Evaluation of 25 genotypes of *Amaranthus cruentus* for leaf yield, iron, zinc and carotenoids content.

Eliel B. Sossou[1], Enoch G. Achigan-Dako[1*], E. O. Deedi Sogbohossou[1, 3], Herbaud P. F. Zohoungbogbo[1, 4], Nicodeme H. Fassinou[1], Happiness O. Oselebe[2]

[1] Laboratory of Genetics, Horticulture and Seed Science, Faculty of Agronomic Sciences, University of Abomey-Calavi, BP 2549 Abomey-Calavi, Republic of Benin [2] Department of Crop Production and Landscape Management, Ebonyi State University, Abakaliki, Nigeria. [3] Biosystematics Group, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, the Netherlands. [4] World Vegetable Center, West and Central Africa, Coastal & Humid Regions, Cotonou, Benin.

Abstract

The present study examined the yield performance of 25 genotypes of *Amaranthus cruentus* for total carotenoids, zinc and iron content as well as leaf yields. Experiments were conducted during the early planting season (April-June) and the late planting season (August-October) 2019 in the experimental field of the Department of Crop production and Landscape Management, Ebonyi State University, Nigeria and the extraction of nutrients content in the Laboratory of Biochemistry of Alex Ekwueme Federal University, Nigeria. The total yield and marketable vegetable yield showed significant differences among all the genotypes. For total carotenoids, zinc and iron content, significant variation was found among the genotypes. Seasonal variation significantly affected all the traits except total carotenoids levels. These results confirmed the hypothesis that selection can be made for leaf yield performance and some nutrients such as iron and zinc. The study provides information on the variation for traits of interest to enhance breeding for leaf yield and nutrient content in *A. cruentus*.

Keywords: Amaranth, Nutrient content, Leaf yield, performance

INTRODUCTION

Over the past half-century, the production and demand of calorically dense staple foods such as cereals have increased accounting for 3.8 % of global production, and remain the major source of energy in response to food security (OECD-FAO, 2016). Production of micronutrient-rich non-staple crops has not increased in equal measure (Bouis and Saltzman, 2017). Although, they are increasingly well-known for their therapeutic value, benefits in health-promoting, and protecting attributes as well as their potential nutritional value essential for food and nutrition security (Arya et al., 2019; Schreinemachers et al., 2018). However, nutrient deficiencies are still common in Africa with overall iron-deficiency, which is a major cause of anemia and vitamin A deficiency, which is the leading cause of blindness (Garnett et al., 2013; WHO, 2010; WHO, 2009) .

Previous studies have proven that *Amaranthus cruentus* (L.) is an important crop that have a high nutritional value for human health with the potential to alleviate malnutrition and sustaining food security (Achigan-Dako et al., 2014; Gerrano et al., 2015). The species is highly appreciated, and one of the most commonly produced and consumed African indigenous vegetables (AIVs) (Smith and Eyzaguirre, 2007). Many studies were performed and their results aimed to guide breeding programs for enhanced leaf yield and to provide a basis for estimating elemental micronutrient variability in vegetable amaranths (Byrnes et al., 2017). Thus, iron and zinc content were assessed to identify genotypes with high levels of those nutrients. According to (Achigan-Dako et al., 2014), intraspecific variation in the genus could be exploited to improve macro and micro-nutrients mostly for vitamin A, iron and zinc contents. So far, the intraspecific variation of *A. cruentus* leaves content in iron, zinc, and carotenoids was only assessed for a limited number of local accessions (Byrnes et al., 2017; Jiménez-Aguilar and Grusak, 2017; Prakash and Pal, 1991). One of the three main challenges to improve the nutrition of one billion people by 2030 relates to the mainstreaming of biofortified traits into public plant breeding programs (Bouis and Saltzman, 2017). These breeding programs might follow a long process for appropriate cultivars development and entails screening of germplasm for available genetic diversity, pre-breeding parental genotypes, developing and testing micronutrient-dense germplasm, conducting genetic studies, and developing molecular markers to lower the costs and quicken the pace of breeding. Facing those challenges, national agricultural research institutes and the private sector should emphasize the participatory evaluation of local cultivars and the commercial varieties together with farmers and consumers for yield stability and identification of promising lines for development of breeding programs or direct release on the market. This study focused on assessing leaf yield, and nutritional performance of a set of 25 genotypes of *A. cruentus* to generate useful information regarding the variation in traits of interest within the species.

Materials and methods

2.1. Experimental location

This study was conducted at the experimental farm of the Faculty of Agriculture and Natural Resources Management, Ebonyi State University (EBSU), Abakaliki, in the South-eastern region of Nigeria at 6°151N latitude and 8°100E longitude. The early seasonal trial was carried out from April until June 2019, with an average temperature of 27.6 °C. The second trial was performed from August until October2019 with an average temperature of 26.3°C. Abakaliki city has a bimodal rainfall with the main rainfall pattern occurring from April to October, with its first peak often occurring in July and the second in September (Diagi and Nwagbara, 2018). The total amount of rainfall received during the two seasons was 152.9 mm and 211.4 mm, respectively. The site had a sandy loam soil with the following characteristics: pH= 5.8, ECEC= 7.828, %N= 0.14, %P= 25.5, %K= 0.164.

2.2. Plant materials

In this study, 25 genotypes of *A. cruentus* from Nigeria, Benin, Tanzania, USA, and Mexico were used of which twelve were from the collection of the Laboratory of Genetics Horticulture and Seed Science (GBioS) and thirteen from EBSU (Table 8). Two commercial cultivars were included as checks: EA2 (Madiira 2) and NIG 10 (ABAKALIKI Local2). 'Madiira 2' was formally released by Horti-Tengeru in Tanzania and 'ABAKALIKI Local2' is commercialized in the local seed market and widely used by farmers in Abakaliki (Nigeria).

2.3. Field layout and agronomic practices

Field experiments were arranged in a simple lattice (5*5) design with two replicates for evaluation during two seasons. All genotypes including the check varieties were germinated in a nursery made on soil and 4 weeks old seedlings were transplanted at a spacing of 0.25 m within and between rows. Each replicate contained 25 plots grouped in five blocks of five genotypes. The distance between plots was 0.5 m and the distance between replicates was 1 m. Each plot consisted of three rows of 9 plants giving a total of 27 plants per genotype and per replicate. Poultry manure was weighted and applied just after bed diggings at a rate of 25 t/ha, 3 days before transplanting. Lambda-cyhalothrin 2.5 EC (Lambdacal) was applied to control caterpillars and Mancozeb combined with metalaxyl to control fungi.

2.4. Data collection

2.4.1. Total yields

Two yield data collected, were respectively, the total yield and marketable fresh vegetable yield. The total yield consisted of weighing the plant above the soil surface. The marketable fresh vegetable yield was obtained from the weight of leaves and tender stems after separation of the thicker central and axial stems.

2.4.2. Nutritional quality traits

An elemental micronutrient analysis for carotenoids, iron, and zinc content was performed in the Laboratory of Chemistry and Biochemistry of Alex Ekweme Federal University, Nigeria.
Fresh leaf samples were collected on foliar subsamples of three fresh leaves randomly selected on five plants from each plot four weeks after transplanting and labelled for chemical analyses. The zinc and iron content were determined by using dry ashing procedure and the analytic data was recorded in replicate as described by the Association of Official Analytical Chemists (AOAC, 1990). Whereas, the total carotenoids were analysed in weighing 2 g of fresh plant leaf sample and homogenise it with 20 ml of acetone 80%. The absorbance of the solution was read at 470 nm, 646 nm, and 663 nm against the solvent (acetone) blank according to the methods used by Sumanta et al. (2014). All the analytic data was recorded in triplicate, and the quantification was expressed in μg/g.

2.5. Data analysis

2.5.1. Analysis of variance

To detect significant differences between genotypes, traits with a normal distribution were subjected to an analysis of variance (ANOVA) across seasons in a linear mixed model (lmer). At first, block, replication, and their interaction were considered as random effects and genotype and season as fixed effects. Secondly, block, replication, season, and their interaction were considered as random effects and only genotype as fixed effect. Afterwards, both models were compared with the R^2 values extracted to select the best model which explained the high proportion of variance for genotypes and seasons. Unless stated, otherwise, all analyses were performed with the R software version 3.5.2 (RCore, 2018).

3. RESULTS

The results of analyses of variance of all the traits across seasons are presented in Table 1. The mean sum of squares, due to genotypes was highly significant ($P < 0.001$) for all traits. No significant difference was found for replicates. The effect of season was significant for all the traits except for the total carotenoids content in both seasons. The best linear mixed-effects model used in this study for all the variables explained the high proportion of variance observed for the fixed factors (R^2 values range from 47.16 – 90.35). Moreover, the coefficient of variation (CV) ranged from 2.9 to 27.88% and the highest values were obtained for marketable vegetable yield (27.88%) and total yield (26.96%).
Due to the highly significant difference between the seasons and interaction genotypes*seasons, the best genotypes for some traits during the early planting season were not necessarily the best ones for the later planting season.

Table 1: Means square value for the analysis of variance in Amaranthus cruentus for quantitative traits during the earlier and the later planting season

Variables	Seasons (ddl=1)	Genotypes (ddl=24)	Rep (ddl=1)	Genotypes*Seasons (ddl=24)	Residual (ddl= 46)	R^2
TY	14423.9***	3288***	0.00ns	6059.8***	601.1	90.35
MFVY	5482.7***	691.4***	0.00ns	1248.2***	181.1	86.48
Caro	1124.2ns	1184.9*	0.00ns	1182.3*	634.5	47.16
Zinc	0.41***	0.037***	9.27e-9ns	0.05***	3.4e-3	91.33
Iron	4.59e-5 ns	0.024***	1.9e-11ns	0.017***	2.24e-3	71.05

***indicates significant at **(P < 0.001)**, ** indicates significant at **(P < 0.01)**, *indicates significant at **(P<0.05)**, **ns** indicates non-significant. TY: Total yield; MFVY: Marketable fresh vegetable yield, Caro: Total carotenoids

3.1. Leaf yield traits

A wide range of performance was observed among the genotypes for the total yield and the marketable fresh vegetable yield over both seasons. The total yield varied significantly among genotypes (P < 0.001) and no genotype was better for leaf yield per plant than the check cultivars (NIG10 and EA2). Among all the genotypes, EA2 from Tanzania was the highest yielding genotype with the average total yield of 176.68±173.09 g/plant, followed by EA2 and NIG12, whereas the lowest yielding genotype was BEN8 from Benin with an average total yield of 30.63±29.66 g/plant.
For the marketable fresh vegetative yield, the check cultivar (EA2) was better than all the genotypes with an average of 83.7±83.87 g/plant. BEN8 was the genotype with the lowest marketable yield (19.09±16.71 g/plant).

3.2. Nutritional quality traits

For nutrient content, about sixty-eight percent (68%) and fifty-two (52%) of the genotypes respectively had higher carotenoid content than the check cultivars (NIG10) and (EA2). The highest carotenoids content was noticed in BEN1 (132.13±25.13 µg/g), followed by BEN7, and the lowest carotenoids content was noticed in NIG7 (70.38±22.29 µg/g). About sixty-four percent (64%) and twenty percent (20%) of the genotypes, respectively, had higher levels of iron than the check cultivars (NIG10 and EA2). The highest iron content was noticed in EA1 (3.35±0.15 mg/100g) and the lowest iron content was noticed in NIG7 (2.64±0.57 mg/100g). As for zinc content, ninety-six percent (96%) and sixty-four (64%) of the genotypes respectively, were better in zinc content than the check cultivars. The highest zinc content was noticed in AM1 and EA2 (2.73 mg/100g) and the lowest zinc content was noticed in NIG10 (1.7±0.72 mg/100g).

Table 2: Combined mean performance of 25 genotypes of Amaranthus cruentus for yields, and some nutrient content across two planting seasons

Genotypes	Caro (µg/g FW)	Iron (mg/100g FW)	Zinc (mg/100g FW)	MFVY (g/plant)	TY (g/plant)
AM1	86.69±51.66	3.07±0.23	2.73±1.21	62.17±36.9	126.33±94.61
AM2	88.9±14.42	2.76±0.44	2.18±0.92	36.73±27.84	70.42±52.49
AM3	110.14±19.03	3.29±0.29	2.03±0.59	41.53±17.76	69.15±36.34
BEN1	132.13±25.13	3.33±0.14	2.28±0.93	83.58±23.01	151.76±31.60
BEN2	116.12±61.72	3.12±0.27	1.97±0.44	52.12±53.06	83.45±84.89
BEN3	92.26±40.82	3.19±0.19	2.59±0.76	41.3±26.1	71.83±40.77
BEN4	83.3±12.21	3.06±0.26	1.68±0.61	32.67±20.07	55.47±38.61
BEN5	101.9±49.26	3.21±0.14	2.29±0.41	53.96±47.32	101.34±92.86
BEN6	90.91±21.59	3.22±0.09	1.97±0.23	48.82±27.88	83.49±50.40
BEN7	128.31±17.65	3.27±0.12	1.9±0.42	45.36±16.69	75.96±29.09
BEN8	80.17±46.86	2.85±0.08	1.79±0.45	19.09±16.71	30.63±29.66
EA1	100.34±40.92	3.35±0.15	1.88±0.4	37.6±26.21	65.88±45.45
EA2[b]	94.9±28.98	3.02±0.06	2.73±0.28	83.7±83.87	176.68±173.09
NIG1	122.2±26	3.27±0.13	1.97±0.35	13.97±13.27	18.42±19.06
NIG10[a]	81.87±26.5	3.12±0.07	1.7±0.72	77.65±74.88	172.01±181.58
NIG11	105.28±24.07	2.8±0.32	1.95±0.38	49.68±32.05	88.28±62.52
NIG12	86.26±34.78	3.06±0.24	1.71±0.3	60.24±52.78	142.34±131.78
NIG2	112.53±53.33	3.03±0.21	2.13±0.64	34.93±16.87	64.29±35.14
NIG3	105.83±52.5	3.24±0.14	1.84±0.56	63.3±31.05	157.95±86.24
NIG4	81.44±42.3	3.19±0.17	2.04±0.15	48.63±29.6	97.46±66.79
NIG5	98.51±38.22	2.94±0.43	1.82±0.39	40.6±18	77.77±36.29
NIG6	112.95±18.68	3.17±0.22	1.91±0.6	34.94±30.6	47.88±28.97
NIG7	70.38±22.29	2.64±0.57	2.16±0.85	34.03±8.26	63.72±19.48
NIG8	123.45±13.46	3.08±0.06	2.07±0.82	57.37±30.16	97.03±50.53
NIG9	88.42±22.95	3.2±0.1	1.74±0.52	40.3±33.08	71.35±64.35
Mean	99.81	3.1	2.04	48.276	90.95
LSD	35.3	0.067	0.08	18.96	34.49
CV	25.24	2.9	5.43	27.88	26.96

TY: Total yield; MFVY: Marketable fresh vegetable yield, Caro: Total carotenoids, FW: Fresh weight, a: control check (Abakaliki local2), b: control check (Madira 2).

4. DISCUSSION

The observation of significant differences (P < 0.05) across two seasons for all the traits, confirmed the hypothesis that selection is possible for total yield, marketable fresh vegetable yield, and some nutrient content such as iron and zinc. Similarly, the two seasons testing revealed at which extent the target traits are influenced by seasons.

In our study, a large and significant variation (P < 0.05) was observed for all the traits among all the 25 genotypes. Such variation was also noticed between seasons except for carotenoids content, and this fact might have resulted from the environmental contrast between both seasons. Indeed, the late planting season was warm and wet (83.3% RH; 211.43 mm rainfall), while the early planting season was cool with less precipitation (152.9 mm rainfall). Due to the influence of the environment on those traits, further investigations must be done including more genotypes, locations, and years to validate these results and also to figure out to which extent the variation in those traits are genetically controlled. The presence of various genotypes from Tanzania and Benin amid the best yielding genotypes shows that tests of cultivars or varieties from abroad can be initiated for distinctiveness, uniformity, and stability. The leaf yield of Madiira 2 used in our study, performed lesser than in previous studies in USA ranging from 218 to 420 g/plant (Byrnes et al., 2017). Low value was reported in Ethiopia for Madiira 2 (78.3 g of fresh leave/plant) across two years (Yosef et al., 2018). Comparatively, these results are more explained by the types of soil (Clay loam, Sandy loam, Nitosols, and Vertisols), fertilization schemes, and locations. Moreover, a large variation was noticed between seasons for leaf yield traits in those studies as we observed in our study. Therefore, as concluded by Dinssa et al. (2018), leaf yield is highly influenced by the environment and might be improved by adequate cultural practices.

A sevenfold variation was observed in carotenoids (27.34 - 188.79 μg/g), with a mean of 99.8 μg/g and a coefficient of variation of 32.62%. Previous studies on the species reported different carotenoids content: (130 - 150 μg/g) in three landraces from Mexico (Prakash and Pal, 1991). This difference between studies may be attributed to the number of genotypes evaluated, the growing conditions and the developmental stages. According to Prakash and Pal (1991), *A. cruentus* is a good source of carotenoids and that statement was confirmed by our study since the levels of carotenoids determined in others vegetables are 36.9 to 95.8 μg/g FW in spider plant, 89.3 to 101.2 μg/g FW in African nightshade and 27 ± 0.8 μg/g FW in jute mallow (Acho et al., 2014; Luoh et al., 2014; Sogbohossou et al., 2019). Further studies should be carried out to determine the levels of some carotenoids components namely β-carotene, α-carotene and β-cryptoxanthin which are the major provitamin A to successfully combat VAD (Vitamin A deficiency) and also other important components such as lutein and retinol known for their antioxidants activities and enhancers of the immune response. Low values were observed for iron content in this study, ranging from 2.15 to 3.61 mg/100g and a coefficient of variation of 2.9%, while the zinc content ranged from 1 to 3.80 mg/100g and a coefficient of variation of 5.43%. The levels of zinc (0.84 ± 0.18 mg/100g) and iron (1.3 ± 0.18 mg/100g) reported by Jiménez-Aguilar and Grusak (2017) was lower comparatively to our study whereas similar ranges of iron (1.78-3.60 mg/100g) was noticed during a micronutrient content evaluation of vegetable amaranth entries at New Jersey (Byrnes et al., 2017). But the results are under the levels of daily requirements. Indeed, the requirements on daily basis of zinc and iron intakes of populations from several countries range respectively from 4.7 to 18.6 mg/day for zinc and 8 mg/day for iron (Maret and Sandstead, 2006; Micronutrients, 2001).

5. CONCLUSION

There is a variation for yield traits, and some nutrient content within *Amaranthus cruentus*. But most of them are highly influenced by environment (Seasons). This is an evidence of the genetic potential and the environmental effect on the crops which can be exploited for selection and growth enhancement. Thus, selection can be made for the yield, iron, zinc and carotenoids contents. But, further studies must be carried out in several environments in order to perform an effective selection base on the effects of genotypes x seasons x location.

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REFERENCES

Achigan-Dako, E.G., Sogbohossou, O.E., and Maundu, P. (2014). Current knowledge on Amaranthus spp.: research avenues for improved nutritional value and yield in leafy amaranths in sub-Saharan Africa. Euphytica 197, 303-317.

Acho, C.F., Zoue, L.T., Akpa, E.E., Yapo, V.G., and Niamké, S.L. (2014). Leafy vegetables consumed in Southern Côte d'Ivoire: a source of high value nutrients. Journal of Animal and Plant Sciences 20, 3159-3170.

AOAC, B.A.M. (1990). Association of official analytical chemists. Official methods of analysis 12.

Arya, M., Reshma, U., Syama, S.T., and Anaswara, S. (2019). Nutraceuticals in vegetables: New breeding approaches for nutrition, food and health: A. Journal of Pharmacognosy and Phytochemistry 8, 677-682.

Bouis, H.E., and Saltzman, A. (2017). Improving nutrition through biofortification: a review of evidence from HarvestPlus, 2003 through 2016. Global food security 12, 49-58.

Byrnes, D.R., Dinssa, F.F., Weller, S.C., and Simon, J.E. (2017). Elemental micronutrient content and horticultural performance of various vegetable amaranth genotypes. Journal of the American Society for Horticultural Science 142, 265-271.

Diagi, B., and Nwagbara, M. (2018). Perceived impact of climate change on swamp rice cultivation by farmers in Ebonyi State, Southeastern Nigeria. Archives of Current Research International, 1-10.

Dinssa, F., Yang, R., Ledesma, D., Mbwambo, O., and Hanson, P. (2018). Effect of leaf harvest on grain yield and nutrient content of diverse amaranth entries. Scientia horticulturae 236, 146-157.

Garnett, T., Appleby, M.C., Balmford, A., Bateman, I.J., Benton, T.G., Bloomer, P., Burlingame, B., Dawkins, M., Dolan, L., and Fraser, D. (2013). Sustainable intensification in agriculture: premises and policies. Science 341, 33-34.

Gerrano, A.S., van Rensburg, W.S.J., and Adebola, P.O. (2015). Genetic diversity of Amaranthus species in South Africa. South African Journal of Plant and Soil 32, 39-46.

Jiménez-Aguilar, D.M., and Grusak, M.A. (2017). Minerals, vitamin C, phenolics, flavonoids and antioxidant activity of Amaranthus leafy vegetables. Journal of Food Composition and Analysis 58, 33-39.

Luoh, J.W., Begg, C.B., Symonds, R.C., Ledesma, D., and Yang, R.-Y. (2014). Nutritional yield of African indigenous vegetables in water-deficient and water-sufficient conditions. Food and Nutrition Sciences 5, 813-822.

Maret, W., and Sandstead, H.H. (2006). Zinc requirements and the risks and benefits of zinc supplementation. Journal of trace elements in medicine and biology 20, 3-18.

Micronutrients, I.o.M.U.P.o. (2001). Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. (Washington (DC): National Academies Press (US)).

OECD-FAO (2016). Agriculture in Sub-Saharan Africa: Prospects and challenges for the next decade (Author Rome).

Organization, W.H. (2010). Indicators for assessing infant and young child feeding practices: part 2: measurement (Geneva, Switzerland: World Health Organization), pp. 82p.

Prakash, D., and Pal, M. (1991). Nutritional and antinutritional composition of vegetable and grain amaranth leaves. Journal of the Science of Food and Agriculture 57, 573-583.

RCore, T. (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Schreinemachers, P., Simmons, E.B., and Wopereis, M.C. (2018). Tapping the economic and nutritional power of vegetables. Global food security 16, 36-45.

Smith, I., and Eyzaguirre, P. (2007). African Leafy Vegetables: their role in the World Health Organization's Global Fruit and Vegetables. African Journal of Food, Agriculture, Nutrition and Development 7, 1-9.

Sogbohossou, E.D., Kortekaas, D., Achigan-Dako, E.G., Maundu, P., Stoilova, T., Van Deynze, A., de Vos, R.C., and Schranz, M.E. (2019). Association between vitamin content, plant morphology and geographical origin in a worldwide collection of the orphan crop Gynandropsis gynandra (Cleomaceae). Planta 250, 933-947.

Sumanta, N., Haque, C.I., Nishika, J., and Suprakash, R. (2014). Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by using various extracting solvents. Research Journal of Chemical Sciences ISSN 2231, 606.

WHO (2009). Global prevalence of vitamin A deficiency in populations at risk 1995-2005: WHO global database on vitamin A deficiency.

Yosef , A., Samuel, T., Habtam, S., Selamawit, K., Melkamu, H., Jibicho, G., Tesfa, B., Shimelis, A., and Gebeyehu, W. (2018). Performance of Amaranth (Amaranthus Cruentus L.) Genotypes for Leaf Yield in Ethiopia. Journal of Biology, Agriculture and Healthcare Vol 8, 47-50.