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Mara Sanders
Rutgers University

Albert O. Ayeni
New Use Agriculture and Natural Plant Products Program, Department of Plant Biology, School of Environmental and Biological Sciences, Rutgers, The State University of New Jersey

James E. Simon
1New Use Agriculture and Natural Plant Products Program, Department of Plant Biology, School of Environmental and Biological Sciences, Rutgers, The State University of New Jersey; 2Rutgers Center for Agricultural Food Ecosystems, New Jersey Institute for Food, Health & Nutrition, Rutgers, The State University of New Jersey, jimsimon@rutgers.edu

Comparison of Yield and Nutritional Composition of Roselle (*Hibiscus sabdariffa*) Genotypes Grown in Central New Jersey

Mara C.D. Sanders[1], Albert O. Ayeni[1] and James E. Simon[*1,2]

[1]New Use Agriculture and Natural Plant Products Program, Department of Plant Biology, School of Environmental and Biological Sciences, Rutgers, The State University of New Jersey, 59 Dudley Rd., New Brunswick, NJ. USA.
[2]Rutgers Center for Agricultural Food Ecosystems, New Jersey Institute for Food, Health & Nutrition, Rutgers, The State University of New Jersey, 61 Dudley Rd., New Brunswick, NJ. USA.

***Corresponding author:** jimsimon@rutgers.edu

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ABSTRACT

When introducing a new crop to an area, assessments of the yield and nutritional composition can assist farmers to determine which crops and varieties are the best for commercialization. In this study, the leaf yield and nutritional composition of four field-grown Roselle genotypes were determined over two growing seasons at the Rutgers University Horticultural Research Farm III in East Brunswick, New Jersey, in 2017 and 2018. The genotypes (African Green, Indian Red, Indian Variegated, and Thai Red) were chosen based on regional demand for ethnic vegetables across the Northeastern United States. African Green Roselle yielded the highest dry weight: 81.89 grams of stem branch tips per plant harvested every two weeks over an 8-week period during two consecutive growing seasons, followed by Indian Variegated (79.94 g), Indian Red (74.23 g) and Thai Red (55.70 g). All four genotypes contained 2% (dry weight) of the daily-recommended dose of calcium and potassium. Therefore, 230 g of dried Roselle leaves would be theoretically able to meet the daily minimum calcium, iron and potassium requirements of children aged between 1-8 years. Additional research is needed to study the impacts of branch tip harvesting on calyx production as well as to develop an early blooming cultivar with high nutritional composition. Such information would help to increase the urban access to fresh ethnic crops. Indigenous vegetables such as Roselle can serve as a model for other ethnic crops in their transition to commercial specialty crops.

INTRODUCTION

African indigenous vegetables (AIVs) such as Roselle (*Hibiscus sabdariffa),* African Spider plant *(Cleome gynandra),* Amaranth (*Amaranthus spp.)*, Moringa *(Moringa oleifera),* and others whose leaves are consumed provide nutritious food items that are eaten locally, can often be commercially sold providing profitable higher value niche crops and are have potential to enter into new market crops around the world (Weller et al., 2015). Ethnic populations in the Northeastern United States including African, Chinese, Asian Indian, Mexican and Puerto Rican were willing to pay more for ethnic vegetables (Ariyawardana et al., 2010). There is great potential for the production and sale of ethnic crops in the eastern United States due to the large ethnic populations in its major cities (Govindasamy et al., 2006). Availability of ethnic foods is low due to phytosanitary concerns that restrict imports and limited local production in the U.S. (Guarnaccia et al., 2012). Ethnic, or traditional, crops can range from grains to vegetables, but are usually native or naturalized crops that are specific to a region and have been maintained close to their center of origin (Gockowski et al., 2003; FAO, 1988). Ethnic or indigenous crops outside the mainstream commercial crops found in supermarkets are also called underutilized and neglected crops (Williams and Huq, 2002).

Roselle (*Hibiscus sabdariffa*, L. Family Malvaceae) is an ethnic crop that is reported to originate from North Africa and Southeast Asia) (Fasoyiro, 2005; Morton, 1987; Mohammed, 2012). Roselle is primarily grown for its calyx (used for tea, jams, jellies, and other beverages) in China, India, Senegal, Sudan, Uganda, Indonesia, Malaysia, Mexico, and more recently in the United States in Florida, California, Louisiana, and Kentucky (Juliani et al., 2009; Mohamed et al., 2012). Roselle has a variety of common names such as Jamaican sorrel, red sorrel, Indian sorrel, rozelle, rozelle hemp, natal sorrel and rosella (Mohamed et al., 2012). Vernacular names include rozelle, jelly okra, lemon bush and Florida cranberry (Small, 1997; Ayeni et al., 2018).

Roselle is an annual, indeterminate, autogamous, dicotyledonous herbaceous subshrub reaching up to 2.4 m tall (Boulanger, 1984). *H. sabdariffa* var. *sabdariffa* has 4 races that breed true to seed: *bhagalpuriensi, intermedius, albus*, and *ruber. Bhagalpuriensi* has green and red streaked inedible calyces. *Intermedius and albus* have yellow-green edible calyces and also produce fiber. The race *ruber* has edible leaves and calyces (used in drinks, wine, beverages, jams, jellies, coloring, and flavoring ingredients in Europe, Africa, Asia, and the Americas (Duke, 1985; Villani et al., 2013, Ayeni et al., 2018). Roselle requires a monthly rainfall from 130-250 mm during the first three to four months of growth (Plotto, et al., 2004). Dry weather is desirable during the latter months of growth and can increase the quality of calyces and aid in post-harvest drying. Roselle is highly photoperiodic (El Afry et al., 1980). Seeds are usually planted 2.5 cm deep, 60-100 cm between rows and 45-60 cm between plants in a row (Mohamed et al., 2012). Diseases that affect Roselle include fungi, viruses, bacteria, nematodes and a number of insect pests (Plotto et al., 2004; Duke, 1983; Adkins et al., 2006).

The leaves are also a product of commerce in some areas where traditionally its' consumed as a leafy green vegetable or added as a condiment to other dishes in some areas in Sub-Sahara Africa (Plotto et al., 2004; Mataa et al., 2020; Zhen, 2016). In India, hibiscus leaves are prepared in wide variety of pickled dishes, dals and curries (Mishra et al., 2018, Ayeni et al., 2018). Nutritional composition data is sparse, and differences between cultivars and land races are recognized (Villani et al., 2013; Mataa et al., 2020, 2018; and see Table 1). Nutritional data is variable because to date much does not originate from standard controlled comparative studies using the same analytical techniques, growing conditions, etc. The phytochemical compounds found in Roselle leaves include polyphenols neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, quercetin, kaempferol and their glycosides, and 5-(hydroxymethyl) furfural (Villani et al., 2013; Zhen et al., 2016). The *in vitro* antioxidant levels varied by cultivar from 17.5 to 152.5 ± 18.8 μmol Trolox/g as expressed by their varied leaf and stem colorations (Zhen et al., 2016). Roselle leaf extract showed potential anti-inflammatory activity when applied to RAW 264.7 cells as it reduced the lipidpolysaccharide induced nitric oxide production (Zhen et al., 2016).

The compound 5-(Hydroxymethyl) furfural (5-HMF) was found in Roselle leaves and was identified as a potential biomarker for assessing the quality of dried Roselle leaves (Zhen et al., 2016). The 5-HMF is usually formed when dehydration occurs in acid environments or when subjected to high temperatures and is commonly found in foods with sugar following drying or baking (Roman-Leshkov et al., 2006). The levels of 5-HMF varied by cultivar and was potentially impacted by the effectiveness of the drying methods (Zhen et al., 2016).

An analysis of the nutritional and secondary metabolites of Zambian Roselle harvested at immature, mature and senescent leaf stages determined that raw, mature leaves had the best nutritional content (Siziya, 2017). The cooking method of Roselle leaves also has an impact on the nutritional content, with raw leaves preserving most of the available nutrients, but water blanching reduced anti-nutritional factors such as alkaloids and oxalates along with water-soluble vitamins (Mataa et al., 2020; Siziya, 2017).

Roselle and other indigenous vegetables are being evaluated for their nutritional qualities as malnutrition, especially of children, is a serious public health concern in Zambia, Tanzania, and Kenya (Fanzo, 2012). In Zambia, it is estimated that 1.5 million children suffer from chronic malnutrition, among children less than five years of age, 47% are stunted, 28% are underweight and 5% are acutely malnourished (Anon, 2009). Micronutrient deficiencies, especially iron and iodine, can have detrimental long-term health and economic consequences (Banerjee et al., 2011). This paper focused on the leaves of roselle among different genotypes for yield and nutritional composition.

MATERIALS AND METHODS

Four Roselle cultivars (Africa Green (AG), Indian Red (IR), Indian Variegated (IV), and Thai Red (TR)) were grown during the 2017 and 2018 field season in New Brunswick, New Jersey. Plant Seeds were acquired as follows: AG (Nigeria), IR (www.seedsofindia.com), IV (sport from IR seeds), TR (Baker Creek Heirloom Seeds, www.rareseeds.com). The African green (sometimes called 'white') genotype has green leaves, stems, veins and calyces. Indian red has red petioles, stems, leaf veins and calyces with green leaf tissue. Indian variegated has red and green striped stems, leaf veins, and calyces with green leaves and red petioles. The Thai red genotype is primarily used for calyx harvesting and has red calyces, veins, and stems with leathery dark green leaves (www.rareseeds.com). These varieties were field trialed in East Brunswick, New Jersey located in Middlesex County (40°27'42.7"N, 74°25'36.8"W). Middlesex County has a mean annual precipitation rate of 45.31in, average temperature of 51.9°F, an average minimum temperature of 41.5°F, and an average maximum temperature of 62.2°F (NOAA, 2020). The longer-term objective was to select the most promising genotypes for introduction in Kenya, Tanzania and Zambia in collaboration with our partners working with AIVs in Eastern Africa.

Plants were started from seed in a standard greenhouse flat filled with Pro-Mix® brand BX general-purpose professional growing medium soil. Seedling trays were grown under greenhouse conditions (12-hour light cycles) for approximately 6 weeks before being transplanted into the field. Field planting was done by hand following a randomized complete block design with 3 replications. Plants were planted under black plastic mulch with drip irrigation and grown for another 6 weeks before harvesting began. In 2017, the seedlings required 6 weeks of greenhouse production, followed by transplanting into the field for an additional 7 weeks before the first harvest. In 2018, the seedlings required 5 weeks of greenhouse production, followed by transplanting into the field for an additional 5 weeks before the first harvest.

The Roselle genotypes were grown in a mix of Nixon Loam and Sassafras soils at Rutgers Horticultural Research Farm III. Using the randomized complete block design accounted for variations in soil types and wetness. Each section was planted with 45 cm distance between plants, 1-meter distance between rows, and with guard plants to reduce edge effects. A combination of herbicides and hand weeding was used to control weeds between rows.

Roselle was harvested every two-weeks by removing the branch tips. After the initial removal of the apical meristem subsequent branches were harvested if they exceeded 11 cm in length (from the main branch). Branch tips were then placed in paper bags after fresh weights were obtained. Plant samples were dried in an electric plant drier at 50°C for 4 days or to constant weight. Samples were then weighed for dry mass and stored. The dried samples were ground to 20 mesh or 0.85 mm sieve size. Replicates from each harvest date were combined and were analyzed at the Pennsylvania State University Agricultural Analytical Services Lab using the standard acid digestion and ICP analysis protocol (Huang and Schulte, 1985).

Nutritional data was combined from the 2-week harvests over the two growing seasons to analyze the average nutritional content in each genotype. The genotypes were analyzed for five macronutrients (phosphorus, potassium, calcium, magnesium and sulfur) and seven micronutrients (manganese, iron, copper, boron, aluminum, zinc, and sodium).

Data was analyzed using Microsoft 2010 Excel (with the add-in XLSTAT by Addinsoft) and SAS University Edition (SAS Institute Inc., 2018). The repeated measures ANOVA test and Tukey's Post-hoc analyses were conducted on the harvest data collected over the 2017 and 2018 growing seasons. Repeated measures ANOVA and Tukey's honestly significant difference post-hoc analysis were chosen to accommodate missing data points due to mold, missing samples and diseased plants. Statistically significant data was determined by a P-value threshold of 0.05.

During the 2017 growing season, planting was delayed due to rainy conditions. Therefore, planting was done 6 weeks after the plants were sown in the greenhouse. Leaf harvesting, in the form of branch tips, started at 7 weeks after transplanting into the field. African green, Indian Red and Indian Variegated genotypes were grown. Diseases present were fusarium, powdery mildew and edema, all appearing at the end of the season. Branch tips were harvested by hand every two weeks. Data on fresh weights, dry weights, plant height and the number of stem branch tips were collected.

During the 2018 growing season, seedlings were grown for 5 weeks in the greenhouse before transplanting into the field. The plants were harvested beginning at 5 weeks after transplant. The African green, Indian Red, Indian Variegated, and Thai Red genotypes were grown. Diseases present were powdery mildew, Tobacco Mosaic Virus, and edema. Powdery mildew was present on seedlings and persisted over the growing season. Branch tips were harvested by hand every two weeks. Data on fresh weights, dry weights, plant height and the number of stem branch tip were collected.

RESULTS

Yield. Results for the two-week interval harvests over the 2017 and 2018 growing seasons when averaged together have a significant difference between the means based on a repeated-measures ANOVA test (Table 2). However, the Tukey's HSD post-hoc analysis shows no significance between the genotypes. Based on the means and small standard deviations the genotypes had a very similar average dry branch tip yields per plant (Table 2). The dry weight harvest averages were all very similar with the first harvest having the lowest yields and very similar yields for the subsequent harvests (Figure 1).

Macronutrients. There were differences in phosphorus levels between the genotypes with AG being significantly lower than IR and IV. AG was also significantly lower in potassium than IR and IV. AG was significantly higher than IR and IV in calcium and aluminum (Figure 2). All genotypes were significantly different from each other for boron levels according to the post-hoc analysis. Zinc levels did not differ among the genotypes.

Micronutrients. Results from the micronutrients analysis of Roselle genotypes were analyzed using the Multivariate Analysis of Variance (MANOVA) (Table 3). All genotypes showed a difference between the nutrients present at each harvest with p-values of <0.0001. The mineral content in the African Green (AG) genotype were similar across all harvests. However, AG showed higher iron content at the first harvest and higher sodium at the third harvest. The Indian Red (IR) genotype showed similar levels in the mineral analysis. IR, however, had slightly higher iron and magnesium at the first harvest and higher sodium at the second and third harvests. Indian Variegated (IV) showed little variation among minerals over the four harvests. The IV has a similar pattern to AG and IR with first harvest plants having higher iron and magnesium content and a higher sodium content at the third harvest. Also, IV had a higher variation in aluminum content over all harvests.

Nutrient contents of macro and micronutrients were similar among African Green, Indian Red, and Indian Variegated. The TR had higher variation across the harvests and did not have a higher iron or magnesium content at the first harvest as was the case for the other genotypes (Figure 3).

DISCUSSION

Roselle provides substantial levels of calcium and iron compared to daily needs. The African Green, Indian Red, and Indian Variegated genotypes used in this study performed very similarly in terms of yield and nutritional content. Choosing a genotype to grow in New Jersey or in Africa should be dependent on the eco-geographical similarities with intended introduction areas, intended market and local demand. Ecophysiological/Farming systems research relating to how these ethnic crops are grown and physiological responses thereof will be critical research themes (Mataa et al., 2018).

Discrepancies between the ANVOA and Tukey's analyses were potentially caused by small sample sizes due to diseases, mold and missing data. Diseases observed included powdery mildew, fusarium wilt, and tobacco mosaic virus. Powdery mildew caused detrimental symptoms on some plants while it caused others to have shorter internodes and a 'witches' broom' effect (a mass of stems growing from a similar point), which caused a decrease in yield. Fusarium wilt caused several plants to split open along the stem and produce a jelly-like substance. The tobacco mosaic virus was also found in one plant sample possibly from pests in the greenhouse before transplanting. All diseases were identified by the Rutgers Plant Diagnostic Laboratory.

Other issues encountered were edema and varietal mixtures included in the seeds. Edema was observed on all genotypes, with AG and TR being the most affected. Edema, or oedema, was possibly due to overwatering of the plants, causing bumps on the epidermis of the stems and petioles. As the season progressed, the bumps caused by edema would rupture, leading to possible increased risk of disease.

Small sample sizes and large variations within genotypes have possibly led to a deceptively low p-value of the MANOVA analysis. Future work should utilize large samples of several genotypes from the same growing region to determine the nutritional composition of Roselle specific to certain areas. Plant breeders should consider metabolites such as antioxidants as well as other bioactive secondary metabolites associated with human health as well as the nutritional composition of Roselle when selecting and breeding more desirable cultivars.

Exclusive focus on biological issues and failure to include socio economic parameters in studies such as ours has led to low uptake of potentially important technologies arising out of applied research. Considering the yield similarities, the profitability of the genotypes in this study and consumer surveys should be conducted to determine the most desirable, and therefore marketable, of the genotypes. The next steps should include breeding Roselle for increased and uniform yields. Since the growing season in New Jersey is too short for proper calyx production, breeding should focus on plants with early flowering in order to maximize farmer profits by having leaf and calyx producing plants. Comparative studies to determine whether varieties are best grown for one product (e.g. leaves or calyces) or both needs to be ascertained.

Future studies should determine the impacts of leaf harvesting intensity on the calyx production in order to determine the best harvest strategy. Given the unique flavor and health benefits of Roselle, potential use as a microgreen and production in vertical farming should be explored. Dual purpose varieties, grown for both leaves and calyces, should also be considered.

Future breeding programs should focus on meeting the demands of consumers in terms of taste, traditional uses and preferred texture. Careful plant breeding can utilize the variation between genotypes like the African, Indian and Thai ones used in this study to create more homogenous crops in terms of yield and nutritional content. Roselle already has a promising nutritional profile that can be enhanced with selective breeding. Further research into the variations of nutritional content over the growing season in Roselle and other indigenous vegetables can lead to insights into impacts on human health, as well as better harvesting and postharvest methods.

Table 1. Nutritional composition of Roselle (*Hibiscus sabdariffa)* leaves according to three different sources*

Component	Nutritional Composition (100g)[1]	Nutritional Composition (100g)[2]	Nutritional Composition from Zambia (ZM 5738) (100g)[3]
Moisture	85.6 g	85.6%	78.73%
Energy	57 Kcal	43 Kcal	nd
Protein	1.7 g	3.3 g	13.63%
Fat	0.1 g	0.3 g	6%
Carbohydrate	12.4 g	9.2 g	41.30%
Ash	0.2 g	1.6 g	13.33%
Vitamin A, RE	133 µg	nd	nd
Vitamin A, RAE	66.5 µg	nd	nd
Beta-carotene	797 µg	4135 mg	nd
Thiamine	0.01 mg	0.17 mg	nd
Vitamin C	44 mg	54 mg	25.23 mg
Calcium	9 mg	93 mg	nd
Fiber	nd[4]	1.6 g	25.73%
Phosphorus	nd	93 mg	nd
Iron	nd	4.8 mg	nd
Riboflavin	nd	0.45 mg	nd
Niacin	nd	1.2 mg	nd

*Data does not originate from a controlled comparative study using same nutritional analytical techniques, growing conditions and laboratory techniques.

[1]Institute of Nutrition, Mahidol University, 2014

[2]Duke and Atchley, 1984

[3]Siziya, 2017

[4] nd= no data

Table 2. Mean weight of dried branch tips in three roselle genotypes harvested at 2-week intervals every two weeks over an 8-week period at Horticultural Farm 3, East Brunswick, New Jersey in 2017 and 2018.

Roselle Genotype	Harvest #				
	1	2	3	4	
	Branch Tip Dry Weight (g)				
African Green (AG)	9.58 ± 4.31*	23.48 ± 12.15	21.31 ± 7.19	27.53 ± 10.75	
Indian Red (IR)	8.58 ± 2.83	19.89 ± 9.94	21.02 ± 15.70	24.75 ± 12.21	
Indian Variegated (IV)	10.24 ± 2.68	23.36 ± 9.83	22.25 ± 7.79	24.09 ± 9.04	
Solution for Fixed Effects					
Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	8.0846	0.8739	60	9.25	<.0001
Tukey (HSD) / Analysis of the differences between the categories with a confidence interval of 95%:					
Contrast	Difference	Standardized difference	Critical value	Pr > Diff	Significant
GENOTYPE-AG vs GENOTYPE-IV	5.050	1.912	2.405	0.144	No
GENOTYPE-AG vs GENOTYPE-IR	3.191	1.208	2.405	0.453	No
GENOTYPE-IR vs GENOTYPE-IV	1.858	0.704	2.405	0.762	No
Tukey's d critical value:			3.402		

*Standard deviation

Table 3. Plant nutrient contents per 100g of dried leaves for four Roselle genotypes harvested at two-week intervals over an 8-week period during two growing seasons (2017 and 2018) in New Jersey. Means shown with standard deviations (St. dev.) for the genotypes African Green (AG), Indian Red (IR), and Indian variegated (IV).

	AG	St. dev.	IR	St. dev.	IV	St. dev.
P %	0.439[1]	0.052	0.454	0.056	0.445	0.054
K %	1.693[1]	0.257	1.903	0.269	1.942	0.324
Ca %	1.578[1]	0.174	1.438	0.277	1.542	0.260
Mg %	0.469	0.058	0.444	0.093	0.463	0.074
S %	0.300	0.057	0.295	0.042	0.295	0.050
Mn mg/kg	50.684	22.081	45.387	16.365	39.622	17.908
Fe mg/kg	73.908	17.350	71.816	11.946	64.729	10.530
Cu mg/kg	12.028	1.770	12.127	1.401	11.886	1.396
B mg/kg	43.391[1]	4.872	39.675[1]	5.873	35.193[1]	3.059
Al mg/kg	18.915[1]	13.218	17.337	8.820	15.084	7.067
Zn mg/kg	30.603	4.175	34.419	3.324	32.783	3.366
Na mg/kg	25.219	18.193	24.819	19.232	20.790	14.291

[1]significant at 95% confidence interval

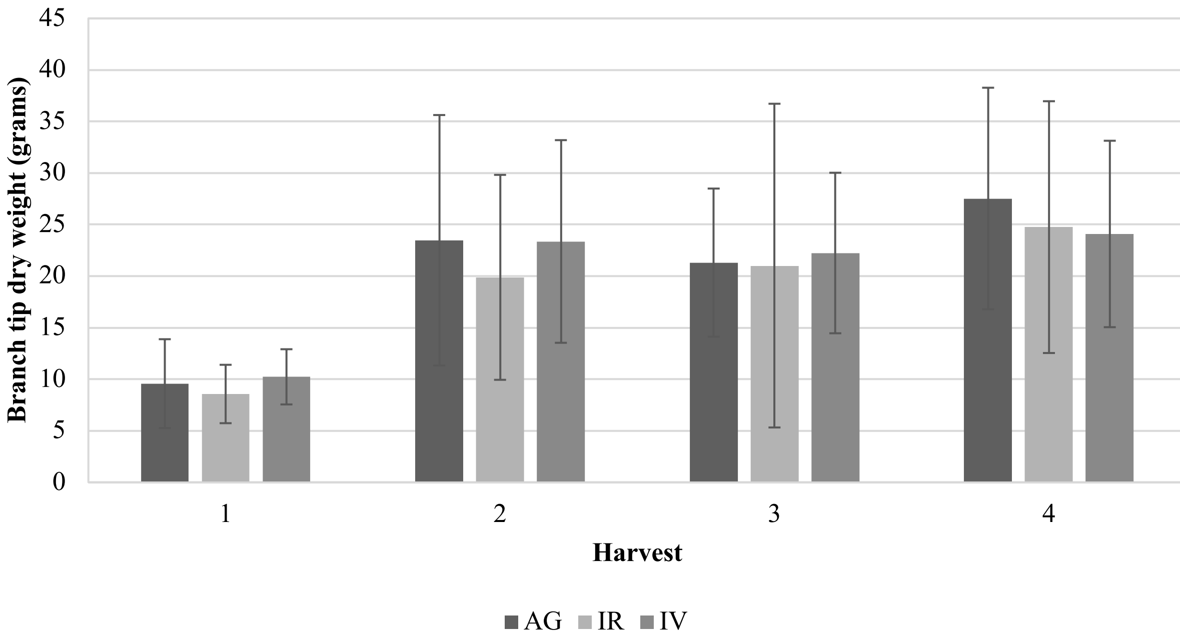

Figure 11. Dry leaf yield per plant means from the African Green (AG), Indian Red (IR), and Indian variegated (IV) genotypes harvested every 2 weeks for 8 weeks. Bars represent ± 1 standard deviation. Plants were grown approximately 6 weeks in the greenhouse and 6 weeks in the field before harvesting began.

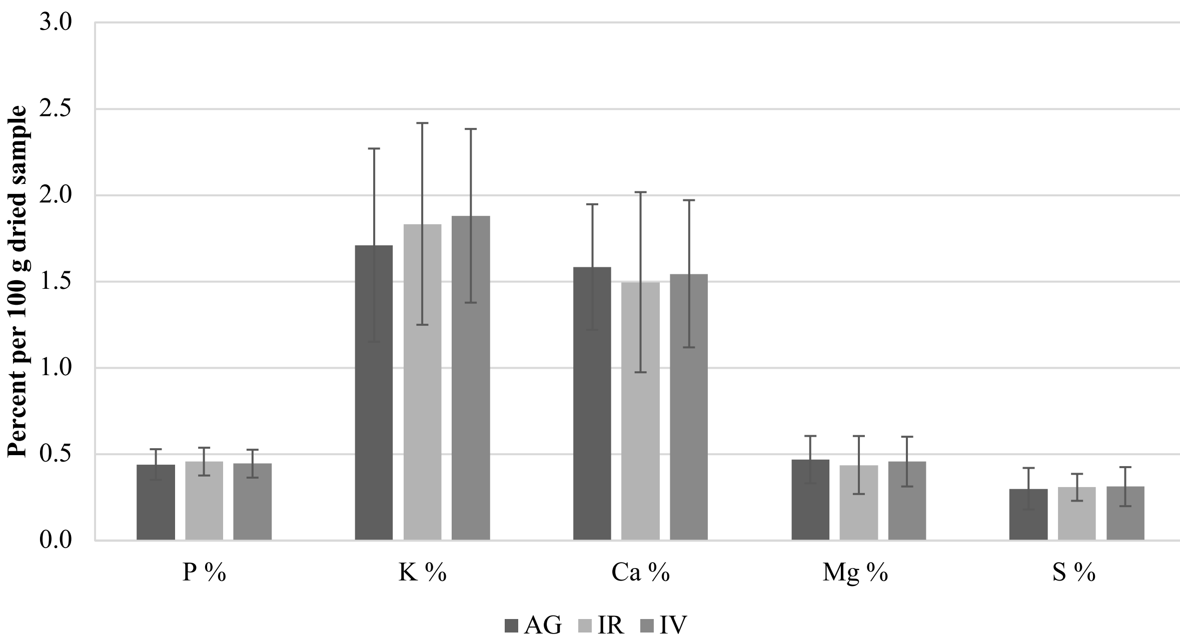

Figure 2. Percentage of phosphorus, potassium, calcium, magnesium and sulfur per 100 g dried leaf sample. Samples means are from 2-week harvests over an 8-week period during two growing seasons (2017 and 2018) in New Jersey. Bars represent ± 1 standard deviation (n=33). African Green (AG), Indian Red (IR), and Indian variegated (IV).

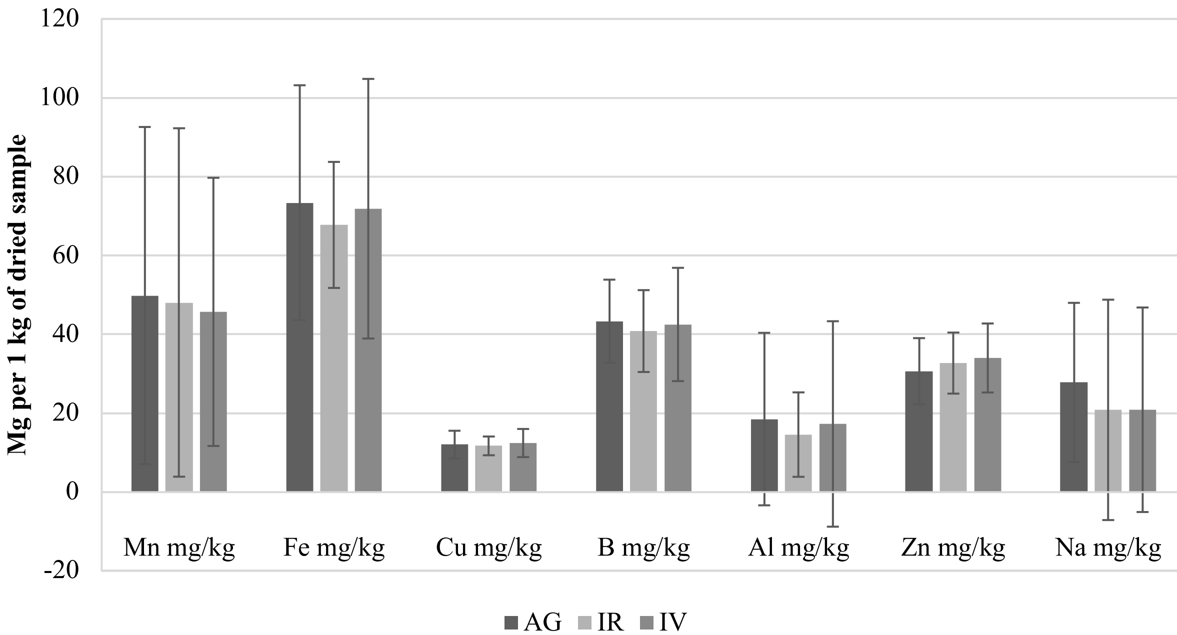

Figure 3. Micronutrients contents (mg/kg) in the African Green (AG), Indian Red (IR), and Indian Variegated (IV) cultivars including manganese, iron, copper, boron, aluminum, zinc, and sodium. Samples means are from 2-week harvests over an 8-week period during two growing seasons (2017 and 2018) in New Jersey. Bars represent ± 1 standard deviation (n=33).

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