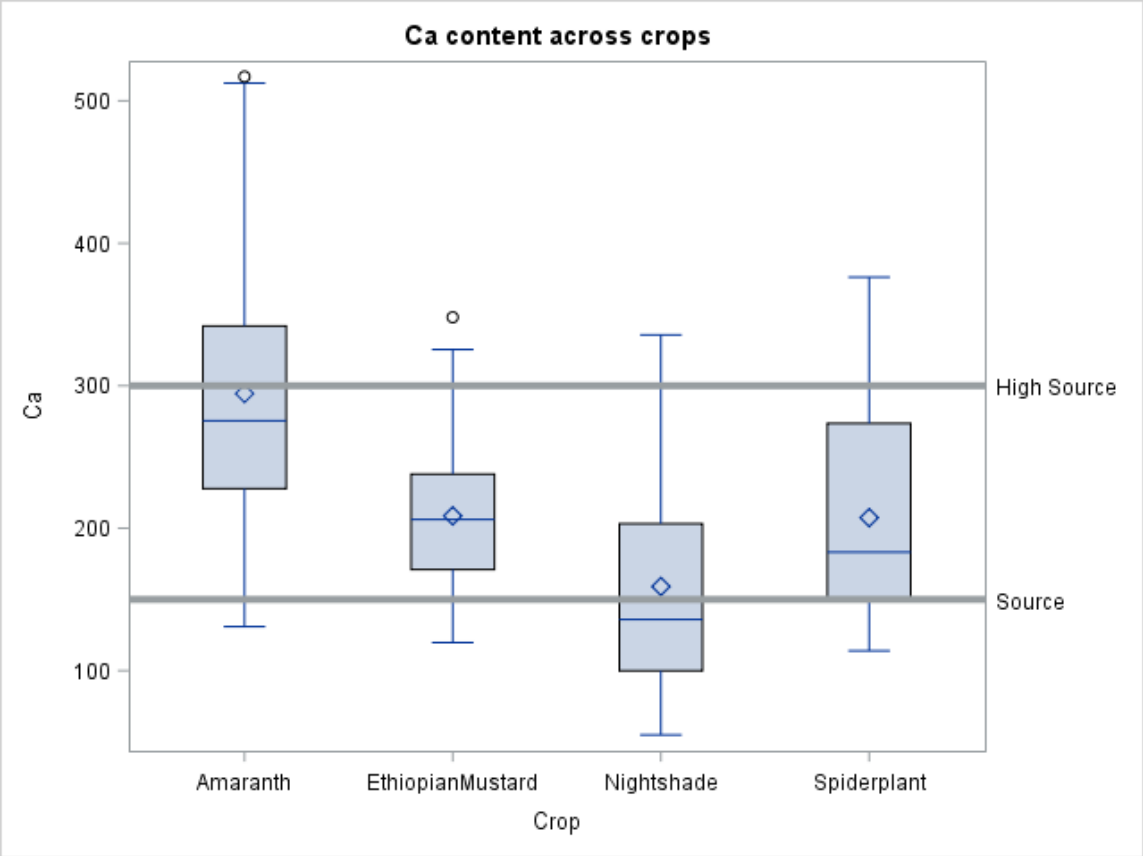

Figure 3.3 Mean Ca content of amaranth, Ethiopian mustard, edible nightshade, and spiderplant with all genetic entries aggregated, across multiple environments.

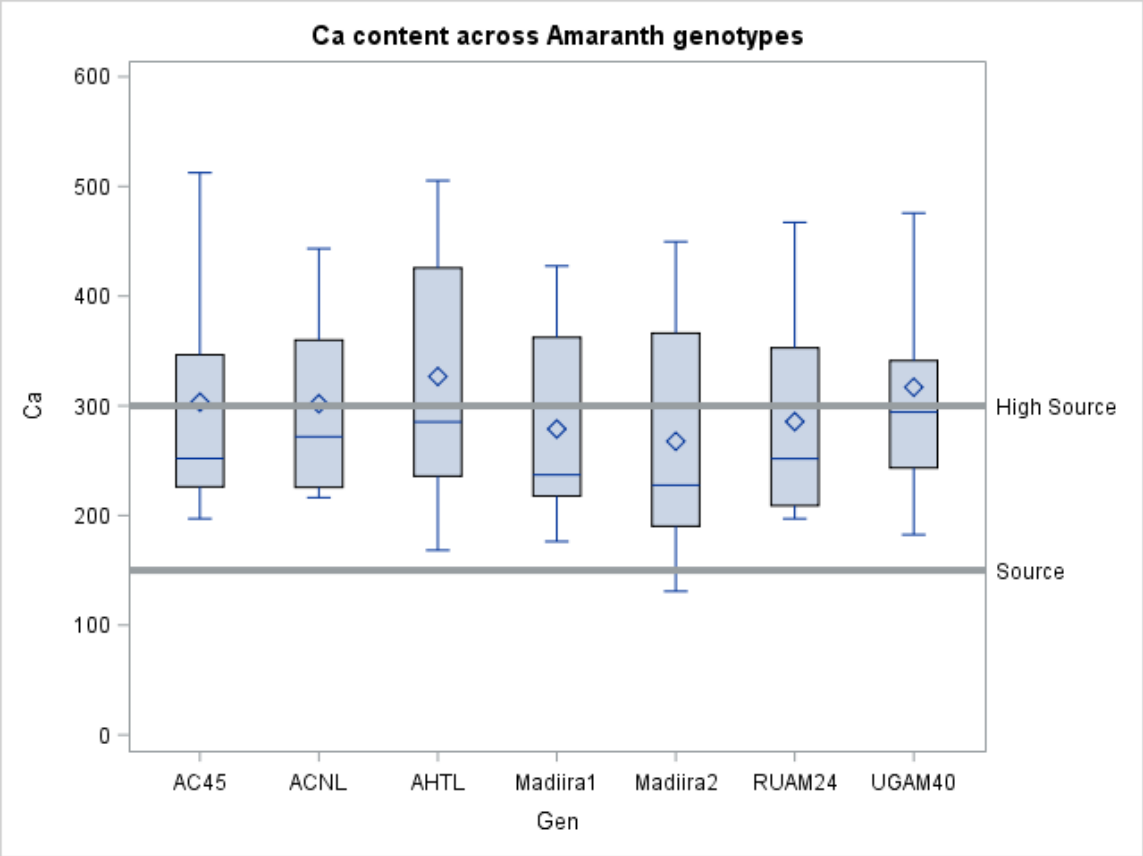

Figure 3.4. Mean Ca content of genetic entries within amaranth across multiple environments.

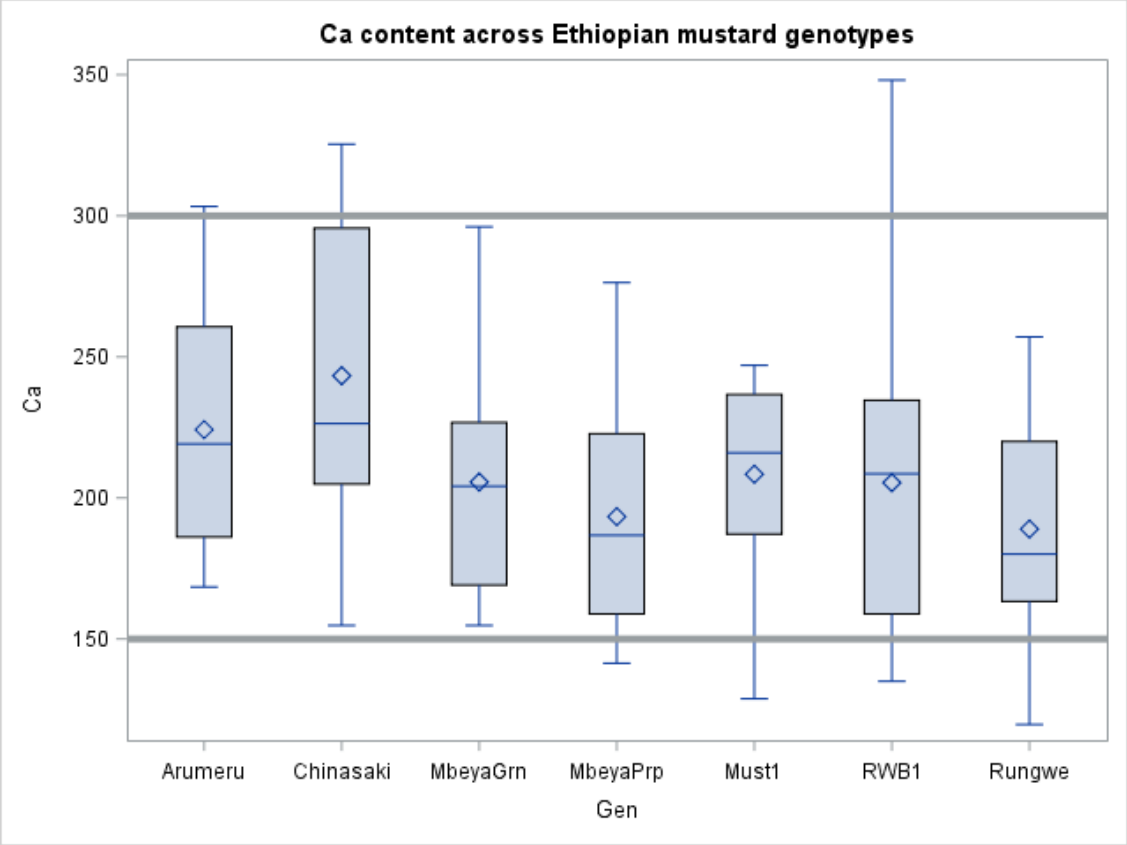

Figure 3.5. Mean Ca content of genetic entries within Ethiopian mustard across multiple environments.

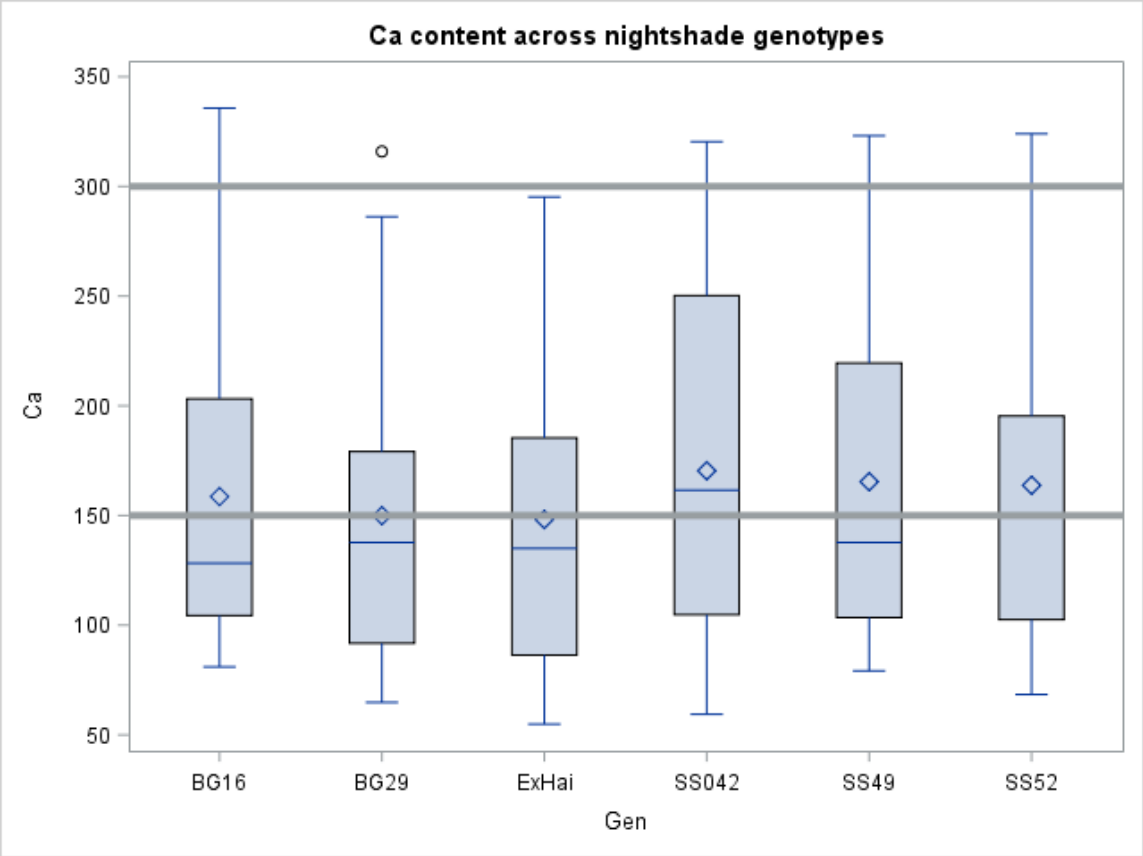

Figure 3.6. Mean Ca content of genetic entries within edible nightshade across multiple environments.

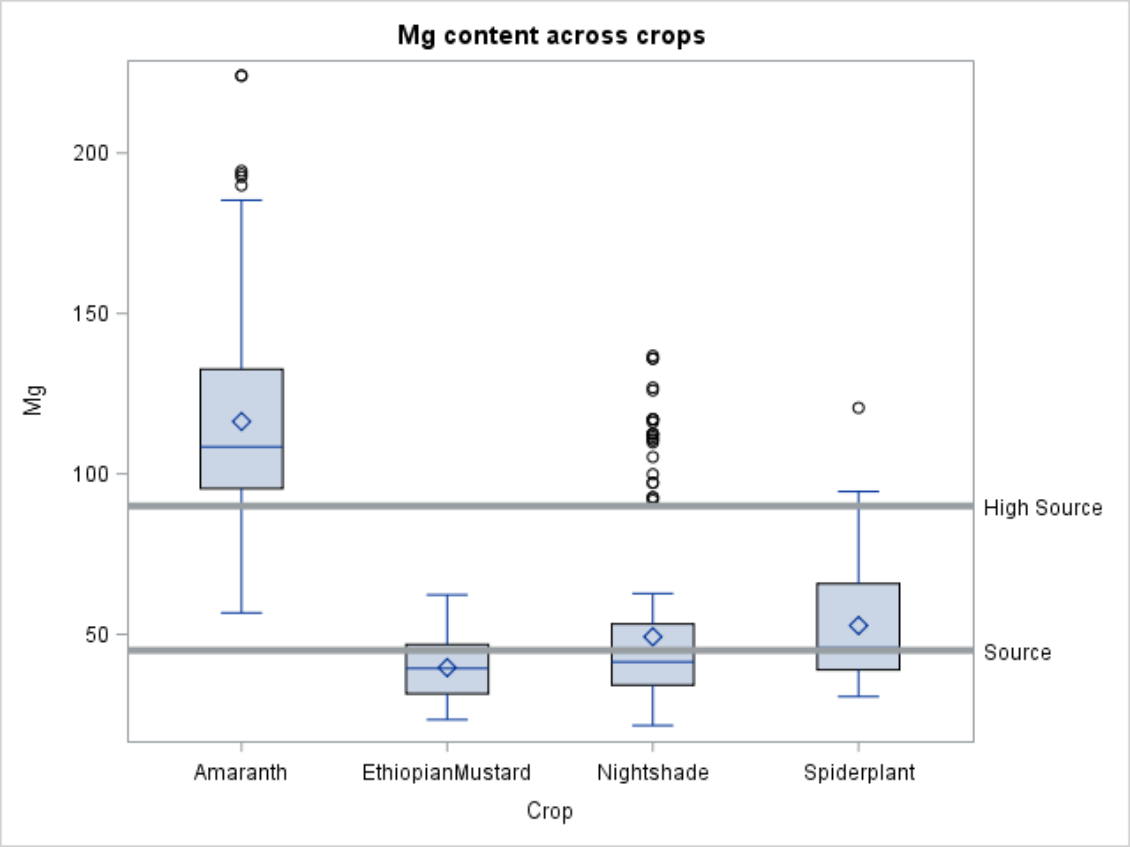

Figure 3.7. Mean Mg content of amaranth, Ethiopian mustard, edible nightshade, and spiderplant with all genetic entries aggregated, across multiple environments.

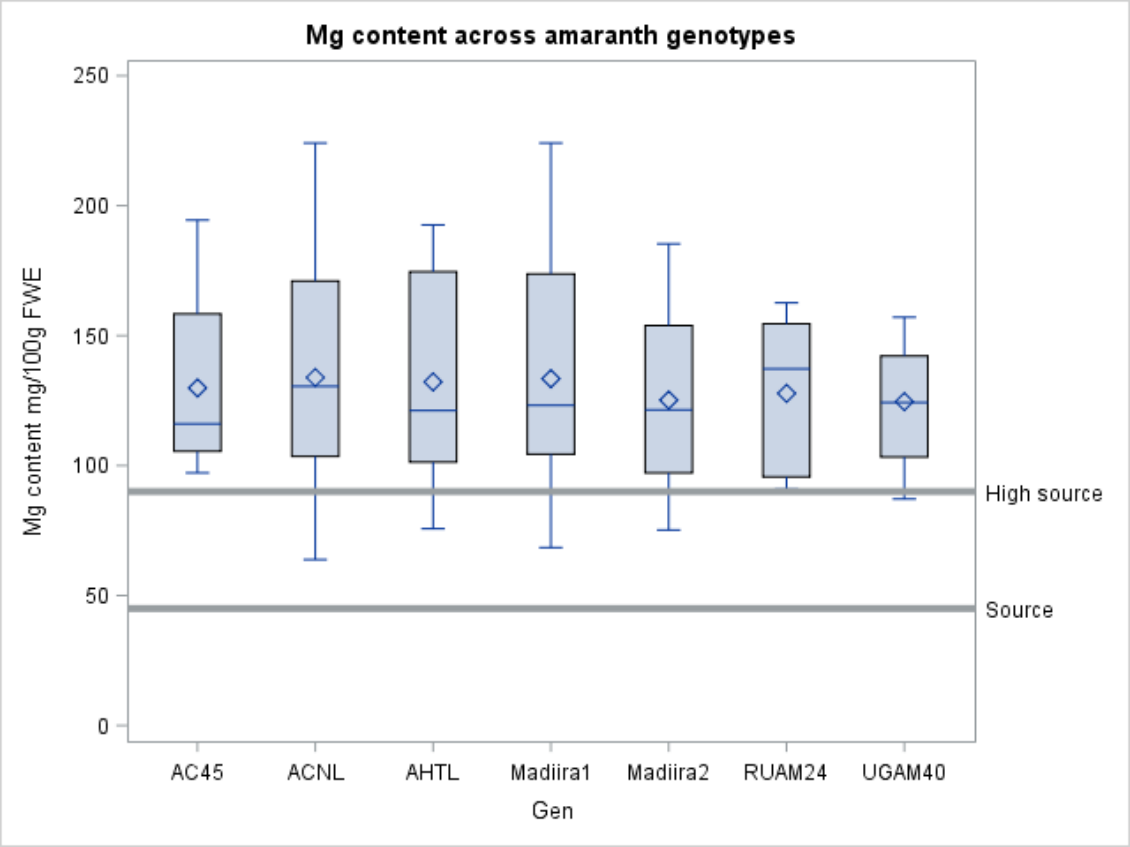

Figure 3.8. Mean Mg content of genetic entries within amaranth across multiple environments.

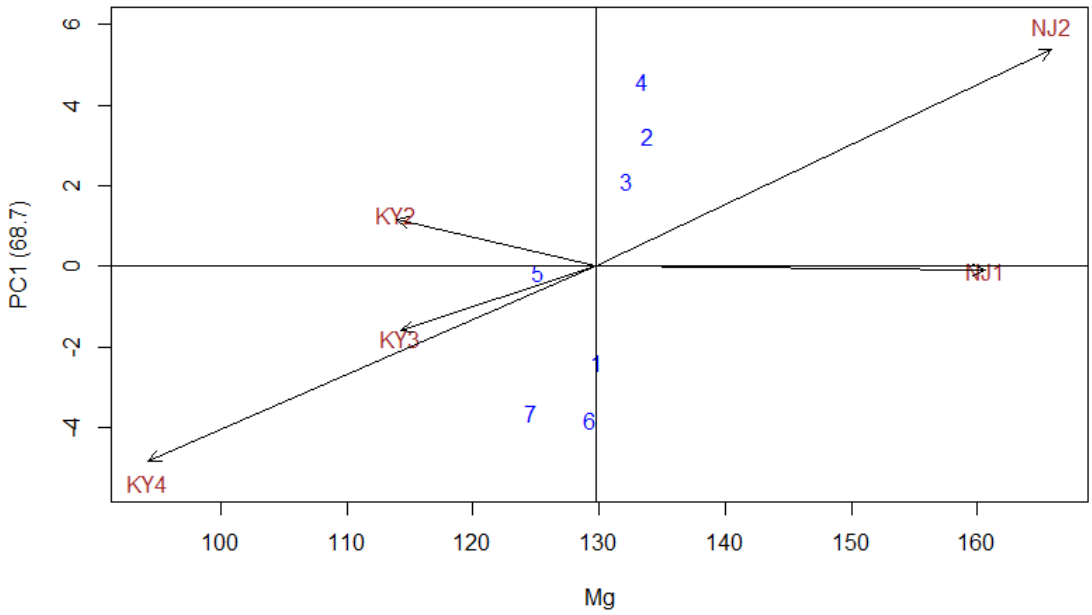

Figure 3.9. AMMI biplot view of PC1 (y-axis) by Mg (mg·100 g^{-1}) (x-axis) content across genetic entries of amaranth in five environments.

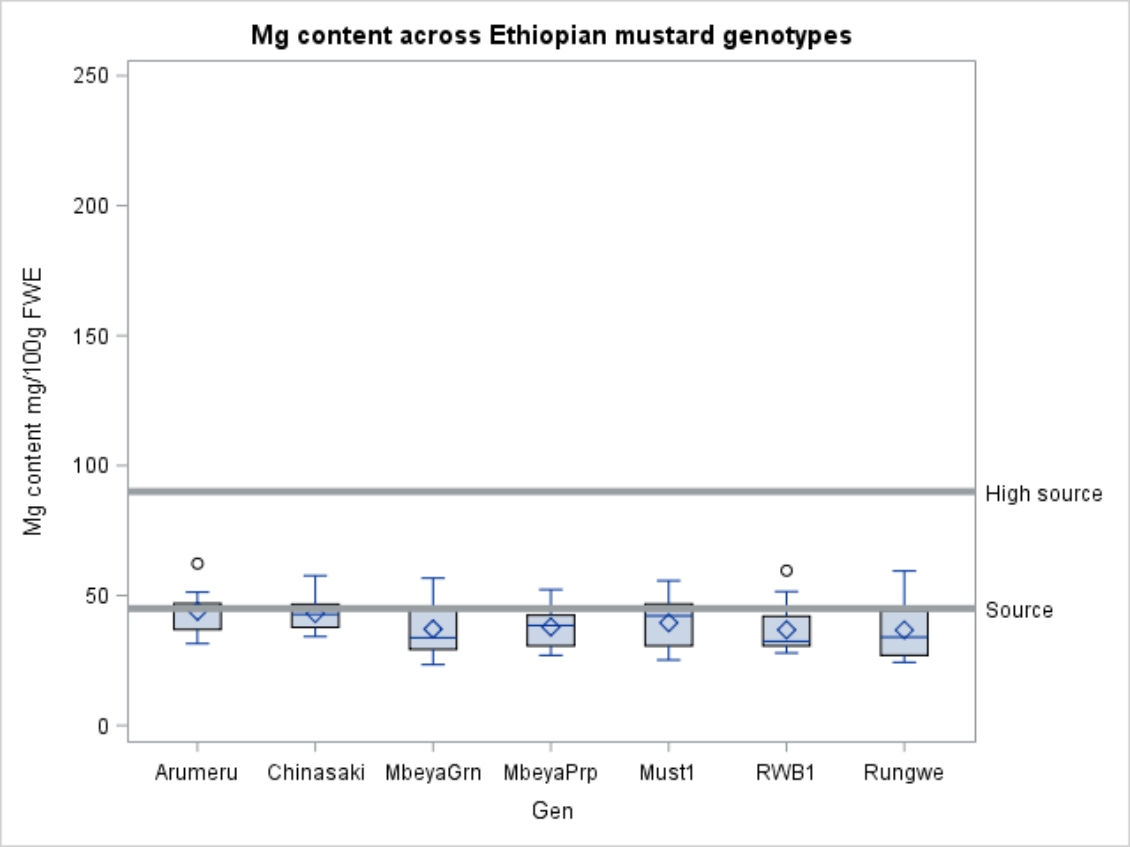

Figure 3.10. Mean Mg content of genetic entries within Ethiopian mustard across multiple environments.

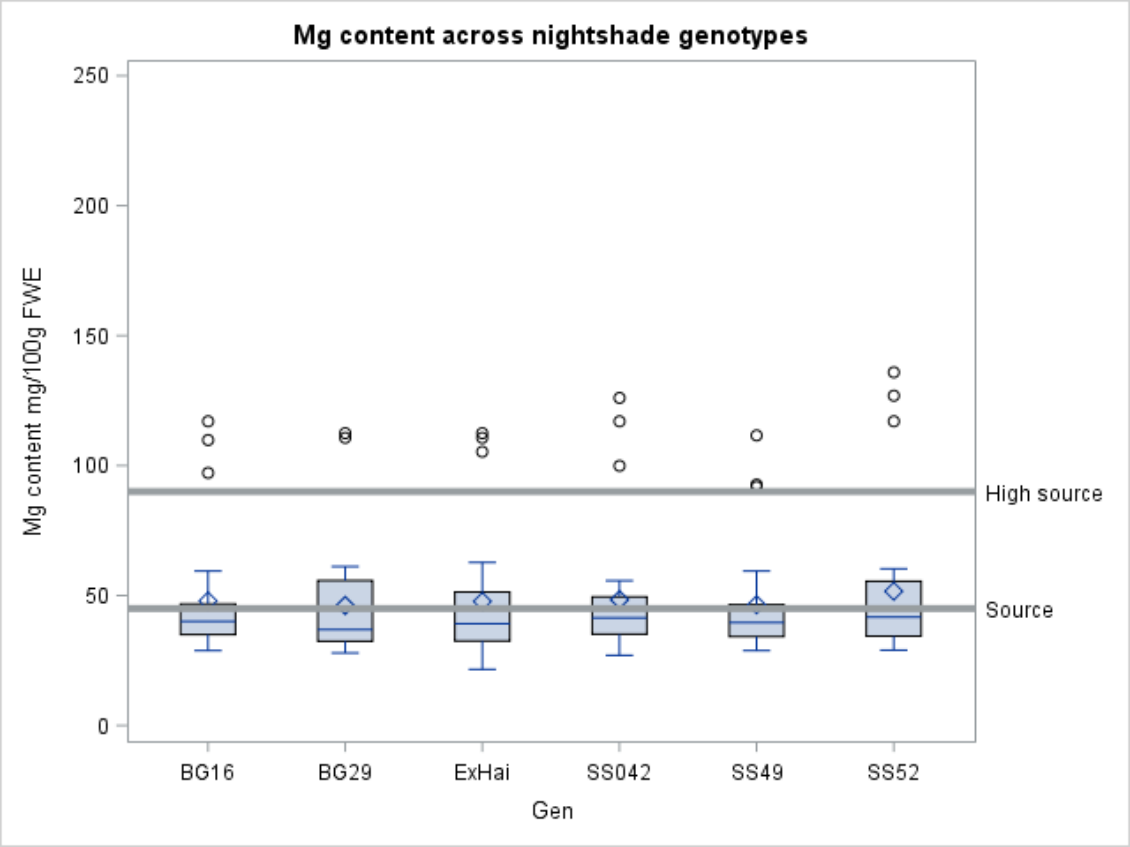

Figure 3.11. Mean Mg content of genetic entries within edible nightshade across multiple environments.

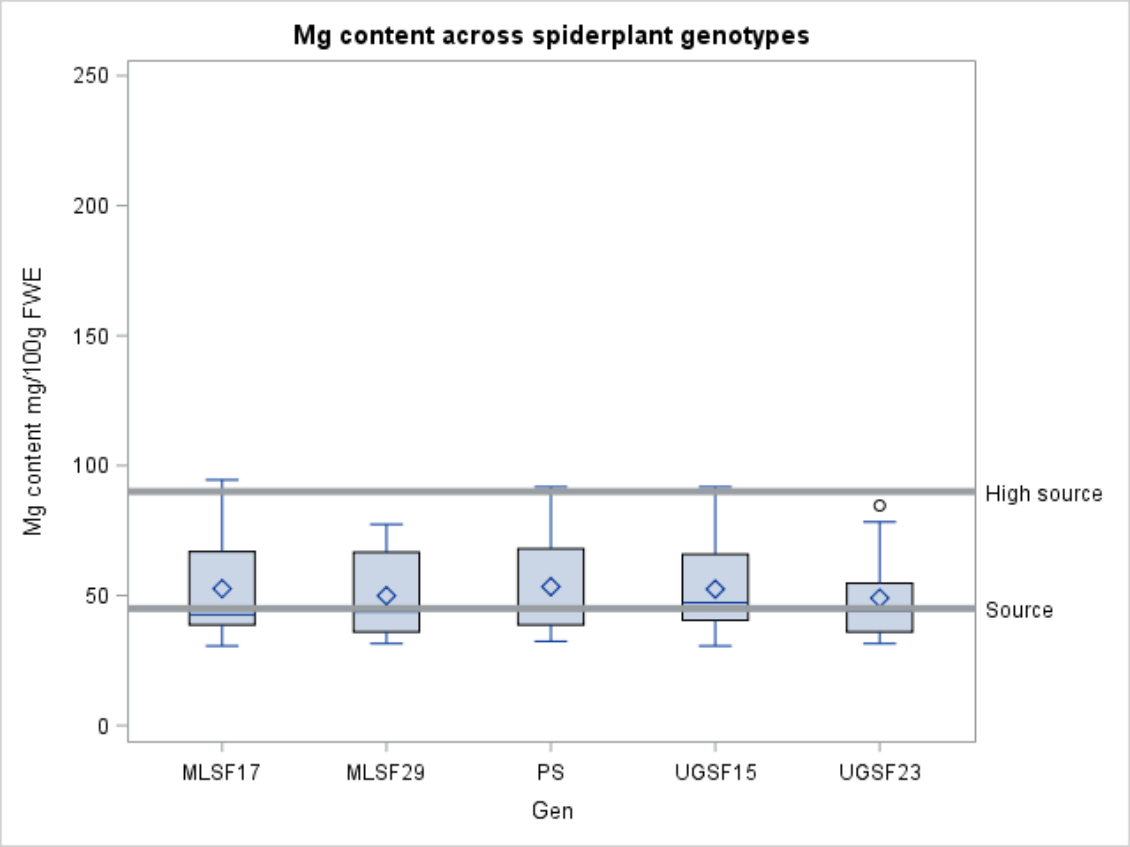

Figure 3.12. Mean Mg content of genetic entries within spiderplant across multiple environments.

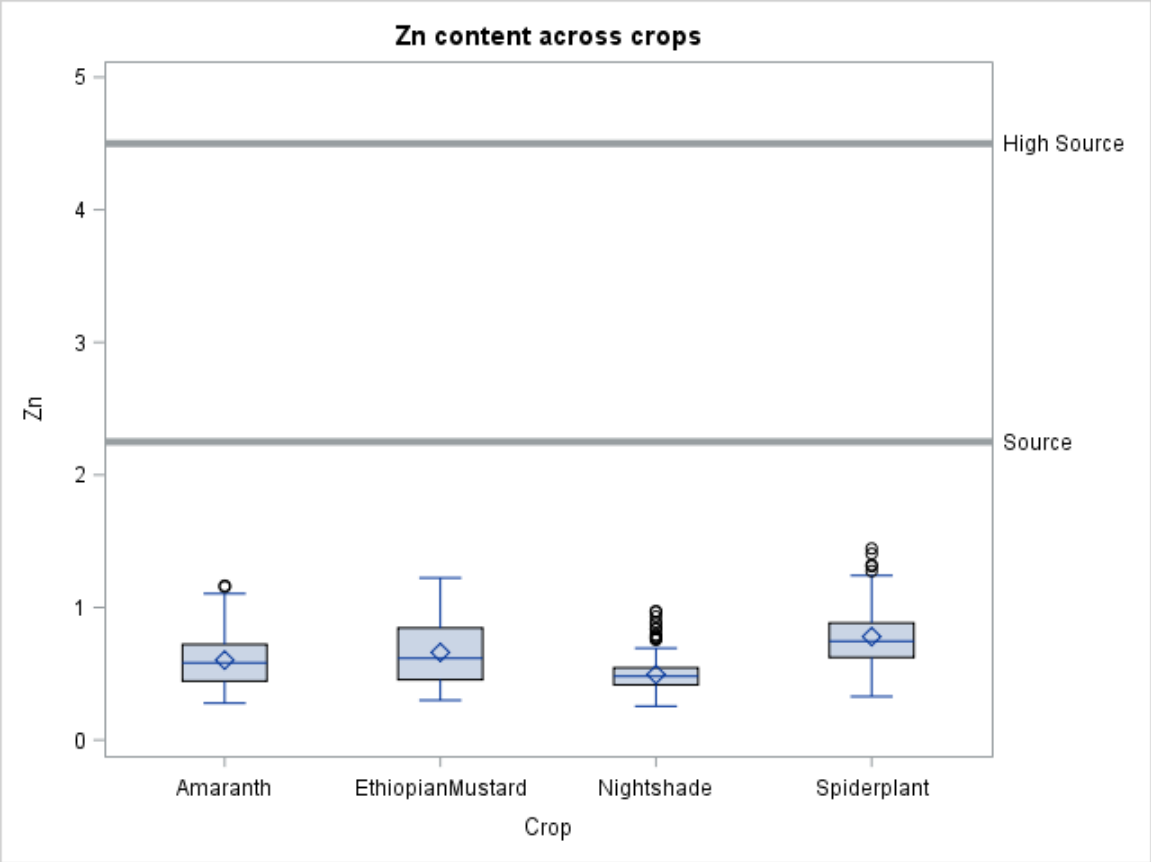

Figure 3.13. Mean Zn content of amaranth, Ethiopian mustard, edible nightshade, and spiderplant with all genetic entries aggregated, across multiple environments.

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Chapter 4 Selection for improved Fe content in vegetable amaranth

4.1 Introduction

Amaranth is a leafy green vegetable crop consumed as part of traditional meals within communities in many of the countries which are most severely and consistently afflicted by iron deficiency anemia, globally (National Resource Council, 1984; World Health Organization, 2010). While leafy green vegetables can often be characterized as a high source of Vitamin A, this class of crops is often found to contain quantities of elemental micronutrients below respective "source" thresholds as discussed in Chapter 1.

Results presented in Chapter 3 demonstrate that stable, high source levels of Fe can be observed within amaranth due to genotype. Entry RUAM24 shows promise as a potential cultivar and breeding line following observations described in Chapters 2 and 3, though it is unlikely to have the capacity for introgression through traditional breeding into currently promoted by the World Vegetable Center (WorldVeg) as RUAM24 has been identified as *Amaranthus tricolor* (2n=32) while 'Madiira 1' has been identified as *Amaranthus cruentus* (2n=34) (Bonasora, Poggio, & Greizerstein, 2013; Pal, 1972).

The amaranth species characterized primarily as vegetable-type i.e. *A. lividus, A. tricolor* have been described as having very low rates of outcrossing in the field (Pal, 1972). In contrast, the outcrossing rates of amaranth species characterized primarily as grain-type i.e, *Cruentus, hyprochondriacus, caudatus* have been described as ranging from 3.5 to 34% (Hauptli & Jain, 1985; Jain, Hauptil, & Vaidya, 1982). Despite often being referred to as "grain-type", most of the cultivars promoted as leafy green vegetables by the World Vegetable Center in East and Southern Africa are of these species.

Response to single plant selection has previously been found to be successful in amaranth (Baltensperger, Weber, & Nelson, 1992) as well as selection within discrete landrace entries (Hauptli & Jain, 1985). To introduce an *A. crutentus*, with improved Fe content complementing a market-ready phenotype, a search for high-performance within the latent segregation of Fe content was initiated.

4.2 Materials and Methods

4.2.1 Initial evaluation

Seeds of cv. Madiira1 from WorldVeg (Arusha, TZ) were sown January 16, 2017 in 128-cell trays with growing mix (Fafard Grow Mix 2; Sun Gro Horticulture, Agawam, MA) under 14 h day high pressure sodium lights at the Rutgers University Agriculture Experiment Station Greenhouses in New Brunswick, NJ. Thirty seedlings were transplanted on Feb 17, 2017 to individual 1-gallon pots with the same growing mix that was used for seed trays. Plants were under automated irrigation with individual lines running to each pot. Samples were obtained on March 24, 2017 using a cutback harvesting method leaving lateral shoots to grow for subsequent seed harvest. Samples were placed in separate paper bags and dried in a convection oven at 40°C for three days, when samples were crisp and easily broken apart by hand yet still green in color. Subsamples of the dried harvested leaves were ground using a shearing-action mill and submitted to Penn State Agricultural Analytical Services Laboratory, University Park, PA for elemental micronutrient analysis by inductively coupled plasma mass spectrometry (ICP-MS).

The Fe content from results of greenhouse evaluation ranged from 5.2 mg Fe/100g FWE to 9.2 mg Fe/100g FWE (Table 4.1). The two individual plants with the highest Fe content, 36-20 and 36-30, with 9.2 and 8.5 mg Fe/100g FWE respectively, were moved to a growth chamber on June 7, 2017 under 10 h light, 14 h dark to induce flowering. Seed was

collected August 17, 2018 from each plant and transferred to WorldVeg in Arusha, Tanzania.

4.2.2 Field evaluation

Twenty progeny entries of each '36-20', '36-30', and of the original 'Madirra1' were sown 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. 20N–4.4P–8.3K fertilizer was applied to beds at 200 kg·ha^{-1} prior to transplanting on January 16, 2018 at the WorldVeg research site in Arusha, Tanzania (lat. 36.8ºE, long. 3.4°S, 1290 m elevation). Plants were grown in plots separated by parent. The site is characterized by well-drained clay loam soil with pH 6.4. Furrow irrigation was applied as needed.

Samples were collected on February 20, 2018 by cutting at the axial stem 10 cm from the soil line to allow grow-back, separating any thick stems included in the harvest from the edible portion as described in Chapter 2. Samples were dried at the WorldVeg Arusha site in a convection oven at 40°C and transferred to Rutgers University where subsamples of the dried harvested leaves were ground using a shearing-action mill and submitted to Penn State Agricultural Analytical Services Laboratory, University Park, PA for elemental micronutrient analysis by ICP-MS.

4.2.3 Statistical analysis

Plots of comparative histograms and boxplots of Fe content for each population were produced using Proc Univariate and descriptive statistical analysis was performed using Proc Means, SAS (version 9.4; SAS Institute, Cary, NC).

4.3 Results and Discussion

The mean, median, and 95% two-sided confidence intervals for Fe content of each population was within the source range for Fe content as defined by *Codex Alimentarius* labeling guidelines (Codex Alimentarius, 1997) (Table 4.2).

Response to selection was limited to a reduction in variance in both selection populations. The variance of Fe content within the original Madiira1 was slightly more than 11 and 20 multiples higher than the progeny of selections AM36-20 and AM36-30, respectively (Table 4.2). This indicates that selection increased homogeneity of loci associated with traits that result in the accumulation of Fe in leaf parts.

The mean and median Fe content was highest in Madiira1 (Table 4.2). Despite the substantially higher variance observed in the Madiira1 population, most observations in this group were higher than the mean Fe contents observed in the AM36-20 and AM36-30 populations (Figures 4.1 and 4.2).

Improvement of mean Fe content was not observed in the field trial of selection populations compared to the originating parent line. This indicates that the greenhouse conditions which were used for initial selection of top-performing individuals was not representative of field conditions at the site in Arusha, Tanzania. The selection of '36-20' and '36-30' produced an increase of precision for Fe content in the progeny populations of each of these entries, though not an increase in mean Fe content. Selection for Fe content would potentially be more accurate for subsequent trials if the initial screening is conducted in environmental conditions representative of end-use conditions or which would otherwise be predictably discriminating. A high-pH environment would generally be informative to identify plants which are genetically adapted to tolerate soils that would

limit Fe uptake non-genetically adapted plants (Kobayashi & Nishizawa, 2012; Oertli & Jacobson, 1969).

4.4 Conclusions

Genetic adaptation for consistent high Fe content has been observed in several plant systems (Brown, 1977; Brown, Chaney, & Ambler, 1971; Römheld & Marschner, 1986). Classic hypotheses for mechanisms of adaptation have been supported by modern genetics (Kobayashi & Nishizawa, 2012).

Selection for improved Fe content should be maintained across environments according to existing hypotheses if the screening environment is discriminant. A distribution of Fe content was observed in the initial greenhouse screening of this study (Table 4.1). However, the progeny of the highest-performing entries in the initial screening were not observed to have a higher Fe content in the field trial than the original 'Madiira1' group (Table 4.2).

Both progeny groups from selection had substantially lower variances than the original 'Madiira1' group. This would suggest that the reduction in genetic diversity in the progeny of '36-20' and '36-30' may have selected for adaptations specific to the initial screening environment. This would generally not agree with current accepted hypotheses for improved Fe uptake in non-grass plants, which does not involve any adaptations which would limit uptake in otherwise non-limiting environments (Kobayashi & Nishizawa, 2012).

Narrowing genetic diversity within a population by selection to increase the precision of performance with respect to Fe content would be a valuable improvement for consumers of amaranth in regions with high rates of iron-deficiency. The reduction in variance in selection populations in this study indicates this is feasible. Screening for diversity of Fe

content in an environment limited due to high pH may result in greater repeatability across varying environments.

Table 4.1. Iron content observations of each individual amaranth plant from greenhouse trial at NJAES research greenhouses, New Brunswick, NJ.

Entry	mg Fe/ 100g FWE
36-1	6.3
36-2	6.6
36-3	5.2
36-4	6.2
36-5	7.2
36-6	5.9
36-7	6.7
36-8	6.4
36-10	6.1
36-11	6.7
36-12	6.7
36-13	6.5
36-14	5.8
36-15	7.0
36-16	6.4
36-17	7.3
36-18	5.8
36-19	5.9
36-20	9.2
36-21	6.0
36-22	6.7
36-23	7.7
36-24	5.8
36-25	5.7
36-26	7.0
36-27	8.1
36-28	6.1
36-29	6.5
36-30	8.5

Table 4.2. Summary statistics of progeny populations from individuals selected for improved Fe content (AM36-20 and AM36-30) and a control population not selected for Fe content (Madiira 1).

Parent entry	N	Min	Median	Max	Mean	Variance	SD	SE	Lower 95% CL for Mean	Upper 95% CL for Mean	Coeff of Variation
AM36-20	20	255.21	301.63	357.07	301.55	832.55	28.854	6.452	288.04	315.05	9.569
AM36-30	20	251.25	290.86	322.61	286.99	467.57	21.623	4.835	276.87	297.11	7.535
Madiira1	20	272.13	349.67	681.59	370.38	9383.7	96.870	21.661	325.04	415.71	26.154

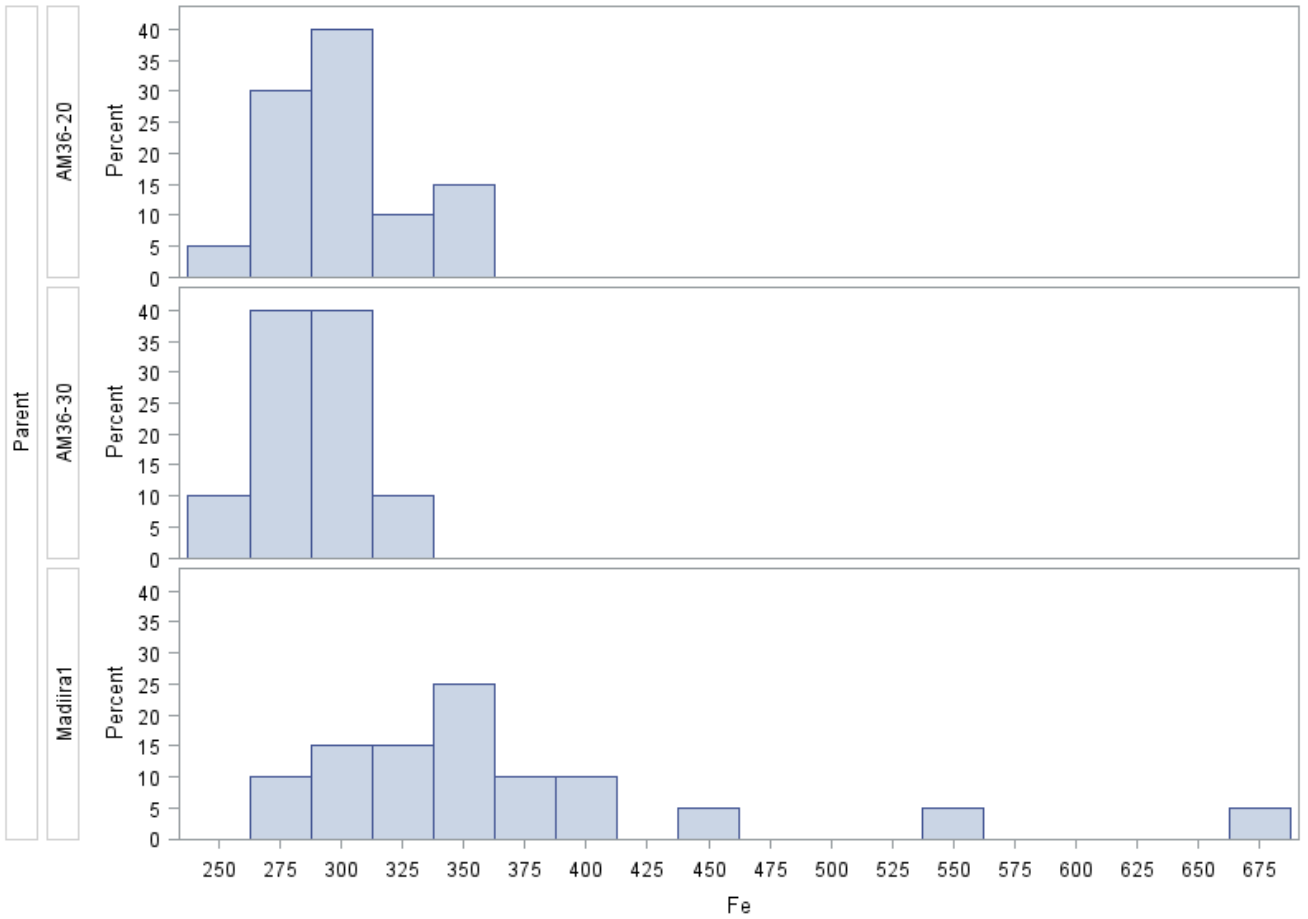

Figure 4.1. Histograms of Fe content by parent line observed from trial in Arusha, Tanzania at World Vegetable Center research station.

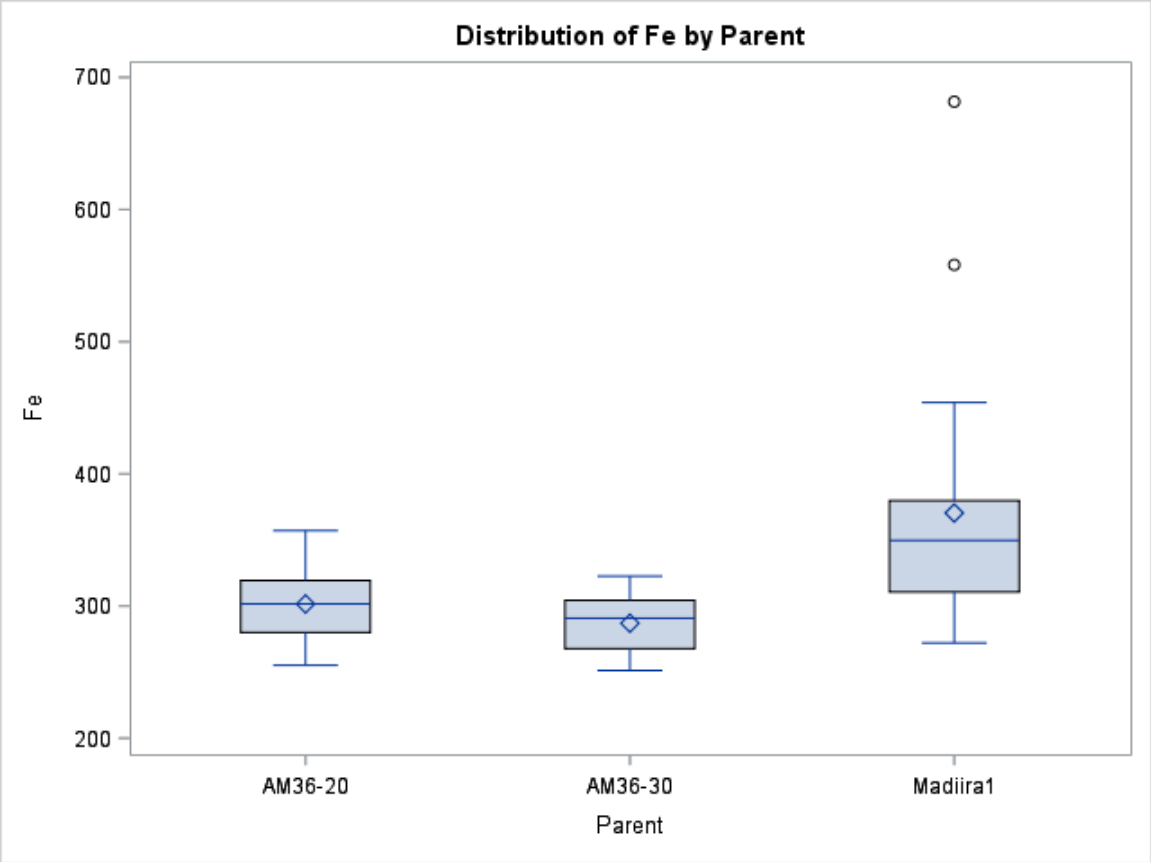

Figure 4.2. Boxplots of Fe content by parent line observed from trial in Arusha, Tanzania at World Vegetable Center research station.

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Chapter 5 Selection for delayed flower time to increase vegetative growth in spideprlant (*Cleome gynandra*)

5.1 Introduction

Spiderplant (*Cleome gynandra*) is an annual herb used primarily as a leafy vegetable in several sub-Sahara African countries: Benin, Botswana, Cameroon, Democratic Republic of Congo, Ghana, Kenya, Malawi, Nigeria, Namibia, South Africa, Swaziland, Tanzania, Uganda, Zambia, Zimbabwe, as well as in India and Thailand (Dansi et al., 2012; Redhead, 1990). The modest development status of this vegetable is disproportionate to the importance of the role it serves as a food source in these regions (Kwarteng et al., 2018; Cecilia M. Onyango, Kunyanga, Ontita, Narla, & Kimenju, 2013).

Modern phylogenetic analysis has characterized *Cleomaceae* as an independent family (Hall, Sytsma, & Iltis, 2002). *Cleome gyndandra* had previously been described as belonging to subfamily Cleomoideae of *Capparaceae* (Chweya & Mnzava, 1997; Hall et al., 2002; Sanchez-Acebo, 2005). There are over 200 species in the genus *Cleome*, with 50 considered to be native to Africa (Iltis, 1960, 1967). The *Cleome* genus is widely distributed throughout the tropics and subtropics, with many growing as weeds in the America, Asia, and Africa, though it is most prevalent in Africa (Iltis, 1960, 1967).

The selection of improved yield directly as a trait has been found to have low heritability in spiderplant due to being quantitative with an environment effect on contributing factors (Chweya & Mnzava, 1997). Late flowering has been identified as a trait to prioritize toward increased vegetative yield, and has been observed to have relatively high heritability (Chweya & Mnzava, 1997; Omondi & Ayeicho, 1992). This is a common genetic improvement strategy for yield increase (Kim et al., 2007; Morales, Maynard, & Janick, 2006; Wallace et al., 1993).

Many producers of spiderplant have been observed to cultivate local selections or landraces of spiderplant and in Zambia, a private seed company sells and promotes a variety of spiderplant (Chweya & Mnzava, 1997). Very few countries where spiderplant is grown wild or cultivated maintain a collection of spiderplant germplasm (Kwarteng et al., 2018). The National Gene Bank of Kenya has been described as holding 45 accessions (Kemei, Wataaru, & Seme, 1997); the National Plant Genetic Resource Center in Arusha, Tanzania, and South Africa reportedly holds 184 accessions (Jan van Rensburg et al., 2009); and 108 accessions are held by the World Vegetable Center (WorldVeg, formerly AVRDC) Genetic Resources Unit of Arusha, Tanzania (Ochieng, Tenkouano, & Yang, 2010).

The spiderplant germplasm held by the National Gene Bank of Kenya and in South Africa are reportedly lacking documentation as well as morphological and agronomic characterization (Wasonga, Ambuko, Chemining 'wa, Odeny, & Crampton, 2015). WorldVeg is actively characterizing spiderplant accessions held by the Genetic Resources Unit for field performance and producer evaluations (Dinssa et al., 2015). One of the most informative studies available in the literature characterizing multiple accessions of spiderplant observed 26 accessions for traits including days to flowering (Cecilia Moraa Onyango, Onwonga, & Kimenju, 2016).

Spiderplant produces perfect flowers on a terminal infloresence which are both self- and outcrossing-compatible and has been observed to be protogynous, which would facilitate greater rates of outcrossing (Chweya & Mnzava, 1997; Solomon Raju & Sandhya Rani, 2016). Spiderplant has been reported to be a majority-outcrossing crop when pollinators are present (Solomon Raju & Sandhya Rani, 2016).

Cytogenetic studies have reported different chromosome numbers within the species *Cleome gynandra.* most often reporting n=17, yet also reporting n=18 , n=16, and

n=10 (Koshy & Mathew, 1985; Rice et al., 2015). This may result in breeding barriers across spiderplant entries.

Deflowering is often predicted to extend vegetative production, indirectly increasing leaf yield in crops with production limited by bolting. Deflowering has had variable results in the limited studies available in the literature (Chweya, 1995; Wangolo, Onyango, Gachene, Mong'are, & Fujita, 2015). Development of genetic resources capable of continuous vegetative production would preclude labor requirements for deflowering and is widely accepted as a reliable method for yield improvement as discussed above.

Flowering time is a widely studied trait given the applications for optimal yield performance and conditional adaptability of cultivars. Maize (*Zea mays*), an outcrossing crop has been described as simple-additive with many genes responsible for small, additive effect on flowering time (Buckler et al., 2009). Self-pollenating crops such as *Arabidopsis* and rice (*Oryza sativa*), have been described as having relatively fewer genes with more substantial interaction effects (Izawa, Takahashi, & Yano, 2003; Yano & Izawa, 2007).

This study was initiated to identify genetic resources of spiderplant with delayed time to flowering. Methodology using greenhouse screening with seedling trays as a viable approach for this purpose can be considered from the results of this study.

5.2 Materials and Methods

5.2.1 Plant materials

Entries for this study included one commercial line from Simlaw Seed (Nairobi, Kenya), and five advanced lines from WorldVeg: UG-SF-23, ML-SF-17, PS, UG-SF-15, ML-SF-29; entries were renamed SP1 through SP7, excluding 'SP2' which no entry was named in this study. Five plants from each variety were grown with growing mix (Fafard Grow Mix 2; Sun Gro Horticulture, Agawam, MA) in 3L plastic containers under 14 h day, high pressure

sodium lights at the Rutgers University New Jersey Agriculture Experiment Station (NJAES) Greenhouses in New Brunswick, NJ from September 5th, 2016 and allowed to openly pollenate to produce seed.

5.2.2 Greenhouse evaluation

Seed collected from one randomly selected individual of each variety was sown in 72-cell trays with growing mix (Fafard Grow Mix 2; Sun Gro Horticulture, Agawam, MA) and germinated at the NJAES Research Greenhouses in New Brunswick, NJ. One seed was planted in each cell, about 1000 seeds in total were planted. The total number of plants emerged was 385. Plants were hand watered and remained in the seedling trays for daily observations of emergence and days to flowering, recorded for each plant individually. Seed was collected from isolated inflorescences of seven individuals selected for early flowering, six for average flowering, and four for delayed flowering.

5.2.3 Field evaluation

A field trial was conducted in Arusha, Tanzania on-station at WorldVeg, eastern and southern Africa (lat. 36.8ºE, long. 3.4°S, 1290 m elevation) to evaluate the time to flowering of the progeny selected from the greenhouse trial. Seedlings 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. Progeny were transplanted on April 21, 2017 and individually evaluated for number of days until flowering from time of transplant. The site is characterized by well-drained clay loam soil with pH 6.4. Furrow irrigation was applied as needed.

5.2.4 Statistics

Data was analyzed from the greenhouse trial and field trial separately, using PROC UNIVARIATE MIXED in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) to create histograms to

demonstrate distributions from observed days to flowering across all observations on individual plants (Figures 5.1 and 5.2). PROC ANOVA in SAS 9.4 was used to conduct mean separation analysis with Fisher's Least Significant Difference (LSD) (Table 5.1), results from field trial effect of days to flowering as an effect of plot was used to created box plot visualization (Figure 5.3).

5.3 Results and discussion

The distribution of days to flowering across individuals included in the greenhouse trial was found to follow a normal distribution excluding a group with highly delayed flowering (Figure 5.1). The mean days to flowering was 52.7, the median days to flowering was 50. After 173 days, twelve individuals had not yet flowered and were moved to a growth chamber under 10h light. Those which did not bolt under reduced daylength were moved back to the main greenhouse room under 14h light. Four individuals with delayed flowering produced seed which was collected from inflorescences isolated with glassine bags. Inflorescences were isolated for seed collection from seven "average" time to flowering individuals, and eight "early" time to flowering individuals. Seed was transferred to WorldVeg in Arusha, Tanzania for field evaluation.

The time to flowering response of individuals in the greenhouse trial had a wide range from a minimum of two days to a maximum of >182 with a standard deviation of 27.9. The median in the greenhouse trial at 50 days was not widely inconsistent from the mean, approximately 53 days (Figure 5.2). The outlier group of plants recorded as taking longer than the approximately six-month period of data collection caused the frequency distribution of responses observed in this trial to be considerably non-normal.

The median days to flowering observed in the field trial in Arusha was 38 days and the mean was about 37 days with a standard deviation of 7.3. The frequency distribution

of days to flowering in the field trial can be considered to fit a normal distribution (Figure 5.2). This distribution of results is more similar to the results found by Onyango et al. (2016) in Kenya; however, the maximum days to flowering in that study was observed to be 42 while the mean days to flowering across entries included in this study was about 47. This comparison of result may represent the beneficial effect of purposefully selecting for delayed flowering time however the results of these studies are not directly comparable having been conducted in different trials and locations (Table 5.1 and Figure 5.3).

There is a wide discrepancy between the observed days to flowering in the greenhouse trial and field trial in Arusha (Figures 5.1 and 5.2). Comparison of these distributions demonstrates days to flowering was more frequently lower in the field trial than in the greenhouse trial, indicating substantial variation due to environment.

Field performance of the progeny collected after greenhouse evaluation was found to be mostly inconsistent with the initial selection observations of the individual parents. The progeny of SP1-1 had delayed flowering in the greenhouse trial but progeny of this individual had the lowest mean days to flowering in the field trial. The individual SP 3-4 was also observed to have delayed flowering in the greenhouse yet the mean days to flowering for the progeny of that individual were only narrowly within the lower threshold of the least significant difference value difference from the overall field trial mean. The progeny of SP3-5 were observed to be within the least significant difference value from the overall field trial mean.

The progeny of SP7-1 was the only plot selected for delayed flower time greater than the least significant difference value from the overall field trial mean. The average days to flowering of the progeny of SP7-1 was found not to be significantly greater than the mean days to flowering for the progeny of SP5-2 and SP4-1, individuals selected from the greenhouse trial as having average days to flowering. The mean days to flowering for the

progeny of SP7-1 was arguably substantially higher than all other plots with a difference of approximately 6 days greater than the plot with the nearest mean observed days to flowering.

Progeny of individuals found to have flowered early in the greenhouse trial did not maintain a distinction of early flowering within the field trial. These plots were all found to be within the least significant difference value of the overall field trial mean days to flowering and can be considered to have average days to flowering in the field trial.

There are multiple potential explanations for the delayed flowering of SP7-1 progeny. If spiderplant follows a simple additive model like that of Maize, SP7-1 may have the greatest homogeneity in loci associated with delayed flowering. Alternatively, SP7-1 may lack expression of genes associated with early flowering time. If spiderplant follows a genetic architecture for flowering like rice or *Arabidopsis*, it may be the case that SP7-1 lacks interaction effect otherwise found in the individuals in this study selected for delayed flowering.

Conclusions

Progeny from SP1-1 were observed to have below-average for days to flowering; having been selected for delayed flowering in the greenhouse, these observations would support an interaction mechanism with the environment. Despite the limited number of progeny observed and large number of potential genes underlying this trait, the distinct contrast between the greenhouse and field performance supports that there is an interaction effect between the environments in this study.

The variation among progeny in SP3-4 and SP3-5 would indicate a distribution consistent with a quantitative trait, though having been derived from individuals observed to flower beyond 180 days in the greenhouse, an interaction effect of environment on the

genetic mechanisms in these entries may exist or a genetic distinction between entries from progeny and parent generations observed may be responsible for the variation of performances. This interaction demonstrates limitation on using a greenhouse screening trial as conducted in this study.

Given the uncontrolled open-pollination origin of seed evaluated in this trial, further evaluation would be appropriate to be confirmed consistent performance with additional trials. Seed from the individuals observed in the field trial in Arusha, Tanzania has been collected for further evaluation. The apparent genetic improvement of SP7-1 represents the longest vegetative production of a spiderplant accession described in the limited observations currently available on this crop.

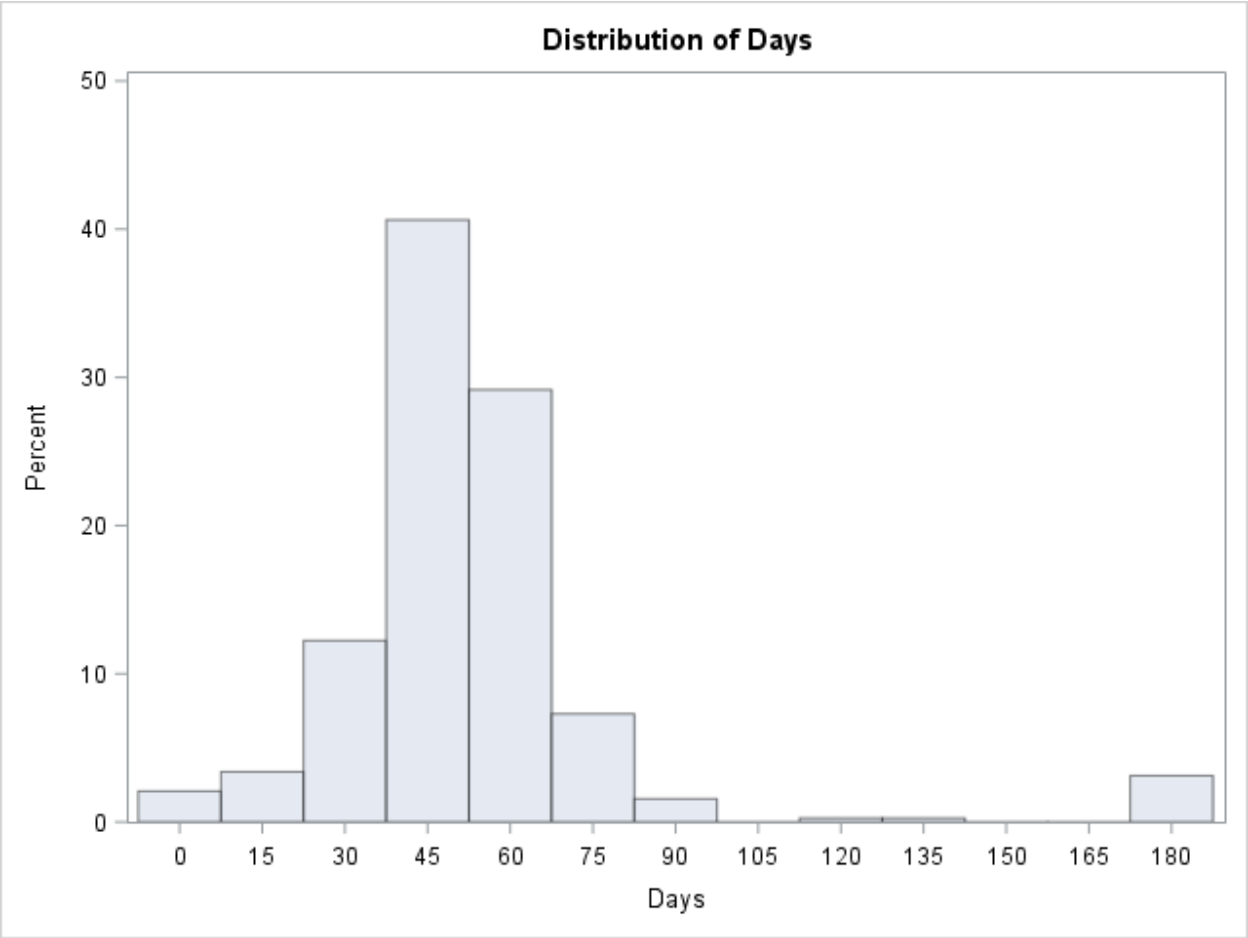

Figure 5.1. Histogram of time to flowering from individuals observed in initial NJAES research greenhouses, New Brunswick, NJ; mean 52.7, median 50.0, minimum 2.0, max 182, standard deviation 27.9.

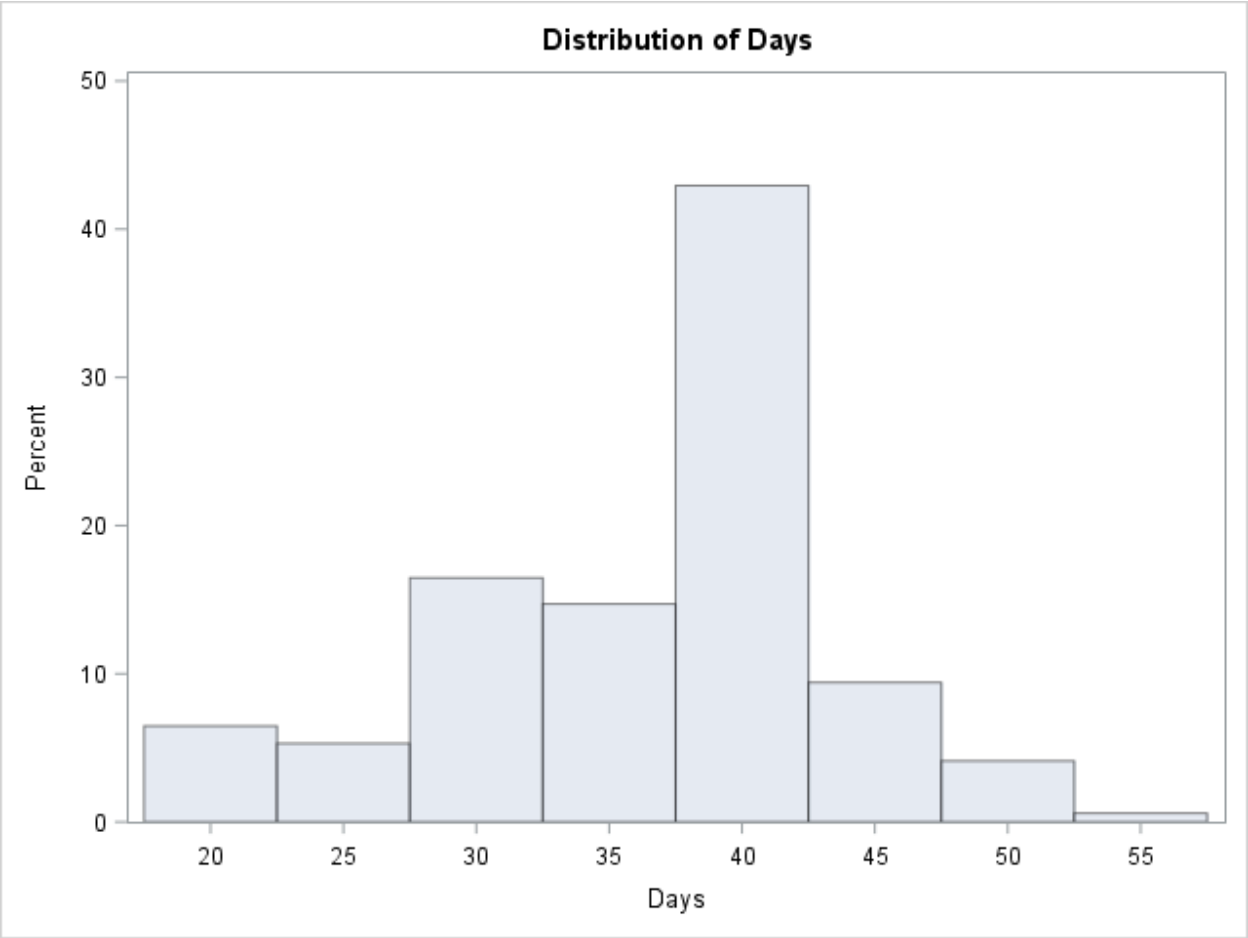

Figure 5.2. Histogram of days to flowering for field trial in Arusha, Tanzania at World Vegetable Center field research station; mean 36.8, Median 38.0, minimum 20.0, maximum 57.0, standard deviation 7.3.

Table 5.1. Field trial results with mean separation of mean days to flower for progeny plots in Arusha, Tanzania.

Entry	n (number of entries in progeny plot)	Mean days to flowering	T-test grouping	Greenhouse characterization of parent entry
SP7-1	7	47.286	A	Delayed
SP5-2	3	41.667	AB	Average
SP4-1	13	41.154	ABC	Average
SP3-5	19	40.579	BC	Delayed
SP1-9	13	39.385	BCD	Early
SP5-3	11	39.273	BCD	Early
SP3-7	14	38.786	BCD	Early
SP2-3	13	36.538	BCDE	Early
SP1-7	23	36.174	BCDEF	Average
SP3-6	10	35.900	BCDEF	Early
SP1-6	6	34.833	CDEF	Average
SP1-10	7	33.714	DEFG	Early
SP1-11	2	33.000	DEFG	Early
SP3-4	5	30.400	EFG	Delayed
SP2-2	10	29.600	FGH	Average
SP1-5	12	27.750	GH	Average
SP1-1	2	23.500	H	Delayed

Means with the same letter are not significantly different. Mean separation performed with Fisher's least significant difference analysis $\alpha=0.05$, error degrees of freedom 153, error mean square 34.94171, critical value of t 1.97559, least significant difference value 6.6524.

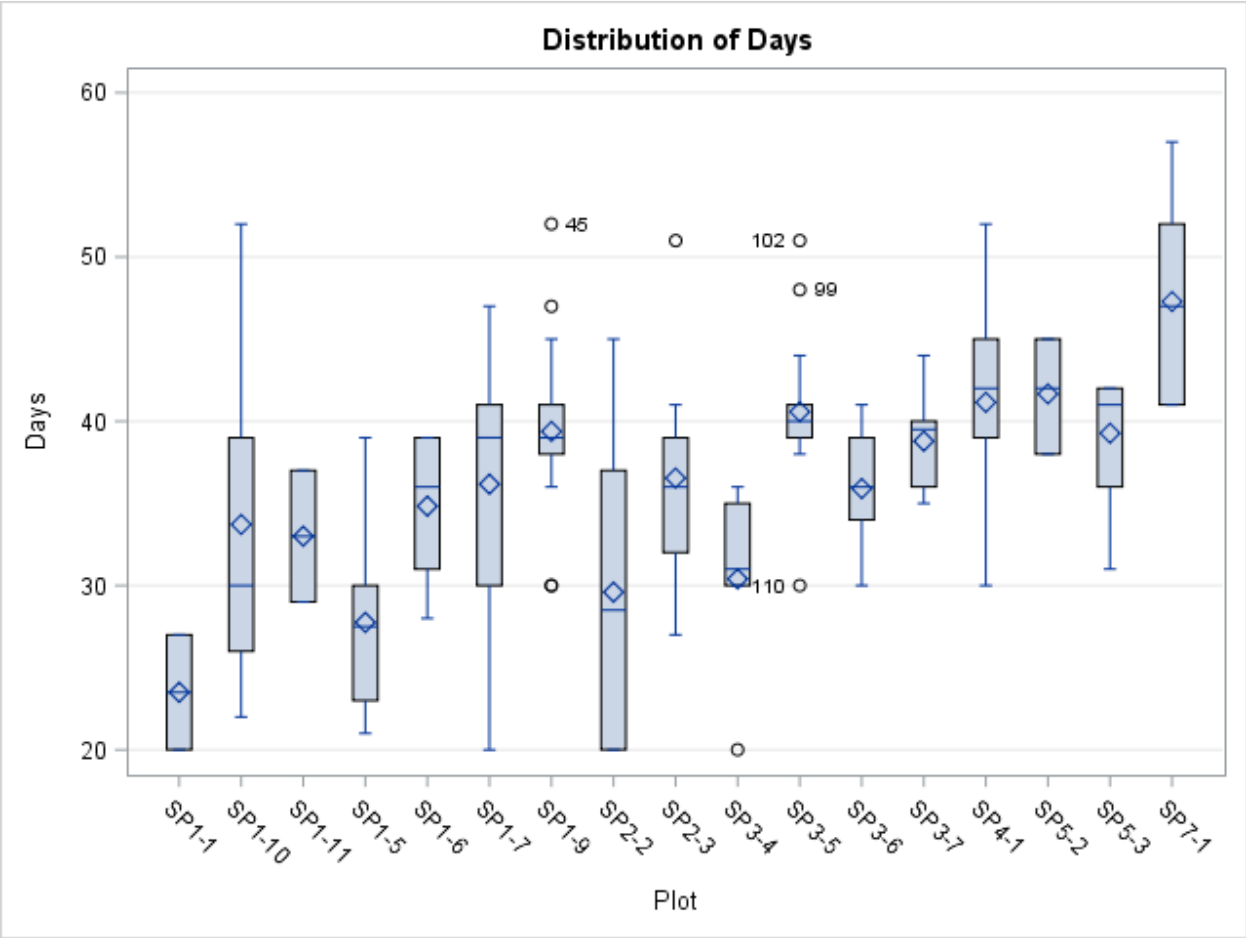

Figure 5.3. Box plot of field trial results for days to flowering as an effect of progeny plot in Arusha, Tanzania.

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Chapter 6 Progeny testing in amaranth using simple sequence repeats

6.1 Introduction

Most species within the genus *Amaranthus* are monoecious, including all of those with any commercial relevance (Brenner, D.M., Myers, R.L., Baltensperger, D.D., Slabbert, M.M., Kulakow, P.A., Sleugh, B.B., Lehmann, 2000). Flowers usually occur in both axial and terminal inflorescences with differences in prevalence between these habits across species (Hauptli & Jain, 1985). Infloresences are indeterminate, each plant often capable of producing over 50,000 seeds (National Resource Council, 1984). Individual flowers are very small, 2-4cm each, requiring at least 10x magnification to observe floral morphology (Brenner, D.M., Myers, R.L., Baltensperger, D.D., Slabbert, M.M., Kulakow, P.A., Sleugh, B.B., Lehmann, 2000; Pal, 1972). Within the infloresence, flowers are organized with both male and female within a glomerule with the exception of two species, *A. spinosus and A. dubius,* which have pistillate and staminate flowers organized into separate glomerules (Pal, 1972).

The very small size of individual amaranth flowers adds complexity to breeding procedures. It is difficult to distinguish between male and female flowers for emasculation prior to the anthers dehiscing, though it has occasionally been described (Jordan, 1996; Murray, 1938). Maintaining record of individual putative female flowers for pollination and seed retrieval is exceptionally challenging. Many amaranth breeders hybridize by dusting with pollen without emasculating and relying on visible traits to distinguish successfully outcrossed progeny (McElroy, 1982; Murray, 1938). Confirmation of successful outcrossing with molecular markers would facilitate amaranth breeding systems and allow researchers and breeders to pursue hybridizations in the absence of visible traits for confirmation.

In this study, a method for employing Simple Sequence Repeats (SSR)s for progeny or paternity testing is tested on *Amaranthus tricolor* to provide a basis to allow breeders working with this crop to bypass criteria for selecting parent lines by the presence of visible traits for identifying successfully outcrossed progeny. Molecular markers offer the means of confirming parentage and genotyping seedlings which would otherwise be ambiguous or indistinguishable phenotypically, facilitating reliable inheritance studies and cultivar development among other applications. This approach been demonstrated in numerous plant systems (Buteler, LaBonte, Jarret, & Macchiavelli, 2002; de la Rosa, James, & Tobutt, 2004; Dow, 1998) (Buteler et al., 2002). Using SSRs is proposed to avoid the additional step of sequencing which would be needed if using single nucleotide polymorphisms (SNPs), another molecular marker system actively being applied to amaranth by researchers (Sunil et al., 2014).

The amaranth entries used to make putative hybridizations were selected for having complementary stem pigmentation previously described as following an inheritance pattern of a single gene with Mendelian dominance, red being dominant to green (Brenner, D.M., Myers, R.L., Baltensperger, D.D., Slabbert, M.M., Kulakow, P.A., Sleugh, B.B., Lehmann, 2000). The pollen parent in this study (AM101) has red pigmentation in the stem and the seed parent (RUAM24) has green pigmentation in the stem. Seedlings resulting from hybridization attempts with red stem pigmentation were considered putative F_1 and tested using SSRs identified to be polymorphic across RUAM24 and Kerala Red.

With emasculation being a relatively unpopular method for breeders of this crop and the limited ability of tracking individual pollination attempts, being able to confirm hybridizations with a molecular technique is an apt approach for this crop.

6.2 Methods

6.2.1 Plant material

Parent lines Kerala Red (USDA NPGS, PI 566897) and RUAM24 (Byrnes, Dinssa, Weller, & Simon, 2017) were selected for being phenotypically distinct in the trait of stem color. Foliar samples from active growth of representative parent lines were collected from four healthy individuals each and maintained separately after tissue collection for DNA extraction in the laboratory.

Hybridization was conducted by dusting pollen from Am101 onto the inflorescence of RUAM24 without emasculation or isolation of seed heads. Seeds were collected from RUAM24 and grown in 72-cell trays containing growing mix (Fafard Grow Mix 2; Sun Gro Horticulture, Agawam, MA) under greenhouse conditions at the Rutgers University Research Greenhouses in New Brunswick, NJ. Foliar samples from ten putative F_1 individuals identified by red stems were collected in liquid nitrogen and maintained separately after tissue collection for DNA extraction in the laboratory.

6.2.2 SSR markers

For all SSR primers, the M13(-21) 18-bp sequence (5′-TGTAAAACGACGGCCAGT-3′) was added to the 5′ end of the forward primer to facilitate fluorescent labeling of the resultant PCR product (Schuelke, 2000). Additionally, all reverse primers were elongated at the 5′ end by using the sequence (5′-GTTTCTT-3′), referred to as “PIG-tailing”, resulting in adenylation of the 3′ end of the forward strand of the PCR product, which served to reduce ambiguities associated with scoring “true” versus “plus A” alleles (Brownstein, Carpten, & Smith, 1996). All primers were synthesized by Integrated DNA Technologies (Coralville, IA).

6.2.3 SSR analysis

Plant genomic DNA was isolated from all samples in this study with a Qiagen Quick-Start DNeasy Plant Mini Kit (Hilden, Germany) by following the manufacturer's instructions. Concentration and quality of DNA extractions were observed with NanoDrop ND-1000 Spectrophotometer analyzed using NanoDrop 1000 3.7.1 software (ThermoFisher, Waltham, MA, USA). PCR was conducted in 96-well plates, in a total reaction volume of 13 µL per sample, using approximately 5 ng genomic DNA, 1 x ImmoBuffer (Bioline, Tauton, MA, USA), 2mM MgCl2, 0.25 mM each dNTP (Bioline), 0.5 U 1 X Immolase DNA polymerase (Bioline), .5 pmol forward primer with M13(-21) addition, 1 pmol reverse primer with "PIG-tailing" addition and 1 pmol forward M13(-21) primer with FAM, NED, PET, or VIC fluorescent labels in each reaction.

Thermalcycling conditions were an initial denaturation of 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 55 °C for 45 s, 72 °C for 45 s, followed by 20 cycles of 94 °C for 30 s, 53 °C for 45 s, 72 °C for 45 s, followed by a final extension of 72 °C for 10 min. Each 96-well plate contained 1 sample of GeneScan Installation Standard DS-33 (ThermoFisher) for assessing consistency of results across PCR plates. PCR products were analyzed by using capillary electrophoresis (ThermoFisher 3500xl Genetic Analyzer) and sized with a LIZ 600 size standard v2.0 (ThermoFisher) and Genemapper 4.1 software (ThermoFisher).

6.3 Results and discussion

6.3.1 Primer selection

Twelve primers identified by Lee et al. (2008) were used in this study (Table 1). Eleven of the twelve primers screened amplified with GB-AMM-078 being the only one that did not amplify (Table 1). Two primers found to be polymorphic between the parent lines, GB-AMM-132 and GB-AMM-123, were used for screening putative F1 entries.

6.3.2 Progeny testing

Putative F_1 individuals were screened with primers GB-AMM-132 and GB-AMM-123; however, GB-AMM-123 did not amplify in the progeny testing. Amplification results from primer GB-AMM-132 in this study support that five out of ten of the putative F1 entries were the result of a hybridization between RUAM24 and Kerala Red (Table 2). The remaining five out of the ten putative F_1 individuals screened were found to have one allele amplify consistently with the seed parent at Locus GB-AMM-132 and another allele which was not consistent with either of the parent lines used in this study (Table 2).

6.4 Conclusions

The results of this study indicated that half of the putative F_1 individuals were the result of unintended hybridization events. Although this is a highly self-pollinated plant with reports of limited outcrossing, the results of this study demonstrate that unintended hybridizations can occur and be difficult if not impossible to visually distinguish from intended hybridizations.

Results of this study are limited by having only one marker that was polymorphic between the parent lines and amplified in the putative F_1 samples. The SSR loci used in this study from Lee et al (2008) were originally identified for variation across species and would likely facilitate progeny detection of inter-specific crosses with greater confidence. An SSR is incapable of distinguishing between an intended hybridization and an unintended outcrossing if the allele of the unintended pollen parent is monomorphic to the intended parent at that SSR locus. Multiple SSRs are typically used for progeny testing in plants (de la Rosa et al., 2004; Lambeth, Lee, O'Malley, & Wheeler, 2001). In the current study, screening with primer GB-AMM-132 was adequate to distinguish between the products of purposeful hybridization and unintended outcrossing without visually observable differences.

This study demonstrates that confirming parentage with molecular markers in amaranth can benefit a breeding agenda both featuring and absent of visible traits to identify progeny of successful crosses. Confirmation with molecular markers should be considered for incorporating into breeding programs for amaranth given the many difficulties specific to this plant system.

Table 6.1. Twelve SSR loci used in this study and the results of amplificant in parent lines.

Locus name	Genebank accession No.	RUAM24	Kerala Red
GB-AMM-013	EF117781	101/178	101/178
GB-AMM-032	EF117782	170/170	170/170
GB-AMM-051	EF117783	257/257	257/257
GB-AMM-071	EF117784	182/182	182/182
GB-AMM-078	EF117785	.	.
GB-AMM-099	EF117786	166/166	166/166
GB-AMM-105	EF117787	170/170	170/170
GB-AMM-123	EF117788	245/145	102/271
GB-AMM-129	EF117789	186/280	186/280
GB-AMM-132	EF117790	177/177	171/171
GB-AMM-136	EF117791	216/216	216/216
GB-AMM-137	EF117792	226/226	226/226

Table 6.2. PCR products using primer GB-AMM-132 across parent lines and ten putative progenies.

Sample	Locus name	Allele 1	Allele 2	Allele 3
Am_101	GB-AMM-132	171		
Am_24	GB-AMM-132		177	
24X101_A	GB-AMM-132	171	177	
24X101_B	GB-AMM-132		177	180
24X101_C	GB-AMM-132		177	180
24X101_D	GB-AMM-132	171	177	
24X101_E	GB-AMM-132	171	177	
24X101_F	GB-AMM-132	171	177	
24X101_G	GB-AMM-132	171	177	
24X101_H	GB-AMM-132		177	180
24X101_I	GB-AMM-132		177	180
24X101_J	GB-AMM-132		177	180

Figure 6.1. Photograph of RUAM24 in NJAES Greenhouse used as seed parent in this study.

Figure 6.2. Photograph of AM101 in NJAES Greenhouse used as pollen parent in this study.

Figure 6.3. Photograph of resulting generation collected from mature inflorescence of seed parent. Green-stemmed seedlings were concluded to be products of self-pollination, samples of red-stemmed seedlings were collected for parentage analysis.

6.5 References

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