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Effect of leaf harvest on grain yield and nutrient content of diverse amaranth entries☆

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ABSTRACT

Amaranth (*Amaranthus* spp.) is a popular crop grown throughout sub-Saharan Africa, where both the grain and leaves are consumed. Amaranth cultivars that offer multiple options to small-scale farmers to produce high foliage yields, high grain yields, or both high foliage and grain yields would be desirable. The objective of this study was to determine the effects of multiple leaf harvests on grain yield and grain nutrient content of diverse amaranth entries (breeding lines and cultivars). Trials were conducted at the World Vegetable Center in Arusha, Tanzania in 2013 and 2014. Seventeen amaranth entries were evaluated for grain yield without defoliation, or after four leaf harvests. Grain samples of 10 entries were evaluated for calcium, iron, zinc, protein, sugar, and fiber content. Highly significant entry and leaf harvest differences in grain yield, leaf yield, and calcium, zinc, and protein contents were detected both years. Differences among years were significant or highly significant only for leaf yield, number of leaves per plant and panicle length. The leaf yields of all the entries were lower in 2013 (year mean = 11 t/ha) than in 2014 (15.3 t/ha). Four leaf harvests reduced grain yield of all entries by at least 50% but did not affect grain nutrient content except for calcium. Under no-leaf harvest PARIS (A)-Sel and BRESIL (B)-Sel gave the highest grain yield in both years. PARIS (A)-Sel suffered the highest grain yield loss (87%) followed by BRESIL (B)-Sel (85%) in 2013, while BRESIL (B)-Sel showed the highest loss (90%) followed by PARIS (A)-Sel (84%) in 2014. 'Madiira 2' was the lowest grain yielder under both leaf harvest and no-leaf harvest in both years. 'Madiira 1' and 'Madiira 2', black seeded cultivars, gave high Fe and fiber contents except the rank of 'Madiira 2' was less consistent for iron cross the years. Three groups of entries could be distinguished: (1) entries that produced high grain yields only if plants were not defoliated; (2) entries that yielded ample leaves but relatively little grain; (3) entries that gave moderate amounts of grain and leaves. Our study suggests there is potential to develop amaranth cultivars for different purposes to meet the needs of amaranth producers.

1. Introduction

Cultivated amaranth includes at least eight species (*Amaranthus blitum L.*, *A. caudatus L., A. cruentus L., A. dubius* (Mart. Ex Thell.), *A. edulis L.*, *A. hybridus L.*, *A. hypochondriacus L.* and *A. tricolor L.*) grown for multiple purposes. In the Americas (South America and Central America) where amaranth is thought to have originated (Sauer, 1950, 1967), the grain is the principal product harvested for human consumption. Amaranth grain is relatively rich in protein (Bressani et al., 1993) and its lysine content is higher than wheat (*Triticum* spp.), rice (*Oryza sativa* L.), maize (*Zea mays* L.), barley (*Hordeum vulgare* L.), rye (*Secale cereal* L.), triticale (x *Triticosecale* Wittmack), oats (*Avena sativa* L.), buckwheat (*Fagopyrum esculentum* L.), and quinoa (*Chenopodium quinoa* W.) (USDA, 2010). A study conducted in Ethiopia concluded that raw amaranth grain can contribute 50–102% of the daily requirements of the essential amino acids (histidine, isoleucine, lysine, threonine and valine) for children 6–23 months-old (Amare et al., 2015). This is as compared to 15–46% contribution from maize and 20–51% from wheat. Some farmers in Kenya, Uganda and Zimbabwe have started supplying amaranth grain to millers and supermarkets (Achigan-Dako et al., 2014), who can use it to fortify and improve protein and micronutrient contents and qualities of milled cereal grains. In parts of Africa and tropical Asia, the tender leaves, shoots and stems of amaranth are consumed as leafy vegetables. Vegetable amaranth farmers harvest once by uprooting the plant, or harvest continuously by pinching leaves, tender branches and shoots over the season. Amaranth vegetative parts contain substantial amounts of calcium, iron, magnesium, and vitamins A and C (Kamga et al., 2013; Mburu et al., 2012;

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Schonfeldt and Pretorius, 2011; Yang et al., 2013). Vegetable amaranth traditionally has been cultivated in small plots or harvested in the wild. Production of amaranth and other African traditional vegetables is expanding and commercial sales are increasing to meet the demand of peri-urban and urban populations (Chelang'a et al., 2013; Dinssa et al., 2016).

Amaranth cultivars that produce both high quality foliage and grain yields (dual purpose cultivars) would be especially useful for small scale farmers in Africa and tropical Asia. Ideally, a dual purpose cultivar would have an extended vegetative period and produce ample leaves, and some tender branches that could be harvested periodically, but the central stems would be allowed to flower and produce grain. Developing dual purpose cultivars requires an understanding of the tradeoffs between vegetative and grain yields and nutrient quality traits. Borras et al. (2004) reported that grain yield of maize, wheat and soybean can be limited by source and/or sink strengths. Roitner-Schobesberger and Kaul (2013) conducted an experiment in Austria to study the effects of source and sink on grain yield of amaranth by artificially altering source or sink size. They reported significant differences among genotypes in the reduction of grain yield due to leaf harvest: complete removal of leaves reduced grain yields by 64.3% and seed number per plant by 63.7%. Similarly, they reported 43%, 53% and 10% reductions in shoot biomass, inflorescence biomass and thousand seed weight, respectively. In Nigeria, cutting back *A. cruentus* at 10 cm from the base of the stem during stem elongation delayed floral initiation, enhanced branching and leaf development, and increased vegetative yield (Awe and Osunlola, 2013).

Mbwambo et al. (2015) evaluated leaf and grain yields of most entries included in this study under 3–4 leaf harvests at two-week intervals starting six weeks after sowing, and under no-leaf harvest. They reported that leaf harvest reduced average grain yields by 25.5%, but no-leaf harvest and leaf harvest treatments were tested in separate experiments and statistical analysis of leaf harvest main effects and interactions of harvest strategy by entry were not possible. Moreover, their study did not assess the effect of leaf harvest on grain nutrient content.

The World Vegetable Center Eastern and Southern Africa located at Tengeru, Arusha, Tanzania hosts a genebank maintaining about 140 amaranth accessions, and an amaranth improvement program focused on development of cultivars adapted mainly to sub-Saharan Africa (Dinssa et al., 2016). Information on the effects of leaf harvest on grain yield and quality parameters of different amaranth accessions and cultivars would help breeders assess the potential for development of dual purpose cultivars or whether distinct vegetable and grain amaranth cultivars are required. The objective of this study was to determine the effects of multiple leaf harvests on grain yield and grain nutrient content of a set of diverse amaranth entries.

2. Materials and methods

2.1. Study site

Experiments were carried out at the station of World Vegetable Center, Eastern and Southern Africa (36.8 °E, 3.4 °S and 1290 masl altitude). The soil type was a well-drained clay loam with pH 7.3. According to soil analysis conducted in 2009 the research station is characterized by 3.2% organic matter, 0.14% total N, 61.8 mg/kg P and 2.9 cmcl/kg K. Trials were conducted March–July 2013, and February–June 2014 during the rainy season. Monthly precipitation data for the 2013 and 2014 growing seasons and for long-term (2005–2014) were extracted from climate hazard infrared rainfall station (CHIRPS) dataset that was developed by U.S. Geological Survey (USGS) and the Climate Hazards Group at the University of California, Santa Barbara (Funk et al., 2015) while mean temperature data were obtained from local meteorology station at the Tanzanian Horticultural Research and Training Institute (Horti-Tengeru) located 3 km from the World Vegetable Center station (Fig. 1).

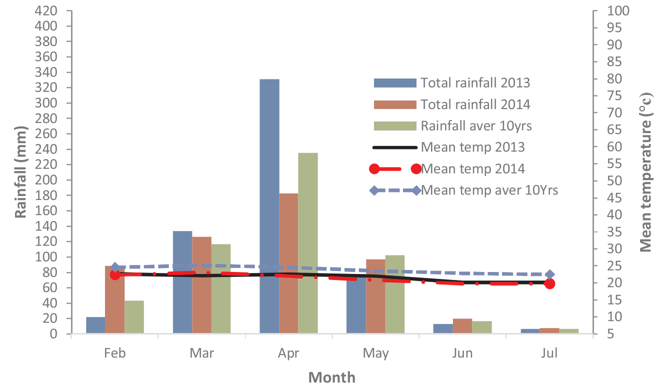

Fig. 1. Monthly total rainfall (Funk et al., 2015), and mean monthly temperatures (from local meteorology station, Horti-Tengeru, Tanzania) February–July, 10-year averages compared to 2013 and 2014 data. The experiments were conducted from 19th March to 31th July in 2013 and from 20th February to 14th June in 2014.

2.2. Treatments and experimental layout

The study included 14 amaranth lines and three commercial cultivars (collectively called entries) (Table 1). The amaranth lines were developed for leaf, grain, or as potential for both grain and leaf through repeated mass selection and self-pollination for more than four generations within 14 different World Vegetable Center accessions. The accessions from which the lines were derived originated from seven countries and included nine *A. cruentus* and eight *A. hypochondriacus* species. Each line code begins with the original accession name followed by 'Sel' to indicate that it is a pure line selection from the accession. The three commercial amaranth cultivars intended mainly for leaf harvest and included as checks were 'Madiira 1', 'Madiira 2', and 'Mchicha'. 'Madiira 1' and 'Madiira 2' were formally released by Horti-Tengeru, and 'Mchicha' is marketed by several seed companies in Tanzania but has not been formally released through a government agency. Seed of 'Mchicha' was obtained from the East African Seed Company, Arusha, Tanzania and seed of other entries was provided by the World Vegetable Center, Eastern and Southern Africa.

The experimental design was a split-plot in a randomized complete block design with three replications. Entries were the main plot. Leaf harvest was the sub-plot treatment with two levels (no-leaf harvest; leaf harvest). Randomizations were conducted independently each year. Main-plots had four 7.2 m-long rows with 24 plants per row. Each sub-plot had two rows. Between and within row spacings were 60 cm and 30 cm, respectively that is 8.64 m^2 sub-plot size and about six plants per m^2. Seeds were sown in plastic trays and three-week-old seedlings were transplanted to the field on 19 March 2013 and 20 February 2014. The central 20 plants per row of each leaf harvest sub-plot were leaf harvested four times at about 10-day intervals between harvests beginning 9 April in 2013 and 17 March in 2014 (about three weeks after transplanting).

2.3. Field management

NPK (20:10:10) fertilizer at the rate of 200 kg/ha was applied manually as a basal application one week after transplanting. Urea (46N) at the rate of 120 kg/ha was applied as side-dressing three weeks after transplanting. Plants were furrow irrigated as needed and weeds were controlled by hoeing. Selecron® (active ingredient (a.i.) Profenofos 720 g/l EC) was sprayed at 10 and 21 days after transplanting at the rate of 1 ml a.i./l water, to control cutworm and whitefly. Aphids and caterpillars were controlled by Actellic (a.i. Pirimiphos-methyl, 1.5 ml a.i /l) sprayed 14 and 45 days after

Table 1
Amaranth entries evaluated for grain yield and plant traits with or without leaf harvest[z], Arusha, Tanzania, 2013 and 2014.

Entry name	Species	Original accession name	Original accession source	Leaf color	Seed color
INCA-Sel	*Amaranthus hypochondriacus*	INCA	–	Green	Creamy-yellow
AH-TL-Sel	*A. hypochondriacus*	AH-TL	Tanzania	Green	Creamy
HTT-Sel	*A. hypochondriacus*	HTT	Kenya	Green	Creamy-yellow
SIMON's FARM-Sel	*A. hypochondriacus*	SIMON's FARM	Sudan	Green	Creamy-yellow
TZSMN82-Sel	*A. hypochondriacus*	TZSMN82	Tanzania	Green	Creamy
TZSMN102-Sel	*A. hypochondriacus*	TZSMN102	Tanzania	Green	Creamy
AH-NL-Sel	*A. hypochondriacus*	AH-NL	Tanzania	Green	Creamy
MELANGE-Sel	*A. cruentus*	MELANGE	Madagascar	Green	White
BRESIL (B)-Sel	*A. cruentus*	BRESIL (B)	Madagascar	Green with a bit of pinkish background	White
PARIS (A)-Sel	*A. cruentus*	PARIS (A)	Madagascar	Green with a bit of pinkish background	White
DB 2006306-Sel	*A. cruentus*	DB 2006306	USA	Green	Creamy-yellow
IP-5-Sel	*A. cruentus*	IP-5	Zambia	Green	Creamy
AM-25-Sel	*A. cruentus*	AM-25	Uganda	Green	Creamy-yellow
RED INFLORESCENCE-Sel	*A. cruentus*	RED INFLORESCENCE	Sudan	Green	Creamy
Mchicha (check)	*A. hypochondriacus*	–	Tanzania	Green	Creamy
Madiira 1 (check)	*A. cruentus*	Ex-Zim	Zimbabwe	Dark green	Black
Madiira 2 (check)	*A. cruentus*	AM 38	Tanzania	Green	Black

[z] The check 'Mchicha' was obtained from East African Seed Company, Arusha, while all the other entries were from the World Vegetable Center, Eastern and Southern Africa.

transplanting. Folicur (a.i. Tebuconazole 430 g/l) at the rate of 1 ml/l of water and Ridomil (a.i. Metalaxyl-M) at 3 g/l of water were sprayed two days after transplanting to control dumping-off. Xantho (a. i. Hexaconazole 5 EC) was applied once during flowering at 50 g a.i./20 l water to control powdery mildew.

2.4. Data collection

2.4.1. Plant height (cm), and number of branches

Plant height and panicle length were measured at grain harvest on four randomly sampled plants per sub-plot. Plant height was measured from ground level to the apex of the plant while panicle length was measured from the base of the panicle to the apex of the plant. The number of branches per plant was counted at grain harvest on ten and eight random plants per sub-plot in 2013 and 2014, respectively.

2.4.2. Leaf yield

Leaf harvest was conducted four times. In the absence of a recommended leaf picking frequency and intensity, all fully developed leaves (leaves ready for harvest as a vegetable) were picked at each harvest date without damaging stems, branches and growing shoots. In 2014 leaf length and width (cm) were measured at every harvest date on three randomly taken well developed leaves per each of four random sample plants per plot. These two traits were not measured in 2013. The first harvest was carried out 21 and 25 days after transplanting in 2013 and 2014, respectively. Subsequent harvests were conducted at about 10-day intervals. The last (fourth) leaf harvest was completed on 6 May 2013 and 22 April 2014. Fresh leaf weight per plot was determined in the field immediately after harvest. Total fresh leaf yields (kg/plot) over harvests were converted to t/ha for statistical analysis.

2.4.3. Crop growth cycle

Days to flowering (50% of the plants per plot flowered) was recorded for the main-plots because there were no clear differences in flowering between with and without leaf harvested sub-plots. Days to maturity was collected from main-plots of the first replication.

2.4.4. Grain yield and 1000-seed weight

Grain yield (kg/plot) was measured on the central 20 plants harvested per row on leaf harvested and no-leaf harvested sub-plots, and converted to t/ha. The last grain harvests were made on 31 July and 14 June in 2013 and 2014, respectively. Thousand-seed weight (g) was calculated from a random sample of 200 seeds per sub-plot.

2.4.5. Grain nutrient content analysis

Due to insufficient lab space and labor to analyze the entire entries and plots, 10 entries were chosen to represent high grain yield, medium and low grain yield entries as identified from the results of statistical analysis, and also to represent different species (*A. cruentus* and *A. hypochondriacus*). The sampled entries also represented different grain color types. The grain samples were taken from two replications per selected entry from no-leaf harvest and leaf harvest sub-plots. Seed samples were placed in foil packs, sealed, and sent to the World Vegetable Center Nutrition Laboratory in Taiwan for analyses of minerals (calcium, iron, and zinc), protein, sugar and crude fiber. Seed samples were ground into a fine powder with a cyclone mill (Pulverisette 14701, FRITSCH, Rhineland-Palatinate, Germany) before analyses. All chemicals used for the analyses in this study, if not specified, were purchased from either Sigma–Aldrich (St. Louis, USA, local supplier: Uni-Onward Corp, New Taipei City, Taiwan) or Merck (Darmstadt, Germany, local supplier: New-A-Star Corp., Tainan, Taiwan). Dry matter was determined from the weight difference of 0.5 g of fine powder before and after placing in an oven at 135 °C for four hours. Protein content was measured with micro-Kjeldahl digestion followed by distillation and titration (AOAC method 979.09; AOAC, 1990). Protein in the sample was digested with concentrated sulfuric acid, using copper sulfuric or H_2O_2 as a catalyst, to convert organic nitrogen to ammonium ions. Alkali was added and the liberated ammonia was distilled into sulfuric acid solution. The distillate was titrated with sodium hydroxide solution to determine the ammonia absorbed in the sulfuric acid solution. The percent protein was calculated from the % nitrogen multiplied by the factor of 6.25 (AOAC method 979.09; AOAC, 1990). The determination of calcium, iron, and zinc contents was performed by washing procedure, strong acid washing, and then detection was with Atomic Absorption Spectroscopy (AOAC method 975.03; AOAC, 1990). Total free sugar content was determined using the Anthrone reagent, which reacts specifically with carbohydrates in concentrated sulfuric acid solution to produce a blue green color at 630 nm (Hanson et al., 2006). The final result was expressed as sucrose equivalents. Crude fiber content was determined according to the AOAC methods with modifications; details were described in Hanson et al. (2006).

2.5. Data analysis

Tests for normality and variance homogeneity were carried out before performing the analyses of variances. Analyses of variances for grain yield, nutrient contents, and horticultural traits appropriate for split-plots with main-plots arranged in randomized complete block design were carried out by year using the Generalized Linear Proc Mixed Method of SAS System for Windows Version 9.4 (SAS Institute, 2013). The following linear model was used for individual analysis of variance conducted in split-plot arrangement: Y = overall Mean + Entry + Block + Block*Entry + leaf harvest + (Entry*Leaf harvest) + Residual Error, where the interaction Block*Entry is the error term used to test the significance of the mainplot factor.

Leaf yield and number of leaves per plant were measured in the leaf harvest sub-plots only, and days to flowering was measured only on main-plots and were analyzed by ANOVA model for a randomized complete block design. The model fitted for this individual analysis of variance was: Y = Overall mean + Entry + Block + Residual error, where Y is response total. Combined analyses of variances over years based on split-plot arrangement with main-plot in a RCB design were carried out for grain yield, thousand seed weight, panicle length, and grain nutrient contents (protein, fiber contents, sugar, calcium, zinc and iron) using the Proc Mixed Method Type III of SAS System for Windows Version 9.4. The model fitted was: Y = Overall mean + Year + rep (Year) + Entry + Year*Entry + rep*Year(Entry) + Leaf harvest + Entry*Leaf harvest + Year*Leaf harvest + Year*Entry*Leaf harvest + Residual error, where year and its interaction with other variables are defined as random, entry is main-plot and leaf harvest treatment is sub-plot. The mean square (MS) of (entry*year) is the error term for entry; MS(leaf*year) for leaf harvest; MS(entry*Leaf*Year) for (entry*leaf harvest) interaction; MS(Rep(entry*Year)) + MS (entry*Leaf*Year) – MS(Residual) for (entry*Year); MS (entry*Leaf*Year) for (Leaf*Year); MS(Residual) for (entry*Leaf*Year). The error term for year was obtained by solving the following equation: MS(Rep*Year) + MS(entry*Year) - MS(Rep(entry*Year)) + MS (Leaf*Year) – MS(entry*Leaf*Year). The combined ANOVA for leaf yield and number of leaves per plant was based on a RCB design using the same procedure. The least significant difference (LSD) test was used for mean separation.

Analysis of covariance (ANCOVA), a general linear model which blends ANOVA and regression analysis, was conducted between leaf yield and number of leaves per plant to evaluate whether the means of the dependent variable leaf yield are equal across levels of the independent variable entry while statistically controlling for the effect of leaf number per plant that was inserted as covariate. The model used was as follow.

$Y_{ij} = M + T_i + B(x_{ij} - \bar{x}) + e_{ij}$, where the dependent variable Y_{ij} is the jth observation under the ith categorical group; x_{ij} – the jth observation of the covariate under the ith group; M – overall mean, $\bar{x}$ - global mean of x; T_i - the effect of the ith level of the independent variable; B – the slope of the line; e_{ij} is the associated unobserved error term for the jth observation in the ith group. The result of ANCOVA showed that there was no linear relationship between leaf yield and leaf number indicating that number of leaves per plant did not affect leaf yield. Therefore, analysis of variance to assess the main effects of both leaf yield and number of leaves were conducted independently.

The amaranth species, *A. cruentus* (nine entries) and *A. hypochondriacus* (eight entries), were compared for their performances in grain and leaf yields using the two-independent sample t-test with unequal sample size using SAS for Windows, Version 9.4. Pearson's correlation analysis was also conducted among the various traits measured under leaf harvest and no-leaf harvest treatments.

3. Results

3.1. Analysis of variance

In individual analyses of variance highly significant entry and leaf harvest mean squares for grain yield and plant height were detected in both years (Table 2). The differences among the entries in thousand-seed weight, leaf yield, leaf number harvested per plant, and days to flowering were highly significant both years. Leaf harvest effects on thousand-seed weight were significant only in 2014. Entry and leaf harvest effects on branch number per plant were non-significant except for entries in 2014. Entry (E) × leaf harvest (LH) interactions were significant in both years for grain yield, and were non-significant for thousand-seed weight. For the other traits, E × LH interactions were

Table 2
Significance of mean squares from individual analyses of variance of 17 amaranth entries evaluated for leaf and grain yields, 1000-seed weight, and horticultural traits, and of 10 entries evaluated for grain nutrient contents (exception nine entries for Fe content) as affected by leaf harvest treatments[z], Arusha, Tanzania, 2013 and 2014.

Trait	Year/Source of variation	Mean squares		
		Entry (E)	Leaf harvest (LH)	E x LH
		Df [y,x] = 16 (9)	Df = 1	Df = 16 (9)
Yields and horticultural traits				
Grain yield (t/ha)	2013	3.103**	112.049**	1.585**
	2014	0.807**	25.538**	0.442**
1000-seed weight (g)	2013	0.171**	0.000NS	0.005NS
	2014	0.252**	0.142**	0.008NS
Leaf yield (t/ha)[w]	2013	15.331**	–	–
	2014	45.232**	–	–
Leaf No. harvested per plant[w]	2013	2232.606**	–	–
	2014	3464.647**	–	–
Days to flowering[v]	2013	501.657**	–	–
	2014	583.157**	–	–
Branch No. per plant	2013	5.480NS	1.000NS	2.438NS
	2014	47.222**	9.544NS	8.266*
Total plant height (cm)	2013	3487.343**	22233.000**	499.963**
	2014	5368.594**	14035.000**	314.898NS
Panicle length (cm)	2013	168.866**	801.361**	63.759*
	2014	133.506*	1302.551**	16.476NS
Nutrient contents				
Ca (mg)	2013	24961. 000**	1551.270**	458.500**
	2014	18800.000*	721.650**	147.190*
Fe (mg)	2013	2015.360**	21.622NS	12.034NS
	2014	396.276NS	0.234NS	5.645NS
Zn (g)	2013	0.460**	0.576**	0.035NS
	2014	0.614*	0.420NS	0.195NS
Protein (g)	2013	2.153**	0.961NS	0.126NS
	2014	2.323*	0.009NS	0.228NS
Sugar (g)	2013	0.521*	0.000NS	0.072NS
	2014	0.968**	0.625*	0.066NS
Fiber (g)	2013	222.987**	0.256NS	0.848NS
	2014	284.804**	0.841NS	0.943NS
DM (g)	2013	0.085NS	0.004NS	0.027NS
	2014	0.645**	0.930**	0.031NS

NS, *, ** differences non-significant ($P > 0.05$), significant ($0.01 \le P < 0.05$), or highly significant ($P < 0.01$), respectively.

[z] Leaf harvest treatments were no-leaf harvest, and leaf harvest (total of four harvests).

[y] Df for entry (degrees of freedom): 16 for yields and horticultural traits, 9 for nutrient contents except for Fe which had Df of 8, and 1 for leaf harvest treatment (sub-plot).

[x] Df for error (a) to test the significance of entry effects = 32 for yields and horticultural traits, 9 for nutrient contents except for Fe that had Df of 8. Df for error (b) used to test the significance of leaf harvest treatments and Entry x Leaf harvest interaction = 34 for yields and horticultural traits, 10 for nutrient contents except for Fe that had Df of 9.

[w] Leaf yield, and Leaf No. harvested per plant were measured in leaf harvest sub-plots only.

[v] Days to flowering was assessed on main-plots only.

Table 3
Significance of mean squares from combined analyses of variance of 17 amaranth entries evaluated for leaf and grain yields, and related traits, and of 10 entries evaluated for grain nutrient contents as affected by leaf harvest treatments, Arusha, Tanzania, 2013 and 2014.

Source	DF[z]	Mean Square									
		Grain Yield (t/ha)	1000 seedwt (g)	Panicle length (cm)	Protein (g)	Fiber (g)	Sugar (g)	Ca (mg)	Zn (mg)	Leaf yield (t/ha)	No. of leaves per plant
Year (Y)	1(1)	101.91NS	0.0096NS	4208.762**	0.648NS	15.664NS	4.608NS	11981NS	0.4961NS	475.95**	40,123.00*
Rep(Year)	4(2)	0.222	0.0474	76.9752	0.8010	4.8500	0.4100	5709.516	0.0486	–	–
Entry (E)	16(9)	2.63NS	0.409**	184.2913NS	3.7186**	505.2081**	1.3508**	42,280**	0.9552**	45.22*	4766.97**
E x Y	16(9)	1.28*	0.0138NS	118.0816NS	0.758NS	2.5831NS	0.138NS	1480.856NS	0.1186NS	15.34**	930.29NS
Rep(Entry x year)	64(18)	0.123	0.0091	53.4342	0.3935	2.1556	0.1458	2956.532	0.0925	–	–
Leaf harvest (LH)	1(1)	122.29NS	0.0784NS	2073.6282NS	0.392NS	1.0125NS	0.3125NS	2194.512NS	0.0061NS	–	–
LH x E	16(9)	1.59**	0.0077NS	36.9337NS	0.392NS	1.4872**	0.0503NS	190.0233NS	0.1103NS	–	–
LH x Y	1(1)	15.30**	0.0635**	30.2841NS	0.578*	0.0845NS	0.3125NS	78.408NS	0.9901*	–	–
LH x E x Y	16(9)	0.43**	0.005274NS	43.302NS	0.1058NS	0.3031NS	0.0875NS	415.6672**	0.1193NS	–	–
Residual	68(20)	0.182	0.0077	29.0720	0.2213	0.6550	0.0768	53.313	0.1301	–	–

NS, *, ** differences non-significant (P > 0.05), significant (0.01 < P < 0.05) or highly significant (P < 0.01), respectively.
z Numbers in bracket are degrees of freedom for nutrient contents (protein, fiber, sugar, Ca and Zn). Residual error degree of freedom for leaf yield and number of leaves per plant is 64.

significant in 2013 or 2014 but not both years. Differences among lines in grain yield and some other traits in response to leaf harvest might have caused the significant E × LH interactions. The E × LH interaction effects were significant for plant height and panicle length in 2013 and for number of branches per plant in 2014.

Entry mean squares were significant or highly significant in both years for grain nutrient contents except iron in 2014 and dry matter in 2013 (Table 2). Differences between leaf harvest treatments were non-significant for all nutrients except calcium in both years, zinc in 2013, and sugar and dry matter in 2014. E × LH interaction effects were highly significant or significant only for calcium content.

In combined analyses of variances the effect of year (Y) was significant or highly significant only in number of leaves per plant, leaf yield and panicle length (Table 3). Differences among entries were significant in leaf yield and highly significant in thousand-seed weight and number of leaves per plant. Differences were also highly significant for grain protein, fiber, sugar, calcium and zinc contents. The effects of leaf harvest treatment were non-significant, and E x LH interaction was significant only for grain yield and fiber content. E × Y and LH × Y interactions were significant or highly significant only in grain and leaf yields (Table 3). For iron content only nine entries were assessed due to a missing value, and only its LH × Y source of variation reached significant level, and therefore not shown in Table 3 to avoid complication in degrees of freedom. The three-way interaction (E × LH × Y) was significant only for grain yield and calcium content. Year is a random variable that is not predictable, and therefore all interactions with year too become random making it difficult to discuss the effects of year and its interaction with other variables. Therefore, only the performances of entries and leaf harvest treatments over the two years have been discussed (Table 4).

3.2. Grain yield, and grain yield as affected by leaf harvest

Entry grain yields under no-leaf harvest were 58.7% greater in 2013 compared to 2014 (Table 4). Similarly grain yields of entries with leaf harvest were 69.7% higher in 2013 versus 2014. Leaf harvest reduced average grain yields of all the entries in both years and the average reductions over entries were 63% and 72% in 2013 and 2014, respectively. The magnitude of the reduction due to leaf harvesting varied among entries and ranged from 46% in 'Mchicha' to 87% in PARIS (A)-Sel in 2013, and from 55% in SIMON'S FARM-Sel to > 90% for BRESIL (B)-Sel in 2014. Averaged over the two years the reduction ranged from 1.26 t/ha (51%) in 'Mchicha' to 3.29 t/ha (87%) in BRESIL (B)-Sel (Table 4). Entry rankings for grain yield with and without leaf harvest tended to differ as shown from both individual analysis of variance (Table 2) and combined over the two years (Tables 3). PARIS (A)-Sel and BRESIL (B)-Sel with yields of 5.12 t and 5.52 t/ha in 2013 and 2.47 t and 2.56 t/ha in 2014, respectively in that order ranked the highest in the no-leaf harvest treatment (Table 4). Averaged over the two years PARIS (A)-Sel (3.89 t/ha) and BRESIL (B)-Sel (3.80 t/ha) yielded significantly higher than other entries under the same treatment. However, they were among the lowest ranked entries in the leaf harvest treatment. AH-TL-Sel (1.97 t/ha) followed by TZSMN82-Sel (1.89 t/ha) and AH-NL-Sel (1.87 t/ha) ranked among the highest grain yielding entries under leaf harvest in 2013. Entries AH-TL-Sel, AH-NL-Sel, and 'Mchicha' ranked relatively high in both harvest treatments and across the two years, and were identified as stable entries for grain yield across the two leaf harvest treatments and years. Commercial cultivars 'Madiira 1' and 'Madiira 2' released as leaf amaranth cultivars consistently ranked among the lowest for grain yield both years in both leaf harvest treatments.

The month of February during which the 2014 transplanting was conducted received the highest rainfall compared to 2013 rainfall and the long-term average, 2005–2014 (Fig. 1). The slightly higher temperatures from April to June with less rainfall in June in 2013 (Fig. 1) may also account for the higher grain yields in 2013 versus 2014 (Table 4). The optimal temperature for amaranth growth ranges from 25 to 30 °C (Grubben, 2004). Grain amaranth performs well under high temperatures and reduced moisture during grain filling and maturation (Chaudhari et al., 2009; Johnson and Henderson, 2002).

3.3. Leaf yield and number of leaves per plant

IP-5-Sel (14.37 t/ha in 2013; 19.65 t/ha in 2014), 'Madiira 2' (13.96 t/ha; 19.63 t/ha) and RED INFLORESCENCE-Sel (12.78 t/ha; 17.89 t/ha) consistently ranked among the top five entries for leaf yield both years (Table 4). BRESIL (B)-Sel, one of the best for grain yield under no-leaf harvest, produced low or mediocre leaf yields, 9.05 t and 14.72 t/ha in 2013 and 2014, respectively. Leaf yields of PARIS (A)-Sel, the other high grain yielding entry, was exceptionally low in 2013 but the highest among all entries in 2014, perhaps due to high rainfall during February in 2014 (Fig. 1) that might have favored high vegetative production in that entry that year.

BRESIL (B)-Sel and PARIS (A)-Sel, top yielding entries under the no-leaf harvest treatment, had the lowest and the second lowest numbers of leaves per plant, respectively both years (Table 4). Mean number of leaves harvested per plant over entries was 39.6% greater in 2014 than 2013 possibly due to higher rainfall in February in 2014 compared to

Table 4
Mean grain yields under no-leaf harvest and leaf harvest treatments, and leaf yield and number of leaves per plant from combined analysis over years of 17 amaranth entries, Arusha, Tanzania, 2013 and 2014.

	Grain yield (t/ha)												
	Without leaf harvest (WO)			With leaf harvest (W)			Differences	Fresh leaf yield (t/ha)			Number of leaves per plant		
Entry	2013	2014	Mean^{z}	2013	2014	Mean^{z}	(WO–W)^{y}	2013	2014	Mean^{z}	2013	2014	Mean^{z}
INCA-Sel	2.98	0.81	1.9	1.25	0.31	0.78	1.12*	9.19	11.06	10.13	121	171	146
AH-TL-Sel	4.09	1.63	2.86	1.97	0.59	1.28	1.58*	12.8	14.54	13.67	103	156	130
HTT-Sel	3.82	1.38	2.6	1.44	0.31	0.88	1.72*	9.63	10.56	10.1	104	138	121
SIMON'S FARM-Sel	3.2	1.08	2.14	1.43	0.48	0.96	1.18*	12.03	17.82	14.93	91	120	106
TZSMN82-Sel	3.55	1.1	2.33	1.89	0.28	1.09	1.24*	10.23	11.46	10.85	126	127	127
TZSMN102-Sel	4.21	1.47	2.84	1.54	0.53	1.04	1.80*	11.85	15.09	13.47	129	171	150
AH-NL-Sel	3.6	1.64	2.62	1.87	0.69	1.28	1.34*	12.73	17.43	15.08	131	170	151
MELANGE-Sel	3.36	0.26	1.81	1.31	0.07	0.69	1.12*	9.35	8.47	8.91	115	186	151
BRESIL (B)-Sel	5.12	2.47	3.8	0.77	0.24	0.51	3.29*	9.05	14.72	11.89	35	58	47
PARIS (A)-Sel	5.52	2.26	3.89	0.74	0.37	0.56	3.33*	9.47	22.11	15.79	48	93	71
DB 2006306-Sel	2.32	1.8	2.06	0.92	0.4	0.66	1.40*	11.78	18.17	14.98	99	144	122
IP-5-Sel	1.78	2.14	1.96	0.41	0.55	0.48	1.48*	14.37	19.65	17.01	107	172	140
AM-25-Sel	3.31	1.18	2.25	1.39	0.27	0.83	1.42*	9.51	10.93	10.22	114	129	122
RED INFLORESCENCE-Sel	3.15	1.32	2.24	1.66	0.38	1.02	1.22*	12.78	17.89	15.34	103	158	131
Mchicha (check)	3.39	1.56	2.48	1.83	0.61	1.22	1.26*	12.75	17.64	15.2	129	130	130
Madiira 1 (check)	2.44	1.09	1.77	0.5	0.24	0.37	1.40*	5.53	13.31	9.42	76	169	123
Madiira 2 (check)	0.91	0.21	0.56	0.19	0.06	0.13	0.43^{ns}	13.96	19.63	16.8	91	103	97
Mean	3.34	1.38	2.36	1.24	0.38	0.81	–	11	15.32	13.16	101	141	121
LSD (5%)			0.45			0.45	0.98			2.17			30

^{z}LSD in the column compares entry means in the respective column.
^{y}LSD (= 0.98) compares leaf harvest means averaged over the two years at each entry, NS = not significantly different and * = significantly different (except for one entry the yield levels of all entries significantly reduced due to the leaf harvest treatment).

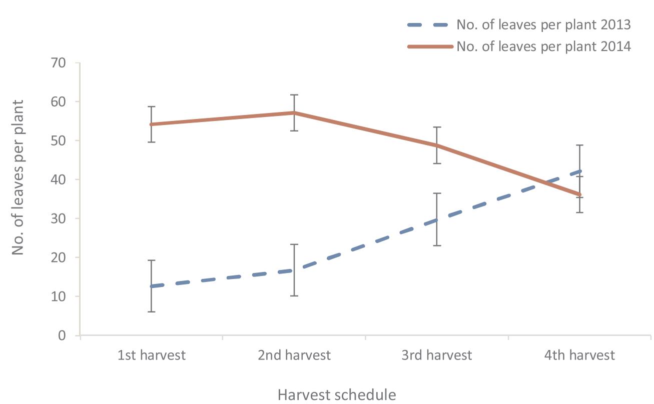

Fig. 2. Mean number of leaves per plant measured on 17 amaranth entries at each of four harvest schedules during the growing period, 2013 and 2014. Bars indicate standard errors.

the same month in 2013. The entries gave higher number of leaves per plant from the first to the third leaf harvest dates in 2014 than 2013 while they produced higher number at the fourth harvest in 2013 than in 2014 (Fig. 2). The high number of leaves produced at the first harvest in 2014 dramatically dropped after the second harvest and became less than the number obtained in 2013 at the fourth harvest. In 2013 the number of leaves counted steadily increased from the first harvest to the fourth harvest. The low grain yield in 2014 had to do, at least partially, with the reduction in number of leaves that was experienced during or close to the reproductive phase. Leaf defoliation decreases photosynthetically active surface area of crops leading to lower solar radiation interception and assimilate production especially if this happens close to flowering phase (Ahmadi and Joudi, 2007; Lauer et al., 2004).

3.4. Number of branches, plant height traits, thousand-seed weight and growth cycle

Although there was a significant E x LH interaction effect in number of branches per plant in 2014 (Table 2), the rankings of the entries that produced high number of branches PARIS (A)-Sel (14.7 branches under no-leaf harvest; 14.4 under leaf harvest), DB 2006306-Sel (14.0; 13.9), IP-5-Sel (17.9; 14.5), 'Madiira 1' (18.6; 15.2), and 'Madiira 2' (19.6; 12.8) differed only slightly across leaf harvest treatments (Table 5). In 2013 'Madiira 1' (246.1 cm) was the tallest entry in the no-leaf harvest, and IP-5-Sel (203.2 cm) in the leaf harvest treatment (Table 5). Although BRESIL (B)-Sel had the longest panicle in both leaf harvest treatments, its panicle length under no-leaf harvest treatment (50.6 cm) was significantly longer than under the leaf harvest treatment (33.9 cm). The panicle lengths of the two other entries, HTT-Sel and PARIS (A)-Sel, in the leaf harvest treatment were significantly less than their respective lengths in the no-leaf harvest treatment. In 2014 'Madiira 1' (227.8 cm) followed by PARIS (A)-Sel (210.3 cm) gave the tallest average plant height (Table 6).

Thousand-seed weights, ranged from 0.38 to 1.02 g in 2013 and from 0.35 to 1.07 g in 2014, were generally consistent for each entry between years (Table 6). TZSMN102-Sel produced consistently the heaviest seed across years. The commercial cultivar 'Madiira 2' followed by the other commercial cultivar 'Madiira 1' gave the smallest seed size in both years. The overall mean seed weights, 0.74 and 0.75 g in 2013 and 2014, respectively, were almost the same in both years.

Days to flowering over entries were not much different between 2013 and 2014 although some entries showed differences of 7–12 days between years (Table 6). Check cultivars, 'Madiira 2' and 'Madiira 1', and entry IP-5-Sel flowered relatively late both years while the check cultivar 'Mchicha' and entry AH-TL-Sel flowered earlier both years. For days to grain maturity collected on the main-plots of the first replication, early maturing entry HTT-Sel and late maturing cultivar 'Madiira 2' differed by about 50 days. Entries IP-5-Sel and SIMON"S FARM-Sel and the cultivar 'Madiira 1' in that order were among the latest maturing entries both years.

3.5. Grain nutrient content

Differences among entries in response to leaf harvest were evident only on grain calcium content (Table 5) as shown by the highly

Table 5
Amaranth entries evaluated for grain calcium content (mg/100 g edible portion on dry weight), and plant traits as affected by leaf harvest treatments, Arusha, Tanzania, 2013 and 2014.[z,y]

	No. of branches		Plant height (cm)		Panicle length (cm)		Calcium (mg/100 g)			
	2014		2013		2013		2013		2014	
Entry	WO	W	WO	W	WO	W	WO	W	WO	W
INCA-Sel	8.5	9.5	161.1	143.7	31.9	27.2	–	–	–	–
AH-TL-Sel	8.9	9.2	170.4	136.2	30.4	24.5	206	221	197	191
HTT-Sel	11.8	9.3	162.9	132.8	35.6	24.9	232	223	196	207
SIMON'S FARM-Sel	10.2	11.4	144.0	122.8	25.1	22.5	–	–	–	–
TZSMN82-Sel	10.1	9.4	138.8	122.4	27.3	28.4	202	222	179	208
TZSMN102-Sel	8.7	11.2	155.1	129.9	30.4	24.2	189	204	177	193
AH-NL-Sel	9.8	11.4	172.3	152.1	36.8	31.4	204	209	191	199
MELANGE-Sel	8.7	9.4	154.4	128.9	36.2	29.4	–	–	–	–
BRESIL (B) -Sel	11.2	10.8	220.5	173.8	50.6	33.9	216	214	180	188
PARIS (A)-Sel	14.7	14.4	218.8	157.9	48.6	29.2	229	217	190	197
DB 2006306-Sel	14.0	13.9	186.9	159.0	38.1	32.3	–	–	–	–
IP-5-Sel	17.9	14.5	195.0	203.2	27.2	26.9	–	–	–	–
AM-25-Sel	9.9	7.7	150.6	130.0	33.9	26.4	–	–	–	–
RED INFLORESCENCE-Sel	9.6	9.8	164.5	130.6	31.0	23.5	–	–	–	–
Mchicha (check)	9.5	11.2	170.4	145.5	35.3	28.4	200	209	184	193
Madiira 1 (check)	18.6	15.2	246.1	172.2	23.0	24.5	349	368	361	379
Madiira 2 (check)	19.6	12.8	178.8	147.6	18.5	26.8	398	462	344	329
Mean	11.9	11.2	175.9	146.4	32.9	27.3	242	255	220	228
LSD (5%)[x]	3.2		22.7		9.2		17.6		14.8	
LSD (5%)[w]	3.7		25.9		9.8		20.3		122.4	

[z] WO = no-leaf harvest, W = leaf harvest.
[y] Values in each year are entry x leaf harvest interaction means. Calcium was analyzed on 10 entries.
[x] The appropriate LSD to compare the two levels of leaf harvest for each entry level each year. For example, for No. of branches the LSD value 3.2 should be used to compare the two levels of leaf harvest treatment for each entry in 2014.
[w] The LSD to compare any two entries at the same leaf harvest level in each year. For example, for the No. of branches the LSD value 3.7 is appropriate to compare two entries at the same leaf harvest level in 2014.

significant or significant E × LH interactions for both years. Calcium means of black-seeded cultivars 'Madiira 1' and 'Madiira 2' were significantly different and significantly higher than other entries both years. Iron contents among entries ranged from 14.6 to 77.7 in 2013 and 17.4 to 52.1 mg/100 g in 2014 (Table 6). Iron contents of 'Madiira 1' and 'Madiira 2' were almost double those of other entries although "Madiira 2' was inconsistent between years. Although significant differences among entries in zinc content were detected, the range of entry means was narrow (3.1–4.1 mg/100 g edible portion on dry weight in 2013 and 3.2–4.5 mg in 2014); content of 'Madiira 2' was slightly elevated but significantly higher compared to other entries. Similarly the ranges of entry means in protein (14.7–17.3 g in 2013 and 14.7–17.3 g in 2014) and sugar (3.0–4.3 g in 2013 and 2.3–4.1 in 2014), and fiber contents were narrow except fiber contents of 'Madiira 1' and 'Madiira 2', which were about three times higher than other entries (Table 6).

3.6. Correlation analysis between grain and leaf yields, and other traits

Results indicated that under leaf harvest treatment in both years grain yield did not correlate with leaf yield except at the first harvest date in 2013 (r = 0.542*) and at the second harvest date in 2014 (r = 0.565*). However, grain yield was positively correlated to number of leaves counted at first harvest (r = 0.502*), second (r = 0.521*) and third (r = 0.706**) harvests, and total number of leaves (r = 0.602*) in 2013. Entries AH-NL-Sel, 'Mchicha', TZSMN102-Sel, TZSMN82-Sel and INCA-Sel that had high number of leaves were among high grain yielders while entries, for example BRESIL (B)-Sel, PARIS (A)-Sel and 'Madiira 2' that had low number of leaves gave low grain yield (Table 4). In 2014 under both leaf harvest treatments grain yield did not correlate to number of leaves per plant. There was no correlation between total number of leaves and leaf yield in both years. In 2014 number of leaves per plant was negatively correlated to each of leaf length (r = −0.524*) and width (r = −0.678**) while leaf yield was positively correlated to each of leaf length (r = 0.594*) and width (r = 0.664**). Both leaf length and width were only measured in 2014. From the correlation result it seems that the increase in number of leaves resulted in decrease in leaf sizes (leaf length and width), and then decrease in leaf area – important trait for photosynthesis activities. In the current study, however, grain yield was not correlated to leaf length and width that are the elements of leaf area. Sarker et al (2015) reported positive correlation between leaf area and leaf yield in amaranth.

Leaf yield was positively correlated (0.674**) to number of branches per plant in 2014. In 2013 thousand-seed weight was positively correlated to grain yield (r = 0.666**), and negatively to plant height (r = −0.687**) and days to flowering (r = −0.841**). Plant height and days to flowering were positively correlated (r = 0.522*). In 2014 thousand-seed weight was negatively correlated to number of branches (−0.654**) and positively to panicle length (0.547*). Panicle length was not correlated to grain yield.

3.7. Species comparison

Significant differences were detected between the two species, *A. cruentus* and *A. hypochondriacus*, in grain and leaf yields, and other traits (Table 7). The differences between the species were highly significant in plant height and thousand-grain weight under no-leaf harvest, and in plant height, grain yield and thousand-grain weight under leaf harvest in 2013. In 2014 their differences were highly significant in plant height, number of branches per plant and thousand-grain weight under no-leaf harvest. Their differences in this year under leaf harvest treatment were significant or highly significant in panicle length, number of branches, plant height, grain yield and thousand-grain weight. The species did not differ in leaf yield and number of leaves per plant except for number of leaves under leaf harvest in 2013 in which *A. hypochondriacus* produced higher number (116 leaves per plant) than

Table 6
Amaranth entry means for 1000-seed weight, plant height and grain nutrient contents averaged over leaf harvest treatments, and growth cycle, Arusha, Tanzania, 2013 and 2014.[z,y,x]

	1000-seed weight (g)		Plant height (cm)	Panicle length (cm)	Days to flowering[w]		Days to maturity[v]		Fe (mg)		Zn (mg)		Protein (g)		Sugar (g)		Fiber (g)	
Year /Entry	2013	2014	2014	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
INCA-Sel	0.73	0.82	130.7	35.7	24	20	74	67	–	–	–	–	–	–	–	–	–	–
AH-TL-Sel	0.78	0.88	148.9	37.7	22	20	74	67	–	–	3.2	3.3	16.2	16.7	3.0	3.0	7.1	6.4
HTT-Sel	0.88	0.89	130.7	35.5	27	24	71	64	16.5	17.4	3.4	3.2	17.3	16.6	3.1	2.3	8.5	6.9
SIMON'S FARM-Sel	0.63	0.69	139.7	41.4	32	37	116	109	–	–	–	–	–	–	–	–	–	–
TZSMN82-Sel	0.88	0.92	136.8	44.4	21	20	72	65	16.6	23.9	3.4	3.5	16.9	17.3	3.3	2.6	7.5	6.2
TZSMN102-Sel	1.02	1.07	139.1	42.3	23	20	72	65	14.6	23.1	3.1	3.5	16.6	16.8	3.4	2.6	7.4	5.6
AH-NL-Sel	0.92	0.95	160.2	40.6	27	20	75	68	21.6	25.8	3.2	3.4	15.9	17.1	3.3	2.8	8.0	6.1
MELANGE-Sel	0.83	0.86	174.5	45.1	23	20	84	77	–	–	–	–	–	–	–	–	–	–
BRESIL (B)-Sel	0.76	0.79	191.4	37.7	35	33	94	87	24.0	21.5	3.9	4.0	16.0	16.9	3.4	2.9	8.0	6.5
PARIS (A)-Sel	0.78	0.69	210.3	44.1	35	35	83	76	15.4	23.7	3.9	3.7	16.1	16.0	3.5	3.0	7.7	7.6
DB 2006306-Sel	0.6	0.59	168.7	36.5	41	36	72	65	–	–	–	–	–	–	–	–	–	–
IP-5-Sel	0.63	0.58	200.6	37.8	45	36	120	113	–	–	–	–	–	–	–	–	–	–
AM-25-Sel	0.80	0.93	129.8	39.1	22	20	75	68	–	–	–	–	–	–	–	–	–	–
RED INFLORESCENCE-Sel	0.69	0.58	149.9	34.6	35	34	89	82	–	–	–	–	–	–	–	–	–	–
Mchicha (check)	0.84	0.85	152.4	43.1	22	20	73	66	21.2	27.6	3.3	3.6	16.8	16.9	3.5	2.9	8.8	6.8
Madiira 1 (check)	0.41	0.36	227.8	44.2	50	62	114	107	57.7	52.1	3.6	4.1	16.8	16.1	4.3	4.1	23.7	24.9
Madiira 2 (check)	0.38	0.35	156.9	26.8	69	63	122	115	77.7	24.7	4.1	4.5	14.7	14.7	3.7	3.4	27.1	27.9
Mean	0.74	0.75	161.7	39.2	33	31	87	80	29.5	26.6	3.5	3.7	16.3	16.5	3.5	3.0	11.4	10.5
LSD (0.05)	0.10	0.12	25.4	9.7	6	4	–	–	19.0	NS	0.3	0.6	0.8	1.2	0.7	0.6	2.78	2.8

[z] Values are main effects of entry for which entry x leaf harvest interactions were non-significant.
[y] Nutrient contents were measured on 10 entries from two replications, and values are per 100 g edible portion on dry weight.
[x] Dash (-) indicates entry not assessed for the traits.
[w] Collected on the main-plots from three replications.
[v] Obtained from the first replication on the main-plot.

Table 7
Amaranth species, *A. cruentus* and *A. hypochondriacus*, compared for various traits under no-leaf harvest and leaf harvest treatments, Arusha, Tanzania, 2013 and 2014.

	Mean		Levene's test for equality of variances		t-test for equality of means			
Trait by leaf harvest treatment and year	*Amaranthus cruentus*	*A. hypochondriacus*	F	Prob.	t	Sig. (2-tailed)	Mean difference	Std. error of difference
2013 with no leaf harvest								
Grain yield (t/ha)	3.1	3.6	7.88	0.007	−1.547	0.128	−0.517	0.334
Thousand-grain weight (g)	0.7	0.8	4.63	0.036	−3.787	0.000	−0.168	0.044
Panicle length (cm)	34.1	31.6	8.36	0.006	0.927	0.359	2.519	2.718
Plant height (cm)	190.6	159.4	13.99	0.000	4.034	0.000	31.255	7.747
No. of branches per plant	8.7	9.1	0.47	0.495	−0.674	0.503	−0.384	0.570
2013 with leaf harvest								
Grain yield (t/ha)	0.9	1.7	4.02	0.051	−6.010	0.000	−0.768	0.128
Thousand-grain weight (g)	0.7	0.8	2.21	0.144	−3.920	0.000	−0.187	0.048
Panicle length (cm)	28.1	26.4	3.87	0.055	1.110	0.272	1.666	1.499
Plant height (cm)	155.9	135.7	15.67	0.000	3.020	0.004	20.240	6.710
Total leaf yields (t/ha)	10.6	11.4	6.40	0.020	−1.090	0.280	−0.760	0.690
Total No. of leaf/plant	87.8	116.6	3.37	0.070	−3.970	0.000	−28.84	7.270
No. of branches per plant	8.6	8.7	0.27	0.605	−0.140	0.890	−0.081	0.586
2014 with no leaf harvest								
Grain yield (t/ha)	1.4	1.3	18.30	0.000	0.458	0.649	0.089	0.194
Thousand-grain weight (g)	0.7	0.9	6.76	0.012	−4.698	0.000	−0.272	0.058
Panicle length (cm)	43.1	42.4	2.20	0.144	0.298	0.767	0.671	2.251
Plant height (cm)	193.4	150.9	15.69	0.000	4.777	0.000	42.429	8.882
No. of branches per plant	13.8	9.7	37.44	0.000	3.962	0.000	4.094	1.033
2014 with leaf harvest								
Grain yield (t/ha)	0.3	0.5	2.06	0.157	−3.050	0.004	−0.183	0.060
Thousand-grain weight (g)	0.6	0.8	3.64	0.062	−5.230	0.000	−0.224	0.043
Panicle length (cm)	33.8	37.7	0.06	0.810	−2.020	0.049	−3.928	1.944
Plant height (cm)	164.4	133.7	12.37	0.001	4.030	0.000	30.753	7.624
No. of branches per plant	12.1	10.3	4.59	0.037	2.280	0.027	1.750	0.767
Total keaf yields (t/ha)	16.1	14.5	1.71	0.200	1.370	0.180	1.650	1.200
Total No. of leaf/plant	136.0	148.8	1.88	0.180	−0.980	0.330	−12.760	13.02

A. cruentus that produced 87 leaves. In seven of 13 significant or highly significant differences *A. hypochondriacus* gave the highest values. It tended to give the heaviest grain weight in both years under both harvest treatments (Table 7). It also gave significantly the higher grain yields than *A. cruentus* under leaf harvest treatment in both years. *A. cruentus* was taller than *A. hypochondriacus* under both leaf harvest treatments in both years, and gave the highest number of branches per plant in 2014.

4. Discussion

Amaranth is commonly grown in Africa as a leafy vegetable and cultivars bred for high leaf yields such as ‘Madiira 1’ and ‘Madiira 2’ have recently been released in Tanzania for commercialization. Amaranth grain is highly nutritious and there is increasing interest to incorporate amaranth flour into maize meal or other cereal products to improve their nutritional quality. Kachiguma et al. (2015) reported variation in mineral and chemical nutrient composition among amaranth accessions collected from different regions in Malawi. Depending on prices and interest, farmers may wish to maximize either leaf yields or grain yields, or harvest both fresh leaves and grain for sale or home consumption. Consequently, several types of amaranth cultivars may be useful including: (1) those that allow one or more harvests of good quality leaves but also produce relatively moderate or high grain yields (dual-purpose); or (2) those grown for high leaf yields or high grain yields, but not both. This study was conducted to investigate the response of amaranth entries to total leaf yield from multiple leaf harvests and assess leaf harvest effects on grain yield, grain nutrient content, and major plant traits. Results offer insight into the potential for development of dual purpose cultivars or whether entries tend to perform well as either grain or leaf amaranth.

Multiple leaf harvests reduced grain yields of all entries in this study by at least 50%. The reduction was more severe in 2014 as compared to in 2013. Leaf removal reduced plant photosynthetic capacity to synthesize carbohydrates needed for foliage restoration and grain-fill. Defoliation treatments have been known to decrease assimilate availability during grain filling period (Echarte et al., 2006). The dramatic reduction of leaf number during or close to the reproductive phase of the crop in 2014 (Fig. 2) could be one of possible reasons for the low grain yield in the year. The high number of leaves produced at early stage of the crop growth in the year got older before the critical time of grain formation and grain-filling. It has been reported that a sink is supplied by the source nearby and as plants continue growth new sources develop and the photosynthetic rate of older leaves declines (Rawson and Hofstra 1969; Wardlaw 1990; Paul and Foyer, 2001). There may be genetic differences among amaranths in photosynthetic efficiency, capacity to restore vegetative tissue, or other recovery mechanisms. In the current study under the leaf harvest treatment lines that produced more leaves for each harvest date tended to produce significantly higher grain yield in 2013, there was positive correlation ($r = 0.602^{**}$) between grain yield and number of leaves per plant. In that year the number of leaves progressively increased from the first harvest to the fourth harvest (Fig. 2). However, with the exception of the fourth harvest, the number counted at each harvest date was less than the number counted at the corresponding dates in 2014. It was possible that the balanced distribution of leaves across the four-leaf harvest dates in 2013 did not negatively reduce leaf size or photosynthetic area of the leaf. Limitations in overall plant photosynthetic capacity leads to trade-offs and competition among plant parts for photosynthate. Reduced leaf harvest number or less intensive leaf picking at each harvest could probably lessen grain yield reductions. Gichunge et al. (2009) reported that a weekly leaf harvest compared to

2–3 harvests per week over six weeks of pot-grown amaranth did not affect leaf and grain yields of *A. hypochondriacus*. They concluded that fewer pickings reduced competition between leaves and grain for nutrients compared to frequent pickings that constrained leaf development and grain-fill. They did not indicate the extent of the leaf removal at each harvest in their trial. Determination of optimum number of leaf harvesting times per season for optimum grain and leaf yields requires leaf harvest frequency study in which both grain and leaf yields will be assessed from the different number of repeat harvests.

Entries differed in their sensitivity to leaf harvest and consequences on grain yield. With the no-leaf harvest treatment, entries BRESIL (B)-Sel and PARIS (A)-Sel, both *A. cruentus* species, produced high grain yields both years and would be suitable as grain amaranth cultivars consistent with the results of Mbwambo et al. (2015). They tended to be stable across years for grain production when their leaves are not defoliated, but suffered high yield loss under leaf defoliation. The grain yields of *A. hypochondriacus* species entries were higher than the grain yields of *A. cruentus* species under the leaf harvest treatment in both years (Table 7). Both BRESIL (B)-Sel and PARIS (A)-Sel had been selected from accessions collected from Madagascar and it is possible that the original accessions were grown in Madagascar as grain amaranth. Both lines have pinkish stems and pinkish-green mixed leaf colors not preferred by vegetable amaranth producers in most African countries except, for example, in Uganda and Zambia. Conversely, IP-5-Sel, SIMON'S FARM-Sel, and 'Madiira 2' were outstanding as leaf cultivars, although 'Madiira 2' showed early leaf yellowing, an undesirable trait. The leaf amaranth cultivar 'Madiira 1' was released by Horti-Tengeru in collaboration with the World Vegetable Center in 2011 and has become popular among Tanzanian farmers because its leaves remain green over a long period (stay-green) and its taste and cooking quality are highly regarded. However, the leaf and grain yields of 'Madiira 1' were below those of most other entries in this study. Assuming the photosynthetic capacity of 'Madiira 1' is similar to other entries, it is possible that relatively more of its photosynthate is allocated to the leaves at the expense of the grain, which may explain its low grain yield and the stay-green leaf trait. Entries AH-TL-Sel, AH-NL-Sel and TZSMN102-Sel had relatively less grain yield reductions after four leaf harvests, and their deep green leaves were acceptable for vegetable amaranth markets in Africa, so they may have potential as dual purpose types. Mbwambo et al. (2015) reported that AH-TL-Sel, TZSMN102-Sel and TZSMN82-Sel produced higher grain yields after leaf harvest versus no-leaf harvest, a result they attributed to higher lodging in the no-leaf harvest treatment. Lodging did not occur in the current study and all entries gave higher grain yield under no-leaf harvest. The commercial cultivar 'Mchicha' is sold as a vegetable amaranth cultivar in Tanzania but it can be considered as dual purpose because it sustained less grain reduction after leaf harvest and its yellow-cream color grain fulfills market quality requirements.

Besides high grain and leaf yields, amaranth cultivars for Africa must satisfy important quality or horticultural criteria. Most African markets prefer pale, creamy or white amaranth seed and large seed size for popping. Black seed is unacceptable for consumption except as a traditional medicine in some communities such as in western Ethiopia (personal observation). Reasons for the rejection of black seed color need to be investigated, but it could be due to unfavorable texture/flavor/mouth feel and aroma. In a taste panel study conducted by Kahlon and Chiu (2015) pasta made from cream colored amaranth seed had significantly higher acceptance for color but lower taste acceptance compared to pasta made from buckwheat, teff (*Eragrostis tef* Zucc.) and quinoa. Pedersen et al. (1987) compared protein digestibility of three pale-seeded and one black-seeded *A. caudatus* cultivars, and found an average of 87.1% digestibility in the pale-seeded cultivars versus 79.2% (significantly lower) in the black-seeded cultivar when raw seed samples were analyzed. The digestibility in the black-seeded cultivar was further reduced to 68.1% after toasting, while it remained unaffected (87.1%) in the pale-seeded cultivars.

Grain amaranth cultivars should resist lodging, produce relatively few branches and develop compact panicles for uniform dry down. Maboko and Du Plooy (2012) reported that plucking growing tips and side branches increased inflorescence number but they did not state whether grain yield was affected. Requirements for leafy amaranth include early plant vigor, a long vegetative period for prolonged leaf harvest, quick recovery from repeated cuttings, and stay-green. Vegetable cultivars suitable for multiple cutting should have more branching potential to yield high biomass per harvest (Sreelathakumary and Peter, 1993). Genotypic variability among vegetable amaranth (*A. tricolor* L.) for foliage yield and its contributing traits over successive cuttings and years has been reported (Shukla et al., 2006). Panicle nature and number of branches per plant could be very useful traits for visual selection to distinguish grain versus leaf type lines at early stages in amaranth breeding nurseries. One may select against high branching and for long-compact panicle for grain type cultivar development, and for high number of branches for vegetable type. A breeding program interested in dual type cultivars may need to undertake two-way selection, selection against vegetable and grain types, to retain dual type entries—entries that possess both vegetable and grain traits.

In the current study, the same amount of inputs used for vegetable production was applied although both vegetable and grain were harvested. The requirement to carefully pick leaves to avoid plant damage may increase time and labor required for leaf harvesting. This in turn may increase costs for large scale commercial producers whose interest is to maximize profit. Therefore, dual purpose amaranth cultivars may mainly fit home and school garden producers who use amaranth for home consumption and sell small volumes in local markets. Commercial grain amaranth farmers are mainly interested in high grain yield, just as large scale vegetable producers value high yields of vegetative parts. Market preferences for leaf color and shape vary among countries and regions. In Kenya and Tanzania consumers for example prefer dark green leaf color which they associate with good nutrition and cooking quality (Adeniji and Aloyce, 2013; Dinssa et al., 2016). Leaf amaranth cultivars must also yield enough seed to be accepted by commercial seed producers. Very late flowering and maturing cultivars are not preferred by seed producers as extended time in the field incurs higher costs for irrigation water, fertilizer, and labor. It may be difficult to incorporate some leaf and grain traits in the same cultivars, such as a prolonged vegetative period and early grain maturity, but capturing many desired traits (such as early growth vigor and fast recovery from repeated leaf cutting) for grain and leaf types in the same cultivar should be possible.

Amaranth leaves are regarded among the most nutritious leaf vegetables (Kamga et al., 2013). Leaf analysis of 30 amaranth accessions of four different leaf colors—light green, green, dark green and purple was previously harvested by the World Vegetable Center and revealed higher carotenoid levels in dark green accessions compared to light green accessions, and higher levels of calcium and iron (Unpublished data, World Vegetable Center's, Nutrition Lab.). Results of the present study indicated presence of substantial levels of iron and calcium in amaranth seed but only modest differences among entries in zinc, as well as protein and sugar. Except for calcium content, leaf harvest had little effect on the grain nutrient contents measured. Mean protein content of amaranth grain ranged from 15 to 17 g per 100 g DW. Correa et al. (1986) analyzed amino acid profiles of amaranth grain and detected high lysine (5.3–6.3% of the protein) and sulfur-containing amino acids contents of 3.4–4.0%. Leucine was the limiting protein with a chemical score of 50–67%. In this study we found relatively high contents of iron, calcium, and fiber in the black-seeded entries 'Madiira 1' and 'Madiira 2'. Pedersen et al. (1987) reported 16% fiber content in black-seeded amaranth compared to 8% in pale seed color type and that the protein digestibility of black seed is low. It would be interesting for nutritionists and breeders to understand the association of nutrient content and digestibility with seed color in detail. It also will be interesting to explore the possibility of improving Ca, Fe and fiber contents of grain amaranth lines using the high contents identified in the black-seeded amaranth cultivars in the current study.

5. Conclusions

Four leaf harvestings reduced grain yield of all entries by at least 50% but did not affect grain nutrient content. Entries varied in their grain yield response to defoliation, more leaf producers had better grain yield performance under leaf harvest treatment. Three groups could be distinguished: (1) PARIS (A)-Sel and BRESIL (B)-Sel that produced high and satble grain yields across environments in the absence of severe defoliation and are of interest to commercial grain amaranth producers; (2) IP-5-Sel, SIMON'S FARM-Sel, and 'Madiira 2' produced high leaf yields but relatively little grain; and (3) entries that yielded moderate to high amounts of grain and leaves such as AH-TL-Sel, AH-NL-Sel, TZSMN102-Sel and 'Mchicha', which may have potential as dual purpose cultivars. Because the same land and resources could be used to harvest both amaranth leaves and grain, dual purpose cultivars could be important for efficient and sustainable use of land and inputs.

Competing interests statement

No potential conflict of interest was reported by the authors.

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