Journal of Applied Botany and Food Quality 98, 110 - 119 (2025), DOI:10.5073/JABFQ.2025.098.013

[1]Faculty of Life Sciences, Division Urban Plant Ecophysiology, Research Group Quality Dynamics/Postharvest Management of Perishable Crops, Humboldt-Universität zu Berlin, Berlin, Germany
[2]Department of Food Technology and Nutrition, School of Food Technology Nutrition and Bioengineering, Makerere University, Uganda

Nutrient composition of leaves from defoliated grain amaranth (*Amaranthus cruentus* L.) plants

Rachel Byarugaba[*1,2], Dorothy Nakimbugwe[2], Susanne Huyskens-Keil[1]

(Submitted: February 22, 2025; Accepted: June 2, 2025)

Summary

Utilization of both leaves and grains of *Amaranthus cruentus* L. could enhance the nutritional and economic benefits of the crop for improved health and livelihoods. However, little is known about the effect of defoliation on leaf composition and grain production in a dual use scenario. The nutrients and health promoting compounds of the leaves from differently defoliated *A. cruentus* plants were studied in a randomized complete block design (n = 4). Fifty percent of leaves were harvested from plants once at 5, 7 and 9 weeks after sowing and twice consecutively at 5, 7 and 9 weeks after sowing. The carotenoids, total polyphenols and flavonoids content increased with growth stage although significantly reduced by twice defoliation. Protein content was not affected by defoliation frequency. Iron content decreased with twice defoliation in older plants. Grain yield and plant height were not significantly affected by timing and frequency of defoliation. Earlier harvested leaves contained more iron whereas later harvested leaves had higher carotenoids and flavonoids content. Therefore, 50% of leaves of *A. cruentus* plants can be harvested twice consecutively without affecting plant growth and grain yield, thus promoting a dual use system.

Key words: *Amaranthus*, defoliation, dual-use, grain yield, health promoting compounds, micronutrients

Introduction

There has been increased interest in leveraging the potential of value chain development to improve nutrition specifically in sub-Saharan Africa (SSA) (Donovan and Gelli, 2019); (Gelli et al., 2015)). The concept of nutrition-sensitive value chains has arisen with the central goal of ensuring that smallholders not only have increased economic value and returns but also have value relevant to nutrition (Fanzo et al., 2017). Investing in value chains of micro-nutrient rich foods, and identifying opportunities to diversify and enrich diet quality is one approach to effect this strategy (Allen and de-Brauw, 2018). Some of the criticisms of such approaches allude to insufficient evidence of the positive impact on nutritional outcomes and the adoption of a narrow approach to malnutrition (Hambloch et al., 2023). However, there is still room to identify opportunities in value chains of micro-nutrient rich foods as a starting point from which system-based approaches can be further developed.

Amaranth (*Amaranthus* spp.) is a crop that can provide high quality nutrition in a wide array of contexts due to its potential to be utilized both as a leafy green vegetable and as a grain, and its ability to grow in adverse conditions (Das, 2016). In parts of Africa and tropical Asia, the tender leaves, shoots and stems of amaranth are consumed as leafy vegetables. Vegetable amaranth farmers either harvest once by uprooting the plant, or harvest continuously by removing leaves, tender branches and shoots over the season (Achigan-Dako et al., 2014). Interest in amaranth leaves as a vegetable and seeds as grain is increasing in rural, peri-urban and urban areas in many countries in Africa. 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 (Dinssa et al., 2018). Efforts have been made to enhance the access of improved amaranth varieties by breeding for high nutrient content and also for increased productivity, production, and availability to consumers (Mbwambo et al., 2015). However, the number of improved cultivars grown by farmers is limited particularly in SSA (Schafleitner et al., 2022).

The grain amaranth (predominantly *Amaranthus hypochondriacus* L., *A. cruentus* L., and *A. caudatus* L.) are considered stress-tolerant species that can readily adapt to drought, severe defoliation, poor soils (Förster et al., 2023; Netshimbupfe et al., 2022; Cisneros-Hernández et al., 2021) insect pests and pathogens (Smith et al., 2018; Casarrubias-Castillo et al., 2014). In this regard, grain amaranths provide a promising opportunity as a dual-use crop for farmers who wish to make a profit from seed production while obtaining nutritional benefits and income from leaves (Hoidal et al., 2020). Promising genotypes for dual use production have been identified through studies that investigated the effect of leaf harvest in grain amaranths focusing on the physiological, biochemical, and molecular processes supporting the response mechanisms for tolerance to defoliation (Cisneros-Hernández et al., 2021; Hoidal et al., 2019; Vargas-Ortiz et al., 2015; Castrillón-Arbeláez et al., 2012) as well as the effect of defoliation on the yield components and nutrient content of the amaranth seeds (Dinssa et al., 2018; Roitner-Schobesberger and Kaul, 2013). The nutritional value of amaranth leaves throughout development has been studied (Manyelo et al., 2020; Peiretti et al., 2018), but there are only few investigations on the effects of continuous defoliation with a view to maximise the nutritional benefits of the leafy greens in dual-use production systems.

Whereas vegetative parts of amaranth contain substantial amounts of zinc, calcium, iron, magnesium, vitamin C and pro-vitamin A (Sarker and Oba, 2020; Jiménez-Aguilar and Grusak, 2017), the amaranth seeds are relatively rich in gluten-free protein with a nutritionally balanced amino-acid composition compared to cereals (Ballabio et al., 2011). Moreover, there is a growing awareness of the health-promoting properties of compounds in both the leaves and grain proteins and oil from the grain, which may be beneficial for the prevention of some types of cancer, hypertension and high-lipid related disorders (Sandoval-Sicairos et al., 2021; Kasozi et al., 2018; Neugart et al., 2017).

The effect of multiple defoliation on the composition of amaranth leaves has only been demonstrated to a limited extent (Hoidal et al., 2020). Thus, there is the need to determine the nutrient concentrations and health promoting compounds in amaranth leaves when twice defoliation is conducted. Tolerance to defoliation in grain amaranth is

* Corresponding author

genetically determined (ROITNER-SCHOBESBERGER and KAUL, 2013), may vary amongst species and amongst varieties within species and is also reported to be highly dependent on the way defoliation was performed, defoliation intensity and on the environment in which defoliation was performed (VARGAS-ORTIZ et al., 2013). Therefore, the objectives of this study were to evaluate the composition of amaranth leaves following defoliation using different frequencies at various plant growth stages and assess the growth parameters of the defoliated plants.

Materials and methods

Field trial

A field trial to investigate the potential of *Amaranthus cruentus* as a dual use crop was conducted. The field trial took place in the locality of Katiti (1160 m above sea level; 00° 34' 50.2" N, 32° 24' 55.9" E) in Makulubita subcounty, Luwero district in central Uganda. The soil type was sandy clay loam with a pH of 6.7 and 3.5% organic matter. Dried chicken house waste from a deep litter system (pH 6, 31% organic matter, 2% N, 0.056 mg/kg S) was used as manure and applied at a rate of 0.5 kg per planting hole before sowing of seeds. Seeds which were obtained from farmers in Katiti, were identified at the Makerere University, Department of Plant Science, Microbiology & Biotechnology, Herbarium and tested for germination (90% after 24 h) in a petri dish. The seeds were sown in October 2022 and harvested in early January 2023 after 12 weeks. The average temperature and rainfall during the experimental period were 22.4 °C and 7.4 mm respectively and min/max were 16.9 °C/27.9 °C and 2.3/10.2 mm respectively (Tab. 1). In each plot of 4.5 m × 2.4 m in size, there were 27 plants excluding the border plants. The spacing between and within rows was 0.6 m and 0.45 m, respectively. Watering of plants (approximately 1.5 L per plant) was done twice a day, in the morning and evening during the dry period, otherwise the plants were rain fed. Weather conditions during the field trial are reported in Tab. 1. The data were obtained from a weather station that is 26.8 km away from the field trial site but within the same agroecological zone, and therefore is only approximate.

Tab. 1: Weather data for the field experiment site located in Katiiti, Makulubita, Luwero district in Central Uganda

Year	Period	Temperature (°C)			Precipitation (mm)
		Mean	Minimum	Maximum	
2022	October	22.5	17.0	27.9	7.6
2022	November	21.8	16.9	26.8	10.2
2022	December	22.0	16.7	27.3	9.5
2023	January	23.3	17.0	29.6	2.3

Source: Uganda National Meteorological Authority Kawanda weather station, Kampala-Uganda.

The field trial was set up in a randomized complete block design with four blocks. Each block consisted of nine plots with treatments randomly assigned to each block. There were nine treatments in each block comprising of six defoliation treatments and three controls in which plants were left intact. The treatments were based on timing and frequency of defoliation of leaves. Plants were defoliated by removing 50% of the leaves either once at 5, 7 and 9 weeks after sowing or defoliated twice consecutively using the same leaf harvest intensity at 5, 7 and 9 weeks after sowing. For the twice defoliated plants, the leaves harvested at the second time point were each 2 weeks older than the leaves harvested at the first time point (Fig. 1). The total number of leaves on a plant were counted before defoliation to inform the 50% defoliation rate. The leaves targeted were from the top to the bottom of the plant and were cut from the base of the petiole using a scalpel. The systematic removal of the targeted leaves was to ensure a relatively equal balance of source and sink tissue removal (HOIDAL et al., 2019). Immediately after each harvest, the fresh amaranth leaves were transported in cool boxes to the laboratory at the Department of

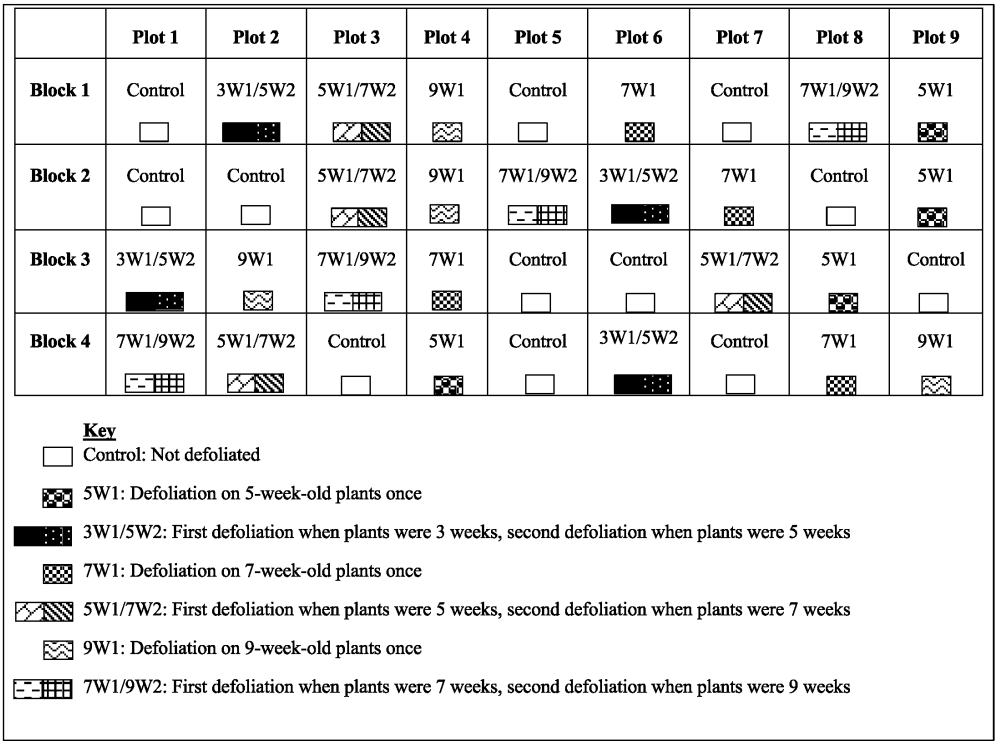

Fig. 1: Experimental design of field trials for amaranth plants used in defoliation experiments

Food Technology and Nutrition, Makerere University where analyses were conducted.

To investigate the effect of the timing and frequency of defoliation, the following parameters were analysed: fresh leaf biomass, plant height, grain yield, dried plant biomass and, protein, total carotenoids, chlorophyll, total phenolics content, flavonoids and iron, zinc, calcium, contents in amaranth leaves.

Determination of plant growth parameters

Fresh leaf biomass was determined by weighing fresh leaves after defoliation of ten randomly selected plants per treatment plot at each scheduled time of defoliation (Hoidal et al., 2020). Following maturation (12 weeks after sowing), the height of six randomly selected plants per plot was measured using a tape measure from the ground level to the top of each flower head (Dinssa et al., 2018). To determine grain yield, mature inflorescence heads from four randomly selected plants per plot were separately hand harvested, threshed and dried to moisture content of 12-13% in an oven at 70 °C (Gallenkamp, United Kingdom) and then weighed (Gimplinger et al., 2008). To obtain the dried plant biomass, three randomly selected plants per plot were chopped, dried separately in a solar dryer for five days and then weighed (Hoidal et al., 2020).

Analytical methods

Determination of protein

Fresh leaves (eight leaves for each of six treatments) were dried in an oven (Gallenkamp, United Kingdom) at 40 °C for 24 h. Dried samples were ground in a stainless-steel blender (Artlon, Daesung Artlon, South Korea). Protein content was determined using the standard Kjeldahl method No. 981.10 (AOAC, 2016). About 0.2 g of dried leaf sample was digested with 3 mL of concentrated sulphuric acid containing copper catalyst powder in a heating block (JP Selecta Bloc digest m 40, JP Selecta, Spain) at 350 °C for 2 h. After cooling, distilled water was added and subsequently steam distillation under alkaline conditions into boric acid (4%) and titration with standardised hydrochloric acid completed. The values of total nitrogen in the samples were multiplied with the conversion factor of 6.25 (Sosulski and Imafidon, 1990) to determine total protein content. Results are presented as percentage protein.

Determination of total carotenoids

The total carotenoids content was determined according to the method described by Rodriguez-Amaya and Kimura (2004) with some modifications. Duplicate extractions were done. About 0.5 g of fresh leaf sample (n=4) was ground with 50 mL 99.5% chilled acetone using a pre-chilled mortar and pestle. The mixture was filtered through glass wool into a 50 mL volumetric flask and made up to volume using the acetone. The filtrate was transferred to a 500 mL separating funnel containing about 30 mL of 90% petroleum ether and washed four times with 250 mL distilled water to remove residual acetone. After the last wash, the upper phase was collected in a volumetric flask after filtering through about 15 g of anhydrous sodium sulphate on a glass wool mounted funnel. The filtrate was made up to 50 mL using petroleum ether. The absorbance of the sample was read at 450 nm using a spectrophotometer (Spectroquant® Pharo 300, EU), and the total carotenoid content calculated using the formula below. Results were presented as μg g^{-1} dry matter.

$$\text{Total carotenoids } (\mu g\ g^{-1}) = \frac{Absorbance \times Total\ volume\ (mL) \times 10^4}{Sample\ weight \times 2592}$$

Determination of total chlorophyll

Chlorophyll content was determined by spectrophotometry of samples prepared by 80% acetone extraction. Duplicate extractions were done. About 0.5 g of fresh leaf sample (n=4) was extracted in 10 mL 80% acetone by centrifugation (Heraeus Megafuge 8, Thermo Scientific, UK) at 3,260 × g for 10 min. This was done several times until the residue became colourless. The supernatants were pooled and made up to 50 mL in a volumetric flask covered with aluminium foil. The absorbance of the supernatant was measured at wavelengths 645, 646 and 663 nm for chlorophyll a, chlorophyll b and total chlorophyll respectively using a spectrophotometer (Spectroquant® Pharo 300, EU). The concentrations of chlorophyll were calculated according to Lichtenthaler (1987) using the equation below. Results were expressed as mg g^{-1} dry matter.

Chlorophyll a (μg mL^{-1}): $11.93(A_{663}) - 1.93(A_{646})$

Chlorophyll b (μg mL^{-1}): $20.36(A_{646}) - 5.50(A_{663})$

Total Chlorophyll (μg mL^{-1}): $6.43(A_{663}) + 18.43(A_{646})$

$$\text{Total Chlorophyll content } (mg\ g^{-1}) = \frac{((6.43 \times A663)+(18.43 \times A646)) \times V}{1000 \times W}$$

Where,

V: Volume of filtrate

W: Sample fresh weight

A: Absorbance value of sample extract

Determination of total polyphenols and flavonoids

The harvested fresh leaf material was frozen in liquid nitrogen, lyophilized (Mini Lyotrap, LTE Scientific Greenfield, England) and milled to fine powder. Phenolic compounds were extracted using the method described by Kim et al. (2003) with slight modification. About 0.1 g of freeze-dried material (n=4) was extracted using 10 mL 80% methanol in a falcon tube by subjecting it to ultrasonic treatment (Bransonic series, M.2800-E; Branson Ultrasonics, Co, Danbury, CT, USA) for approximately 20 min at room temperature. The extract was cooled at -20 °C in a freezer for 10 min and there after centrifuged (Heraeus Megafuge 8, Thermo Scientific, UK) at 3,260 × g for 10 min. The supernatant was decanted from the mixture and collected into a separate falcon tube and stored at -20 °C. The sediment was further re-extracted the second time under the conditions previously described to ensure efficient extraction. The supernatants were pooled and kept in falcon tubes and stored at -20 °C to be used for further determination of total polyphenols and flavonoid contents. Results were calculated from two independent extractions.

The total polyphenols content was determined using spectrophotometry following the method described by Singleton et al. (1999) with slight modification. To 0.3 mL of sample extract, 3 mL distilled water was added. This was followed by addition of 0.3 mL, 0.25 N Folin Ciocalteu reagent and 0.6 mL of 10% sodium carbonate solution. After 40 min of incubation at 25 °C, the absorbance of the samples was read at 765 nm in a spectrophotometer (Spectroquant® Pharo 300, EU) against a blank solution containing 0.3 mL, 80% methanol instead of the sample. A calibration curve with a concentration range of 0.01-0.10 mg mL^{-1} using gallic acid was used. The total polyphenols content was expressed in milligrams gallic acid equivalents per gram of dry matter (mg GAE g^{-1} DM).

The flavonoids content was determined using the method described by Muanda et al. (2011) with slight modification. To 0.5 mL of the sample extract, 2 mL distilled water and 0.15 mL, 5% sodium nitrite solution was added. After 5 min, 0.15 mL, 10% aluminium chloride solution was added followed by the addition of 1 mL, 1M sodium hydroxide solution after another 6 min. After 40 min of incubation at 25 °C, the absorbance of the samples was read at 510 nm in a spectrophotometer (Spectroquant® Pharo 300, EU) against a blank solution containing 0.5 mL, 80% methanol. A standard calibration curve was

plotted using different concentrations of quercetin (0.05-0.5 mg/mL). The flavonoid content was expressed in milligrams quercetin equivalents per gram of dry matter (mg QE g^{-1} DM).

Determination of minerals

The determination of iron, zinc and calcium was done using the method described by Jiménez-Aguilar and Grusak (2015) with modification. Dried leaf samples (n=8) each weighing 0.5 g were soaked in 20-25 mL of 98% nitric acid for 12 hours. Each sample was digested with 10 mL of 98% nitric acid in a heating block (JP Selecta Bloc digest m 40, JP Selecta, Spain) at 360 °C for one hour with occasional addition of drops of 30% hydrogen peroxide to clear precipitates. After cooling, deionised water was added to the sample followed by filtering (Whatman filter paper, 125 mm) and making up to a known volume in a volumetric flask. For determination of calcium, samples were diluted with 10% lanthanum chloride solution. Commercially available standards of 1000 ppm (BDH, VWR International LLC, USA) for iron, zinc and calcium were used as references. An Atomic Absorption Spectrometer (Agilent AA Series, Agilent Technologies Australia Pty Ltd, Australia) was used to read the absorbance of samples at 213.9 nm for zinc, 422.7 nm for calcium and 248.3 nm for iron. Results were expressed as mg g^{-1} dry matter.

Statistical analysis

One-way and two-way Analysis of Variance (ANOVA) was done to determine the significant differences among means generated for the different parameters investigated. This was followed by Tukey's HSD test which was used to separate the means. The differences were considered statistically significant at $p \leq 0.05$. Analyses were performed using the statistical software SPSS (IBM SPSS Statistics 29).

Results

The effect of defoliation timing and frequency on the growth parameters of *A. cruentus* plants and the nutrient and health promoting compounds composition of the leaves harvested was investigated. Results showed that the growth parameters (grain yield, plant height and dried plant biomass) were in general not significantly affected by defoliation frequency and timing. However, the composition of leaves showed significant changes in terms of a number of parameters following defoliation.

Effect of twice defoliation on grain yield and growth parameters in *A. cruentus* plants

Grain yield and plant height were not affected by the twice defoliation regardless of the plant age, except, plant height at the end of the growth season was significantly higher in plants that had been defoliated once at 9 weeks compared to those in which defoliation had been done once at 5 weeks. Dried plant biomass was significantly higher following single defoliation at 5 weeks, was unaffected at 9 weeks but was significantly less at 7 weeks (Tab. 2). In the twice defoliated plants, dried biomass was significantly lower at 5 weeks and 9 weeks, compared to 7 weeks.

Effect of twice defoliation on fresh leaf biomass, nutritional and health promoting compounds in leaves of *A. cruentus* plants

The fresh leaf biomass was significantly higher from 5 through to 9 weeks as expected. There was a significant reduction in the fresh leaf biomass only at 9 weeks when defoliation was conducted twice consecutively (Fig. 2). The protein content was not affected by defoliation frequency, however, it was significantly lower with advancing growth only in plants defoliated once (Fig. 3). The total carotenoids content increased with increase in growth stage in plants defoliated once, but was significantly lower following twice defoliation when conducted at 5 and 9 weeks whereas it was not affected at 7 weeks (Fig. 4). The total chlorophyll content peaked at 7 weeks but was significantly less when twice defoliation was conducted at 5, 7 and 9 weeks in comparison to plants defoliated once (Fig. 5). The total polyphenols content increased with growth stage but was significantly lower when twice defoliation was conducted at 5 and 9 weeks (Fig. 6). The flavonoids content peaked in the 9-week-old plants but was significantly lower in general following twice defoliation at 5, 7 and 9 weeks (Fig. 7).

Effect of twice defoliation on iron, zinc and calcium content in leaves of *A. cruentus* plants

The iron content peaked at 7 weeks in plants defoliated once in comparison to the twice defoliated plants. Following twice defoliation, the iron content was significantly higher in the 5-week-old plants but was significantly less in the older plants (Fig. 8). The zinc content was highest at 9 weeks amongst plants that were defoliated once. In the twice defoliated plants, the zinc was significantly higher at 7 weeks but was again low at 5 and 9 weeks (Fig. 9). Calcium content was stable during the experimental period. However, it was slightly significantly higher only at 9 weeks following twice defoliation (Fig. 10).

Tab. 2: Effect of defoliation on grain yield and growth parameters of *A. cruentus* plants

		Weeks after sowing		
	Defoliation frequency	**5**	**7**	**9**
Grain yield (g/plant)	Control	58.50 ± 10.79^{aA}	56.33 ± 11.91^{aA}	60.46 ± 11.37^{aA}
	Once	65.83 ± 12.50^{aA}	61.26 ± 16.38^{aA}	70.80 ± 15.96^{aA}
	Twice	65.89 ± 9.33^{aA}	60.09 ± 12.84^{aA}	64.01 ± 14.57^{aA}
Plant height (cm)	Control	235.67 ± 14.47^{aA}	235.34 ± 9.66^{aA}	231.94 ± 15.80^{aA}
	Once	225.26 ± 12.63^{aB}	230.50 ± 18.58^{aAB}	238.67 ± 13.84^{aA}
	Twice	226.41 ± 18.22^{aA}	224.43 ± 21.32^{aA}	231.79 ± 18.31^{aA}
Dried plant biomass (g)	Control	263.90 ± 10.13^{bA}	284.64 ± 30.03^{aA}	281.63 ± 19.06^{aA}
	Once	280.52 ± 13.69^{aB}	230.78 ± 14.25^{bC}	300.76 ± 23.79^{aA}
	Twice	224.78 ± 14.71^{cC}	283.58 ± 21.24^{aA}	245.41 ± 14.52^{bB}

Mean ± standard deviation; different lower-case and upper-case letters indicate significant differences between treatments within the same column and row respectively for each parameter (Tukey's HSD test, $p \leq 0.05$).

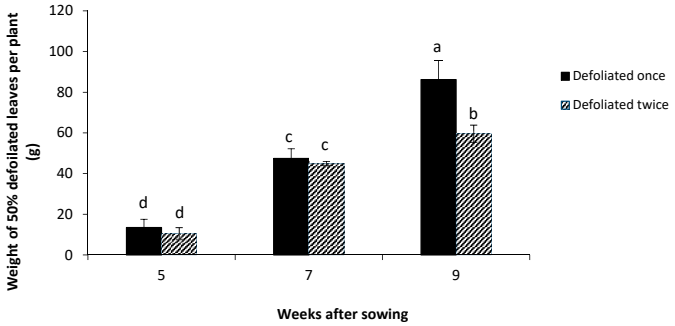

Fig. 2: Effect of defoliation on fresh leaf biomass of *A. cruentus* plants. Mean values (± standard deviation) with the same letter are not significantly different according to Tukey's HSD test ($p < 0.05$)

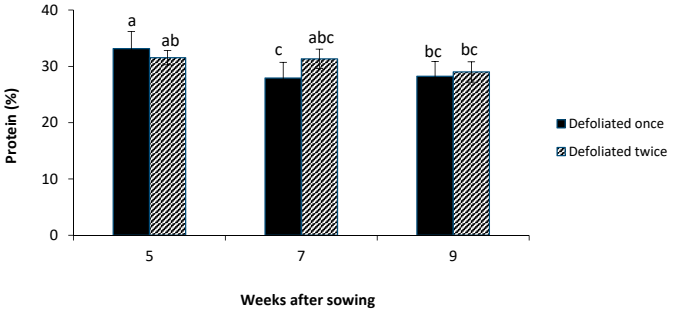

Fig. 3: Effect of defoliation on protein content of leaves of *A. cruentus* plants. Mean values (± standard deviation) with the same letter are not significantly different according to Tukey's HSD test ($p \leq 0.05$)

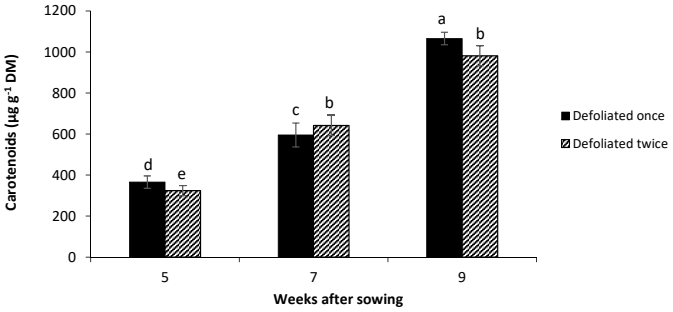

Fig. 4: Effect of defoliation on carotenoid content of leaves of *A. cruentus* plants. Mean values (± standard deviation) with the same letter are not significantly different according to Tukey's HSD test ($p \leq 0.05$)

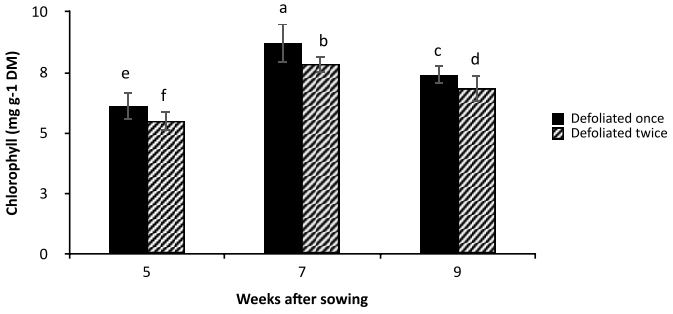

Fig. 5: Effect of defoliation on total chlorophyll content of leaves of *A. cruentus* plants. Mean values (± standard deviation) with the same letter are not significantly different according to Tukey's HSD test ($p \leq 0.05$)

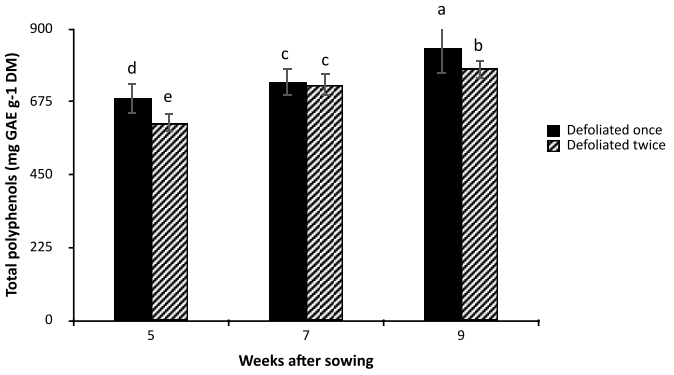

Fig. 6: Effect of defoliation on total polyphenols content of leaves of *A. cruentus* plants. Mean values (± standard deviation) with the same letter are not significantly different according to Tukey's HSD test ($p \leq 0.05$)

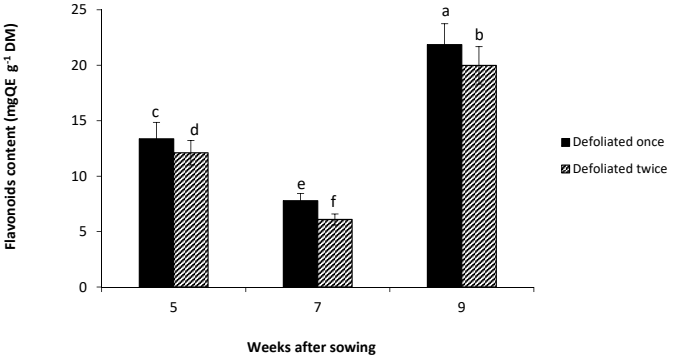

Fig. 7: Effect of defoliation on flavonoids content of leaves of *A. cruentus* plants. Mean values (± standard deviation) with the same letter are not significantly different according to Tukey's HSD test ($p \leq 0.05$)

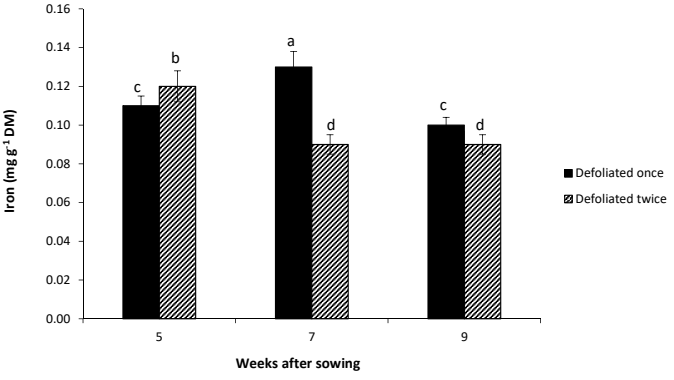

Fig. 8: Effect of defoliation on iron content of leaves in *A. cruentus* plants. Mean values (± standard deviation) with the same letter are not significantly different according to Tukey's HSD test ($p \leq 0.05$)

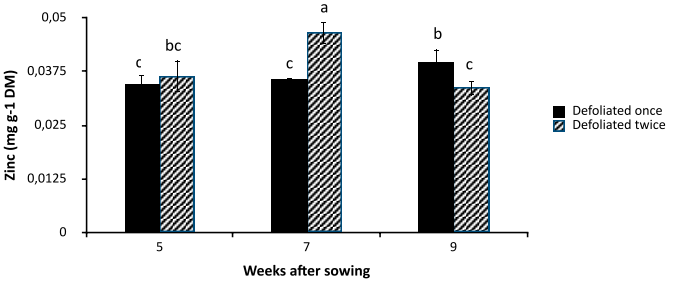

Fig. 9: Effect of defoliation on zinc content of leaves of *A. cruentus* plants. Mean values (± standard deviation) with the same letter are not significantly different according to Tukey's HSD test ($p \leq 0.05$)

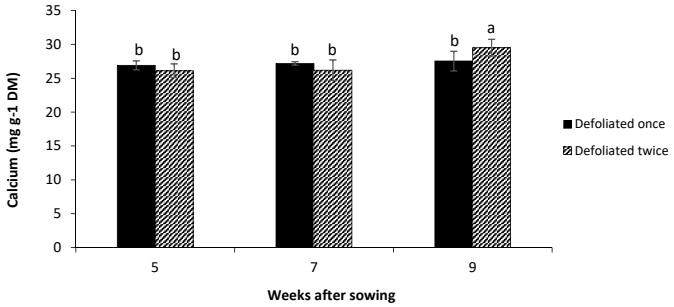

Fig. 10: Effect of defoliation on calcium content of leaves of *A. cruentus* plants. Mean values (± standard deviation) with the same letter are not significantly different according to Tukey's HSD test ($p \leq 0.05$)

Discussion

Grain yield and growth parameters in defoliated *A. cruentus* plants

There was an average grain yield level of about 2.98 tonnes per ha on control plots which was comparable to other studies on amaranth that reported 3.5 (Ochieng et al., 2019), 2.36 (Dinssa et al., 2018) and 5.3 tons/ha (Roitner-Schobesberger and Kaul, 2013), and indicated favourable growth conditions for amaranth in general. Grain yield was not affected by multiple defoliation in field conditions as reported by Hoidal et al. (2019). Furthermore, present results showed that the timing of the twice defoliation did not affect grain yield. The timing of defoliation had been reported to affect grain yield contingent on season (Vargas-Ortiz et al., 2015). In summer-spring, *A. cruentus* plants that underwent a single 100% defoliation event earlier during the vegetative stage and bud formation as compared to later in development did not exhibit a significant reduction in grain yield (Vargas-Ortiz et al., 2015). Hoidal et al. (2020) had contrary results for a single 100% defoliation event that showed a reduction in grain yield regardless of when the defoliation was done. However, in the same study, timing of defoliation for the single 50% defoliation event did not significantly impact grain yield. Roitner-Schobesberger and Kaul (2013) also reported no significant impact on grain yield when a single 50% defoliation event was conducted at the mid flowering stage. The results of the present study showed that even when twice 50% defoliation was done, regardless of when it was initiated, grain yield was not affected. Multiple 50% defoliation of up to two and three times has been reported to produce stable grain yields in *A. cruentus* in field conditions when defoliation was initiated in the vegetative stage, at the 10-leaf and 8-leaf, stage respectively (Hoidal et al., 2019). Therefore, present results suggest that 50% defoliation of up to two times consecutively could be initiated even later during plant development without negative consequences to grain yield.

The response of plant biomass production to defoliation varies across growing environments and genotypes (Hoidal et al., 2019; Moreno et al., 1999). Present results suggest that the developmental stage or timing may also be influential as evidenced by the adverse effect of twice defoliation in plants that were defoliated earlier at 5 weeks and later at 9 weeks. After defoliation, plants may initially experience a reduction in photosynthetic capacity but can recover by mobilizing non-structural carbohydrates from stems and roots, which supports regrowth after leaf loss (Vargas-Ortiz et al., 2013; Castrillón-Arbeláez et al., 2012). In undamaged plants, source-sink relationships direct resource allocation among organs, with the relative strength of sinks determining which will accumulate more resources (Marcelis, 1996). Defoliation or mechanical damage often removes sinks and/or sources, thereby altering source-sink relationships and modifying allocation patterns (Stowe et al., 2000). Vargas-Ortiz et al. (2013) reported that in order to sustain recovery and productivity in defoliated amaranth plants, it was mostly starch reserves stored in stems and roots that were mobilized to different organs according to priorities of vegetative and reproductive development. Given the differences in developmental stages and corresponding sink strength, the higher priority of supporting bud formation at 5 weeks and flowering at 9 weeks could explain the pattern of plant dried biomass decrease at these stages as a response to the twice defoliation.

Fresh leaf biomass, nutritional and health promoting compounds in leaves of defoliated *A. cruentus* plants

In contrast to growth chamber conditions where fresh leaf biomass was reported to be boosted by 50% multiple defoliation of up to three times (Hoidal et al., 2019), the present study showed that fresh leaf biomass was significantly lower after twice defoliation at 9 weeks indicating that sufficient compensatory leaf regrowth was not possible at this stage in comparison to 5 and 7 weeks. Since the defoliation treatment involved removal of leaves from the top to the bottom of the plant, both source and sink leaves were reduced. The combined effect of reducing competition by removal of some sink leaves, stimulation of photosynthetic capacity in remaining leaves and mobilization of internal carbon reserves could have contributed to regrowth of leaves (Délano-Frier et al., 2012). However, due to the high assimilate demands of flowering at 9 weeks, with continuous defoliation, there was insufficient assimilate to support the stimulation of leaf production and photosynthesis to quickly compensate for the stored carbon and nutrient relocation. Moreover, there was maintenance of grain yield observed at this stage in comparison to non-defoliated plants. This points to the fact that defoliation tolerance in terms of seed yield was at the cost of increase in leaf biomass. This is corroborated by results by Moreno et al. (1999) which revealed that in clipped *A. cruentus* plants, more biomass was allocated to seed production according to patterns of whole plant biomass allocation following partial defoliation. Furthermore, the effect of recycling mobilizable nutrients at the onset of senescence in the mature leaves at 9 weeks could have further limited the resources available for leaf regrowth. Whereas the appearance of delayed senescence phenomena after partial defoliation has been reported in some plants (Délano-Frier et al., 2012), at 9 weeks, it is most probable that it is the mature and older senescing leaves that behaved as source leaves.

There was no significant change in protein content following twice defoliation at any of the growth stages although it was lower in older leaves than in the younger leaves (Manyelo et al., 2020; Peiretti et al., 2018). This is consistent with results by Hoidal et al. (2020) for the same defoliation rate in plants grown under green-house conditions. However, for 75% multiple defoliation of up to three times in the same study, leaf nitrogen was reported to increase owing to remobilization of resources in stem and root tissues but with a resultant decrease in grain yield. Similarly, in some plants such as tomato, under specific conditions of limited assimilate, initiating inflorescences were aborted in preference for the higher priority of sustaining developing leaves (Ho, 1988) while in wheat, vegetative organs were stronger sinks relative to grain albeit in conditions of increased source-sink ratio (Ershadimanesh et al., 2024). In this study, grain yield was not significantly affected compared to non-defoliated plants showing that the 50% defoliation rate was not sufficient to disrupt the necessary nitrogen prioritised for seed development hence there was no requirement for increased remobilization into the leaves. With regard to plant age, a decrease in protein is normally inversely related to structural components of plants such as lignin, hemi-cellulose, and cellulose which explains the decrease in palatability associated with older leaves (Hoidal et al., 2020; Pospišil et al., 2006). Nevertheless, more importantly, the protein results showed no significant variation in composition regardless of the frequency of harvesting at a rate of 50%.

The range of carotenoid content observed in leaves of plants that were defoliated once is similar to those reported in other studies on amaranth (ELOLU et al., 2024; TANG et al., 2014). The trend of increasing content in older leaves also matches that observed for ß-carotene content from other studies (HOIDAL et al., 2020; NAWIRI, 2018). Twice defoliation was associated with a significantly lower carotenoid content at all stages except at 7 weeks. The chlorophyll content ranges were similar to those reported in other studies on amaranth (ELOLU et al., 2024; HOWARD, 2019), and the chlorophyll content experienced an increase until anthesis (SKWARYŁO-BEDNARZ and KRZEPIŁKO, 2009). However, in the present study, the chlorophyll content was significantly lower following twice defoliation at all stages of growth. This could have been due to the extra demands on nitrogen to support regrowth of leaves especially because chlorophyll content depends on plant nitrogen status (CASTELLI et al., 1996). Foliar chlorophyll content is a good indicator of plant stress (CARTER and KNAPP, 2001) which is reflected by the present results that show that the decrease was attributed to stress. Leaf pigment composition is sensitive to plant stress, with a range of abiotic and biotic factors responsible for either degradation of photosynthetic pigments like chlorophylls or the synthesis of photoprotective pigments such as zeaxanthin or ß-carotene (BLANCHFIELD et al., 2006; DEMMIG-ADAMS and ADAMS, 1992). In this case, the stress could have resulted from the continuous defoliation which exacerbated the energy imbalance between sink and source tissues.

In general, the total polyphenols and flavonoids are higher in older leaves than in younger leaves (KARAMAĆ et al., 2019) although the levels could be reduced significantly as an effect of twice defoliation. Phenolics are carbon-based compounds that serve multiple functions in leaves including chemical defences against herbivores and pathogen resistance (ZAYNAB et al., 2018), ultraviolet protection due to their absorption properties (NEUGART et al., 2021) and protection against oxidative stress and free radicals (HAJAM et al., 2023). It has been suggested that the synthesis of polyphenolic compounds is constrained by the external availability of resources and internal trade offs in resource allocation (CIPOLLINI et al., 2014). Several partially overlapping hypotheses have been proposed to predict the relationship between nutrient availability, plant growth rate, and allocation to phenolic compounds (STAMP, 2003; JONES and HARTLEY, 1999). There have also been reports that phenolics including both flavonoids and hydroxycinnamic acids increased under nitrogen deprivation (CARTELAT et al., 2005; NØRBAEK et al., 2003). According to the carbon-nutrient balance hypothesis, the production of carbon or nitrogen-based secondary metabolites is related to the carbon-nutrient ratio of a plant (STAMP, 2003). Since there was reduced nitrogen availability with advanced plant growth, the relatively excess carbon could have been allocated to the synthesis of phenolic compounds which explains the increased content in the older leaves. Nitrogen supply influences both protein synthesis as well as the synthesis of polyphenolics. The amino acid phenylalanine, which is the precursor of all phenolics is the branching point between the two pathways (HERRMANN and WEAVER, 1999). Furthermore, the protein competition model postulates that protein-phenolic competition for limiting phenylalanine results in a process-level trade-off between rates of protein versus phenolic synthesis, and an inverse relation between protein and phenolic allocation (JONES and HARTLEY, 1999). However, with continuous defoliation resulting in changes in resource allocation, there was a subsequent decrease in the carbon-nutrient ratio, thereby reducing excess carbon production which explains the observed decrease in the phenolic compounds.

Iron, zinc and calcium content in leaves of defoliated *A. cruentus* plants

The trends observed for iron, zinc and calcium in the leaves of plants that were defoliated once are similar to results from other studies (HOIDAL et al., 2020; MASEKO et al., 2019) and the ranges observed are also comparable (ELOLU et al., 2024; KACHIGUMA et al., 2015). Calcium accumulated with advancing plant growth due to its immobile nature. Once deposited in leaves, relatively low amounts of calcium can be re-mobilized to other organs in most plant species (TANG and LUAN, 2017). Although present results indicate that calcium levels remained constant with increasing plant growth, there was significantly higher calcium content in the older plants following twice defoliation. This could be explained by the fact that with continuous defoliation and subsequent lower leaf regrowth, as reflected by the decrease in leaf biomass particularly at 9 weeks, the proportion of older leaves that were richer in calcium was higher than the younger ones amongst the harvested leaves.

In plants, iron is involved in chlorophyll synthesis and stabilization (BRIAT et al., 2007) which is why the pattern of chlorophyll content is similar to that of iron in the amaranth plants that were defoliated once. Iron increased with the increased need for vegetative growth in the plants but revealed lower content when there was a change in priority to the reproductive phase of the plant. Leaves and seeds are two crucial sinks of iron. However, its storage in the seed is of higher priority as iron is essential for future seed germination (RAI et al., 2021). At 5 weeks, when nitrogen levels were still fairly relatively high, compensatory leaf growth was induced following twice defoliation, and the iron levels also increased possibly in response to this regrowth. However, subsequently, due to the channelling of resources to initiate and sustain reproduction, the iron levels also decreased.

Zinc is associated with the activity of numerous enzymes as a co-factor, and is thus vital for the mediation of plant metabolic reactions. It's structural and catalytic role in antioxidant enzymes contribute to plant adaptation to stress (FARIDUDDIN et al., 2022; NATASHA et al., 2022). Transportation of zinc towards aerial tissues in plants is primarily via bulk flow in xylem tissues (NATASHA et al., 2022). In plants that had been defoliated once, zinc increased with age as more mature leaves tend to have higher transpiration rates facilitating movement from the roots via the xylem, compared to new immature leaves that are principally supplied by the phloem (GRUSAK et al., 1999). The significant reduction in zinc as an effect of twice defoliation at 9 weeks may be attributed to senescence (BUCHANAN-WOLLASTON et al., 2003) involving massive mobilization of resources from the mature leaves to other parts of the plant.

Conclusion and limitations

The results of this study reinforce that *Amaranthus cruentus* is a defoliation tolerant amaranth grain specie which can be utilised both for grain and leaves when a rate of 50% leaf removal for up to two consecutive times is applied. The grain yield is maintained unaffected under these conditions even when defoliation is initiated later during the reproductive development of the plant at flowering. Under the same conditions, the leaf yield is unaffected in the younger plants and therefore leaf utilization can proceed without detriment to plant growth. However, the defoliation tolerance observed by the maintained grain yield happens at the expense of leaf nutrient composition. Grain yield is maintained but with a relative higher cost of stress associated with continuous defoliation on the composition of leaves in terms of the micronutrients and health promoting compounds. The question is whether the reductions observed in these nutrients and compounds translate into reduced availability in terms of potential intake by consumers. It would be beneficial to have a follow up study dealing with the bioavailability of nutrients and health promoting compounds in the leaves of plants cultivated under a similar dual use production system.

The results and conclusions of this study are based on field data for one season and are limited to only one species that was grown in a specific geographic location. However, they confirm the conclusions

of other authors that demonstrated that moderate defoliation does not affect grain yield in *A. cruentus* and further throw more light on the source sink dynamic in terms of the composition of leaves. The results are valuable in that they support the promotion of dual use amaranth production systems as a basis for diet diversification strategy specifically to enrich micronutrient supply which is important in many SSA countries.

Acknowledgement
The study is part of the project Inclusive Nutrition-Sensitive Value Chains in Kenya and Uganda – Upgrading strategies for underutilized horticultural crops (InNuSens) which is funded by the German Federal Ministry of Education and Research (BMBF) and the German Academic Exchange Service (DAAD). We gratefully acknowledge the financial support of BMBF and DAAD.

Conflict of interest
No potential conflict of interest was reported by the authors.

References

Achigan-Dako, E.G., Sogbohossou, O.E.D., 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. DOI: 10.1007/s10681-014-1081-9

Allen, S., de-Brauw, A., 2018: Nutrition sensitive value chains: Theory, progress, and open questions. Glob. Food Sec. 16, 22-28. DOI: 10.1016/j.gfs.2017.07.002

AOAC, 2016: Official Methods of Analysis of AOAC INTERNATIONAL. 20th Edition, 20th edition, Washington DC: AOAC INTERNATIONAL.

Ballabio, C., Uberti, F., Di Lorenzo, C., Brandolini, A., Penas, E., Restani, P., 2011: Biochemical and immunochemical characterization of different varieties of amaranth (*Amaranthus* L. ssp.) as a safe ingredient for gluten-free products. J. Agric. Food Chem. 59, 12969-12974. DOI: 10.1021/jf2041824

Blanchfield, L.A., Robinson, A.S., Renzullo, J.L., Powell, S.K., 2006: Phylloxera-infested grapevines have reduced chlorophyll and increased photoprotective pigment content—can leaf pigment composition aid pest detection? Funct. Plant Biol. 33, 507-514.

Briat, J.-F., Curie, C., Gaymard, F., 2007: Iron utilization and metabolism in plants. Curr. Opin. Plant Biol. 10, 276-282. DOI: 10.1016/j.pbi.2007.04.003

Buchanan-Wollaston, V., Earl, S., Harrison, E., Mathas, E., Navabpour, S., Page, T., Pink, D., 2003: The molecular analysis of leaf senescence-a genomics approach. Plant Biotechnol. J. 1, 3-22.

Cartelat, A., Cerovic, Z.G., Goulas, Y., Meyer, S., Lelarge, C., Prioul, J.-L., Barbottin, A., Jeuffroy, M.-H., Gate, P., Agati, G., Moya, I., 2005: Optically assessed contents of leaf polyphenolics and chlorophyll as indicators of nitrogen deficiency in wheat (*Triticum aestivum* L.). Field Crops Res. 91, 35-49. DOI: 10.1016/j.fcr.2004.05.002

Carter, A.G., Knapp, K.A., 2001: Leaf optical properties in higher plants: linking spectral characteristics to stress and chlorophyll concentration. Am. J. Bot. 88, 677-684.

Casarrubias-Castillo, K., Martínez-Gallardo, N.A., Délano-Frier, J.P., 2014: Treatment of *Amaranthus cruentus* with chemical and biological inducers of resistance has contrasting effects on fitness and protection against compatible Gram positive and Gram negative bacterial pathogens. J. Plant Physiol. 171, 927-939. DOI: 10.1016/j.jplph.2014.02.004

Castelli, F., Contillo, R., Miceli, F., 1996: Non-destructive Determination of Leaf Chlorophyll Content in Four Crop Species. J. Agron. Crop Sci. 177, 275-283.

Castrillón-Arbeláez, P.A., Martínez-Gallardo, N., Arnaut, H.A., Tiessen, A., Délano-Frier, J.P., 2012: Metabolic and enzymatic changes associated with carbon mobilization, utilization and replenishment triggered in grain amaranth (*Amaranthus cruentus*) in response to partial defoliation by mechanical injury or insect herbivory. BMC Plant Biol. 12.

Cipollini, D., Walters, D., Voelckel, C., 2014: Costs of Resistance in Plants: From Theory to Evidence, 263-307. In: Reinecke, A., Hilker, M., Voelckel, C., Jander, G. (ed.), Annual Plant Reviews Volume 47: Insect-Plant Interactions. Wiley-Blackwell. DOI: 10.1002/9781119312994.apr0512

Cisneros-Hernández, I., Vargas-Ortiz, E., Sánchez-Martínez, E.S., Martínez-Gallardo, N., Soto González, D., Délano-Frier, J.P., 2021: Highest Defoliation Tolerance in *Amaranthus cruentus* Plants at Panicle Development is Associated with Sugar Starvation Responses. Front. Plant Sci. 12, 658977. DOI: 10.3389/fpls.2021.658977

Das, S., 2016: Future Prospects in Amaranth Research, 167-172. In: Das, S. (ed.), *Amaranthus*: A Promising Crop of Future. Singapore: Springer Singapore. DOI: 10.1007/978-981-10-1469-7_11

Délano-Frier, J.P., Hernández, V.S.C., Tiessen, A., 2012: Friend or foe? Exploring the factors that determine the difference between positive and negative effects on photosynthesis in response to insect herbivory, 155-206. In: Najafpour, M.M. (ed.), Artificial Photosynthesis. Rijeka, Croatia: InTech.

Demmig-Adams, B., Adams, W.W.-I., 1992: Carotenoid composition in sun and shade leaves of plants with different life forms. Plant Cell Environ. 15, 411-419.

Dinssa, F.F., Yang, R.-Y., Ledesma, R.D., Mbwambo, O., Hanson, P., 2018: Effect of leaf harvest on grain yield and nutrient content of diverse amaranth entries. Sci. Hortic. (Amsterdam) 236, 146-157. DOI: 10.1016/j.scienta.2018.03.028

Donovan, J., Gelli, A., 2019: Designing interventions in local value chains for improved health and nutrition: Insights from Malawi. World Dev. Perspect. 16, 100149. DOI: 10.1016/j.wdp.2019.100149

Elolu, S., Förster, N., Baier, A.K., Opiyo, A.M., Huyskens-Keil, S., 2024: Impact of postharvest osmodehydration treatments and drying processes on the nutritional quality, bioactive compounds and preservation of leaf amaranth (*Amaranthus cruentus*). J. Appl. Bot. Food Qual. 97, 92-101. DOI: 10.5073/JABFQ.2024.097.012

Ershadimanesh, K., Siosemardeh, A., Hoseeinpanahi, F., 2024: Evaluation of source–sink manipulation through defoliation treatments in promising bread wheat lines under optimal irrigation and rainfed conditions. Front. Agron. 6. DOI: 10.3389/fagro.2024.1393267

Fanzo, J.C., Downs, S., Marshall, Q.E., Pee, S. de, Bloem, M.W., 2017: Value Chain Focus on Food and Nutrition Security, 753-770. In: Pee, S. de, Taren, D., Bloem, M.W. (eds.), Nutrition and Health in a Developing World. Cham: Springer International Publishing. DOI: 10.1007/978-3-319-43739-2_34

Fariduddin, Q., Saleem, M., Khan, A., Hayat, S., 2022: Zinc as a versatile element in plants: an overview on its uptake, translocation, assimilatory roles, deficiency and toxicity symptoms, 137-158. In: Khan, S.T., Malik, A. (eds.), Microbial Biofertilizers and Micronutrient Availability. Cham: Springer International Publishing.

Förster, N., Dilling, S., Ulrichs, C., Huyskens-Keil, S., 2023: Nutritional diversity in leaves of various amaranth (*Amaranthus* spp.) genotypes and its resilience to drought stress. J. Appl. Bot. Food Qual. 96. DOI: 10.5073/JABFQ.2023.096.001

Gelli, A., Hawkes, C., Donovan, J., Harris, J., Allen, S., de-Brauw, A., Henson, S., Johnson, N., Garrett, J., Ryckembusch, D., 2015: Value Chains and Nutrition: A Framework to Support the Identification, Design, and Evaluation of Interventions, Washington, DC: International Food Policy Research Institute.

Gimplinger, D.M., Schulte auf'm Erley, G., Dobos, G., Kaul, H.-P., 2008: Optimum crop densities for potential yield and harvestable yield of grain amaranth are conflicting. Eur. J. Agron. 28, 119-125. DOI: 10.1016/j.eja.2007.05.007

Grusak, A.M., Pearson, N.J., Marentes, E., 1999: The physiology of micronutrient homeostasis in field crops. Field Crops Res. 60, 41-56.

Hajam, A.Y., Lone, R., Kumar, R., 2023: Role of plant phenolics against

reactive oxygen species (ROS) induced oxidative stress and biochemical alterations, 125-147. In: Lone, R., Khan, S., Mohammed Al-Sadi, A. (eds.), Plant Phenolics in Abiotic Stress Management. Singapore: Springer Nature Singapore.

Hambloch, C., Mausch, K., Conti, C., Hall, A., 2023: Simple solutions for complex problems? What is missing in agriculture for nutrition interventions. Food Sec. 15, 363-379. DOI: 10.1007/s12571-022-01324-6

Herrmann, M.K., Weaver, M.L., 1999: The shikimate pathway. Annu. Rev. Plant Biol. 50, 473-503.

Ho, L.C., 1988: Metabolism and Compartmentation of Imported Sugars in Sink Organs in Relation to Sink Strength. Annu. Rev. Plant Physiol. Plant Mol. Biol. 39, 355-378.

Hoidal, N., Díaz Gallardo, M., Jacobsen, S.-E., Alandia, G., 2019: Amaranth as a Dual-Use Crop for Leafy Greens and Seeds: Stable Responses to Leaf Harvest Across Genotypes and Environments. Front. Plant Sci. 10, 817. DOI: 10.3389/fpls.2019.00817

Hoidal, N., Jacobsen, S.-E., Odone, A., Alandia, G., 2020: Defoliation timing for optimal leaf nutrition in dual-use amaranth production systems. J. Sci. Food Agric. 100, 4745-4755. DOI: 10.1002/jsfa.10533

Howard, J.E., 2019: Characterization of pigments from cultivated *Amaranthus* species and assessment of their commercial applications as natural food colorants. Master's thesis, University of Illinois, Urbana, Illinois.

Jiménez-Aguilar, D.M., Grusak, M.A., 2015: Evaluation of Minerals, Phytochemical Compounds and Antioxidant Activity of Mexican, Central American, and African Green Leafy Vegetables. Plant Foods Hum. Nutr. 70, 357-364. DOI: 10.1007/s11130-015-0512-7

Jiménez-Aguilar, D.M., Grusak, M.A., 2017: Minerals, vitamin C, phenolics, flavonoids and antioxidant activity of *Amaranthus* leafy vegetables. J. Food Compos. Anal. 58, 33-39. DOI: 10.1016/j.jfca.2017.01.005.

Jones, G.C., Hartley, E.S., 1999: A Protein Competition Model of Phenolic Allocation. Oikos 86, 27-44.

Kachiguma, N.A., Mwase, W., Maliro, M., Damaliphetsa, A., 2015: Chemical and Mineral Composition of Amaranth (*Amaranthus* L.) Species Collected from Central Malawi. JFR 4, 92. DOI: 10.5539/jfr.v4n4p92

Karamać, M., Gai, F., Longato, E., Meineri, G., Janiak, M.A., Amarowicz, R., Peiretti, Pier, Giorgio, 2019: Antioxidant Activity and Phenolic Composition of Amaranth (*Amaranthus caudatus*) during Plant Growth. Antioxidants (Basel) 8. DOI: 10.3390/antiox8060173

Kasozi, K.I., Namubiru, S., Safiriyu, A.A., Ninsiima, H.I., Nakimbugwe, D., Namayanja, M., Valladares, M.B., 2018: Grain Amaranth is Associated with Improved Hepatic and Renal Calcium Metabolism in Type 2 Diabetes Mellitus of Male Wistar Rats. Evid. Based Complement. Alternat. Med. 2018, 4098942. DOI: 10.1155/2018/4098942

Kim, D.-O., Chun, O.K., Kim, Y.J., Moon, H.-Y., Lee, C.Y., 2003: Quantification of polyphenolics and their antioxidant capacity in fresh plums. J. Agric. Food Chem. 51, 6509-6515. DOI: 10.1021/jf0343074

Lichtenthaler, H., 1987: Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods Enzymol. 148, 350-382.

Manyelo, T.G., Sebola, N.A., Mabelebele, M., 2020: Nutritional and Phenolic Profile of Early and Late Harvested Amaranth Leaves Grown under Cultivated Conditions. Agriculture 10, 432. DOI: 10.3390/agriculture10100432

Marcelis, L.F.M., 1996: Sink strength as a determinant of dry matter partitioning in the whole plant. J. Exp. Bot. 47 (Special_Issue), 1281-1291.

Maseko, I., Ncube, B., Mabhaudhi, T., Tesfay, S., Chimonyo, V., Araya, H.T., Fessehazion, M., Du Plooy, C.P., 2019: Nutritional quality of selected African leafy vegetables cultivated under varying water regimes and different harvests. S. Afr. J. Bot. 126, 78-84. DOI: 10.1016/j.sajb.2019.06.016

Mbwambo, O., Abukutsa Onyango, M.O., Dinssa, F.F., Ojiewo, C., 2015: Performances of elite amaranth genotypes in grain and leaf yields in Northern Tanzania. J. Hortic. For. 7, 16-23. DOI: 10.5897/JHF2014.0377

Moreno, M.D., Núñez-Farfán, J., Terrazas, T., Lucero del Mar, P., Ruiz, Trinidad-Santos, A., Carlos, L.T., Larque-Saavedra, A., 1999: Plastic responses to clipping in two species of *Amaranthus* from the Sierra Norte de Puebla, Mexico. Gen. Resour. Crop Evol. 46, 225-234.

Muanda, F., Koné, D., Dicko, A., Soulimani, R., Younos, C., 2011: Phytochemical composition and antioxidant capacity of three malian medicinal plant parts. Evid. Based Complement. Alternat. Med. 2011, 674320. DOI: 10.1093/ecam/nep109

Natasha, N., Shahid, M., Bibi, I., Iqbal, J., Khalid, S., Murtaza, B., Bakhat, H.F., Farooq, A.B.U., Amjad, M., Hammad, H.M., Niazi, N.K., Arshad, M., 2022: Zinc in soil-plant-human system: A data-analysis review. Sci. Total Environ. 808, 152024. DOI: 10.1016/j.scitotenv.2021.152024

Nawiri, M.P., 2018: Beta-Carotene Levels Increase with Leaf Maturity of *Amaranthus hybridus* (L.) Grown in Different Soil-Types of Kwale County, Kenya. EC Nutrition 13, 299-304.

Netshimbupfe, M.H., Berner, J., Gouws, C., 2022: The interactive effects of drought and heat stress on photosynthetic efficiency and biochemical defense mechanisms of *Amaranthus* species. Plant Environ. Interact. 3, 212-225. DOI: 10.1002/pei3.10092

Neugart, S., Baldermann, S., Ngwene, B., Wesonga, J., Schreiner, M., 2017: Indigenous leafy vegetables of Eastern Africa - A source of extraordinary secondary plant metabolites. Food Res. Int. 100, 411-422. DOI: 10.1016/j.foodres.2017.02.014

Neugart, S., Majer, P., Schreiner, M., Hideg, É., 2021: Blue Light Treatment but Not Green Light Treatment After Pre-exposure to UV-B Stabilizes Flavonoid Glycoside Changes and Corresponding Biological Effects in Three Different Brassicaceae Sprouts. Front. Plant Sci. 11, 611247. DOI: 10.3389/fpls.2020.611247

Nørbaek, R., Aaboer, D.B.F., Bleeg, I.S., Christensen, B.T., Kondo, T., Brandt, K., 2003: Flavone C-glycoside, phenolic acid, and nitrogen contents in leaves of barley subject to organic fertilization treatments. J. Agric. Food Chem. 51, 809-813. DOI: 10.1021/jf0258914

Ochieng, J., Schreinemachers, P., Ogada, M., Dinssa, F.F., Barnos, W., Mndiga, H., 2019: Adoption of improved amaranth varieties and good agricultural practices in East Africa. Land Use Policy 83, 187-194. DOI: 10.1016/j.landusepol.2019.02.002

Peiretti, P.G., Meineri, G., Longato, E., Tassone, S., 2018: Chemical Composition, In Vitro Digestibility and Fatty Acid Profile of *Amaranthus caudatus* Herbage During its Growth Cycle. Anim. Nutr. Feed Technol. 18, 107. DOI: 10.5958/0974-181X.2018.00010.0

Pospišil, A., Pospišil, M., Varga, B., Svečnjak, Z., 2006: Grain yield and protein concentration of two amaranth species (*Amaranthus* spp.) as influenced by the nitrogen fertilization. Eur. J. Agron. 25, 250-253. DOI: 10.1016/j.eja.2006.06.001

Rai, S., Singh, P.K., Mankotia, S., Swain, J., Satbhai, S.B., 2021: Iron homeostasis in plants and its crosstalk with copper, zinc, and manganese. Plant Stress 1. DOI: 10.1016/j.stress.2021.100008

Rodriguez-Amaya, B.D., Kimura, M., 2004: HarvestPlus Handbook for Carotenoid Analysis. HarvestPlus Technical Monograph 2, Washington, DC.

Roitner-Schobesberger, B., Kaul, H.-P., 2013: Source capacity during flowering affects grain yield of amaranth (*Amaranthus* sp.). Plant Soil Environ. 59, 472-477. DOI: 10.17221/528/2013-PSE

Sandoval-Sicairos, E.S., Milán-Noris, A.K., Luna-Vital, D.A., Milán-Carrillo, J., Montoya-Rodríguez, A., 2021: Anti-inflammatory and antioxidant effects of peptides released from germinated amaranth during in vitro simulated gastrointestinal digestion. Food Chem. 343, 128394. DOI: 10.1016/j.foodchem.2020.128394

Sarker, U., Oba, S., 2020: Nutritional and bioactive constituents and scavenging capacity of radicals in *Amaranthus hypochondriacus*. Sci. Rep. 10, 19962. DOI: 10.1038/s41598-020-71714-3

Schafleitner, R., Lin, Y., Dinssa, F.F., N'Danikou, S., Finkers, R., Minja, R., Abukutsa-Onyango, M., Nyonje, W.A., Lin, C., Wu, T., Sigalla, J.P., van Zonneveld, M., Hsiao, Y., Kumar, S., Wu, W., Wang, H.-I., Lin, S., Yang, R., 2022: The World Vegetable Center *Amaranthus* germplasm collection: Core collection development and evaluation of agro-

nomic and nutritional traits. Crop Sci. 62, 1173-1187. DOI: 10.1002/csc2.20715

Singleton, L.V., Orthofer, R., Lamuela-Raventós, M.R., 1999: Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299, 152-178.

Skwaryło-Bednarz, B., Krzepiłko, A., 2009: Effect of various doses of NPK fertilizers on chlorophyll content in the leaves of two varieties of amaranth (*Amaranthus cruentus* L.). Ecol. Chem. Eng. 16, 1373-1378.

Smith, J.D., Dinssa, F.F., Anderson, R.S., Su, F., Srinivasan, R., 2018: Identification of major insect pests of *Amaranthus* spp. and germplasm screening for insect resistance in Tanzania. Int. J. Trop. Insect Sci. 38, 261-273. DOI: 10.1017/S1742758418000115

Sosulski, W.F., Imafidon, I.G., 1990: Amino acid composition and nitrogen-to-protein conversion factors for animal and plant foods. J. Agric. Food Chem. 38, 1351-1356.

Stamp, N., 2003: Out of the quagmire of plant defense hypotheses. Q. Rev. Biol. 78, 23-55.

Stowe, A.K., Marquis, J.R., Hochwender, G.C., Simms, L.E., 2000: The Evolutionary Ecology of Tolerance to Consumer Damage. Ann. Rev. Ecol. Syst. 31, 565-595.

Tang, R.-J., Luan, S., 2017: Regulation of calcium and magnesium homeostasis in plants: from transporters to signaling network. Curr. Opinion Plant Biol. 39, 97-105. DOI: 10.1016/j.pbi.2017.06.009

Tang, Y., Li, X., Chen, P.X., Zhang, B., Hernandez, M., Zhang, H., Marcone, M.F., Liu, R., Tsao, R., 2014: Lipids, tocopherols, and carotenoids in leaves of amaranth and quinoa cultivars and a new approach to overall evaluation of nutritional quality traits. J. Agric. Food Chem. 62, 12610-12619. DOI: 10.1021/jf5046377

Vargas-Ortiz, E., Délano-Frier, J.P., Tiessen, A., 2015: The tolerance of grain amaranth (*Amaranthus cruentus* L.) to defoliation during vegetative growth is compromised during flowering. Plant Physiol. Biochem. 91, 36-40. DOI: 10.1016/j.plaphy.2015.03.007

Vargas-Ortiz, E., Espitia-Rangel, E., Tiessen, A., Délano-Frier, J.P., 2013: Grain amaranths are defoliation tolerant crop species capable of utilizing stem and root carbohydrate reserves to sustain vegetative and reproductive growth after leaf loss. PloS One 8, e67879. DOI: 10.1371/journal.pone.0067879

Zaynab, M., Fatima, M., Abbas, S., Sharif, Y., Umair, M., Zafar, M.H., Bahadar, K., 2018: Role of secondary metabolites in plant defense against pathogens. Microb. Pathog. 124, 198-202. DOI: 10.1016/j.micpath.2018.08.034

ORCID
Rachel Byarugaba https://orcid.org/0000-0001-6519-0317
Dorothy Nakimbugwe https://orcid.org/0000-0003-0801-1049
Susanne Huyskens-Keil http://orcid.org/0000-0002-7174-4176

Address of the corresponding author
Rachel Byarugaba, Division Urban Plant Ecophysiology, Faculty of Life Sciences, Humboldt Universität zu Berlin, Lentzeallee 55/57, 14195 Berlin, Germany
E-mail: rachel.byarugaba@hu-berlin.de, byarugabarachel@gmail.com