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The World Vegetable Center *Amaranthus* germplasm collection: Core collection development and evaluation of agronomic and nutritional traits

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Abstract

Amaranth (*Amaranthus* spp.) is an underutilized crop increasing in popularity as a grain and as a leafy vegetable. It is rich in protein, minerals, and vitamins, and adapts well to a range of production systems. Currently, the lack of improved cultivars limits the use of the crop. Breeding-improved cultivars requires access to large collections of amaranth biodiversity stored in genebanks. The task of searching such vast collections for traits of interest can be eased by generating core collections, which display the diversity of large collections in a much smaller germplasm set. The World Vegetable Center amaranth collection contains around 1,000 accessions of 13 species; among them, there are 281 accessions of four species important for use as vegetable amaranth in Africa (*A. cruentus*, *A. hypochondriacus*, *A. caudatus*, and *A. dubius*). Based on single nucleotide polymorphism (SNP) marker genotype diversity, a core collection (CC) of 76 accessions, cultivars, and selections was assembled. To a large extent, it represents the diversity of the whole collection. The CC was evaluated for yield and nutritional parameters during the cool and warm seasons in Tanzania and Taiwan and a pretest for variation of drought tolerance in

Abbreviations: CC, core collection; HQ, headquarters; SNP, single nucleotide polymorphism; WC, whole collection; WorldVeg, World Vegetable Center.

the CC has been performed. Cultivar Madiira 2, an improved cultivar developed for vegetable production in Africa, outperformed all other tested cultivars in terms of yield stability, but several CC accessions had higher yield, lower wilting score, and higher nutrient content than Madiira 2. This indicates the core collection can be used for further improvement of amaranth cultivars.

1 | INTRODUCTION

Several of the 40 to 50 species of the genus *Amaranthus* (Bonasora et al., 2013) are known as weeds, and Palmer amaranth is a particularly noxious one (Koo et al., 2018). But amaranth is also a food crop and is consumed in Africa and much of the world. It is cultivated for its grains (or rather for seeds, as amaranth is a pseudocereal), as well as for its leaves (vegetable use). Grain amaranth is an underutilized niche crop (Early, 1992; Sauer, 1967), whereas amaranth leaves are consumed in South and Southeast Asia, Brazil, Italy, Greece, the Caribbean, and the Pacific Islands (Singh & Whitehead, 1996). Amaranth is particularly popular as a traditional vegetable in sub-Saharan Africa (Ochieng et al., 2019; Oke, 1980). The usual separation of *Amaranthus* species into grain and vegetable types (Joshi et al., 2018) does not hold in Africa, where in addition to tetraploid *A. dubius* L., the leaves of grain type amaranths such as *A. hypochondriacus* L.*, A. caudatus* L., and *A. cruentus* L. are consumed as a vegetable. Due to its rapid growth and low production cost, amaranth is one of the cheapest leafy vegetables in tropical markets, yet profitable for small-scale producers. As a C4 plant it grows well during hot and dry summers, when other green vegetables become rare on the market (Singh & Whitehead, 1996). Amaranth leaves are rich in essential vitamins (pro-vitamin A, vitamin C) and minerals (calcium, Ca; iron, Fe; zinc, Zn; Chakrabarty et al., 2018; Sarkar et al., 2020) that are often low in local diets in Africa (Yang & Keding, 2009). The grain contains about 15% of high-quality, gluten-free protein with a balanced content of essential amino acids for human nutrition. Leaves, like the grains, are also a good source of Ca, magnesium (Mg), potassium (K), Fe, Zn (Bressani et al., 1993; Kachiguma et al., 2015; Mota et al., 2016), and antioxidants (Sarkar et al., 2020). Amaranth is a neglected crop species with high potential to contribute to the prevention of malnutrition, obesity, diet-related disorders, and hidden hunger (Pichop et al., 2014).

Amaranth breeding has mostly focused on grain types to improve yield, reduce plant height and seed shattering, and generate plants suitable for mechanical harvest (Das, 2016; Joshi et al., 2018). Vegetable amaranth breeding mainly targets cultivars of the species *A. tricolor* L. for the Asian market; much less effort has been made to improve *A. cruentus*, *A. dubius*, *A. caudatus*, or *A. hypochondriacus*, which are preferred as leafy vegetables in Africa (Dinssa et al., 2020; Shukla et al., 2004). Vegetable amaranth can be produced in short rotation by thin broadcast sowing and uprooting of about 3-wk-old plants, which avoids most pests and diseases. An alternative production method is repeated harvest of larger plants which increases yields per surface but makes the crop more susceptible to pests and diseases that require specific management methods (Nampeera et al., 2019) and generates demand for improved cultivars. Small-scale farmers increasingly seek dual use amaranth cultivars for multiple leaf collection during plant development and a final grain harvest (Hoidal et al., 2019). In collaboration with smallholder farmers in East Africa, the World Vegetable Center (WorldVeg) and partners have developed product profiles for improved cultivars. These cultivars were bred through selection from segregating accessions followed by cross breeding and were released in several countries in sub-Saharan Africa (Dinssa et al., 2020). Current amaranth breeding at WorldVeg focuses on rapid growth, taste, and nutritional quality of leaves of vegetable and dual use types, broad spectrum resistance to leaf diseases, and drought tolerance.

Breeding success depends on access to crop biodiversity. Amaranth biodiversity is maintained in several genebanks, and the largest amaranth ex situ germplasm collections are held by the North Central Regional Plant Introduction Station of USDA and by the National Bureau of Plant Genetic Resources in India (Genesys, 2021). The WorldVeg genebank currently holds a publicly accessible amaranth collection of more than 1,000 accessions of 13 species (https://genebank.worldveg.org/#/?filter=v2z1YE963mb&p=0); however, this collection is too large for small breeding programs to screen for traits of interest. Core collections (CCs) have been recognized as an efficient approach to promote the use of new biodiversity in breeding and research (van Hintum et al., 2000). Core collections display a large fraction of the diversity present in larger collections in a germplasm set small enough to be efficiently screened for favorable agronomic and nutritional traits by smaller breeding programs with limited funding. Here we present the development of a CC for four amaranth species. The variation in yield and nutrient levels found in this germplasm is described in comparison to current vegetable amaranth cultivars, with the aim to promote use of the CC to breed improved leafy and dual use amaranth cultivars specifically for sub-Saharan Africa.

2 | MATERIALS AND METHODS

2.1 | Plant material and genotyping

In 2018, out of 885 amaranth accessions available in the WorldVeg genebank, plants of 834 accessions were successfully grown in seedling trays. Leaf samples of two plants per accession were lyophilized and DNA was extracted using a column-based extraction kit (Favorgen). The DNA was provided to Diversity Array Technology for genotyping by sequencing and bioinformatic analysis using the Diversity Array Technology analysis pipeline, and around 76,000 single nucleotide markers (SNP) were obtained for 803 accessions and processed in Tassel 5 (Glaubitz et al., 2014). The raw reads of this genotyping effort are available in NCBI BioProject PRJNA734966. The dataset was filtered for ≤50% missing data and ≥0.25% minimum allele frequency. The species annotations of the amaranth accessions of the WorldVeg genebank were verified, and if necessary corrected (Lin et al., 2021). After taxonomic correction, in total 260 accessions and 21 subaccessions (resulting from the separation of segregating accessions into subaccessions) belonged to *A. hypochondriacus*, *A. cruentus*, *A. caudatus*, and *A. dubius*. Characterization data for 11 quantitative and 22 qualitative descriptors of the WorldVeg amaranth germplasm collected during seed regeneration over various years were obtained from the WorldVeg genebank (Lin et al., 2021; Supplemental Table S1). Characterization data were missing for 39 (sub)-accessions; for 16 accessions data of more than one season were available.

Core Ideas

- *Amaranthus* is a neglected crop with high nutritional value but limited access to improved cultivars.
- Breeding-improved amaranth cultivars requires access to trait variation.
- Core collections make it easier to search large genebank collections for desirable traits.
- An amaranth core collection will support breeding of vegetable and dual use (grain and vegetable) cultivars.
- The core collection contains accessions with superior yield, yield stability, and nutritional value.

2.2 | CC establishment, quality control, and phylogenetic and population genetic analysis

A CC representing the diversity of four amaranth species commonly used in Africa as vegetable and dual use types (*A. cruentus*, *A. hypochondriacus*, *A. dubius*, and *A. caudatus*) held by the WorldVeg genebank was established according to Odong et al., 2013. The genotypic data of the accessions were used to calculate a Euclidian distance matrix between accessions using the statistical analysis software R (https://www.r-project.org). An accession was randomly chosen, and all other accessions were grouped around it according to their genetic distance. This process was repeated for the remaining accessions until 70 groups for the planned size of the CC of about 70 accessions were established by iteratively adjusting the chosen radius. From these groups, a set of representative entries for the CC was created by minimizing the average distance between each of the accessions and its nearest entry (A-NE principle; Odong 2013). The core set was complemented by adding improved cultivars such as Madiira 1 and Madiira 2 (Dinssa et al., 2020) and breeding materials from the WorldVeg breeding program (Supplemental Table S1). A set of 1,860 markers with a minimum allele frequency of 1 and 0% missing data in the whole collection (WC) and CC was selected and used to calculate the Shannon's Diversity Index and Nei's expected heterozygosity in the WC and CC, as well as diversity in the various amaranth species of the WorldVeg genebank in PopGene (Yeh, 1999).

The representativeness of the CC on the phenotypic level was assessed by comparing the trait values in the WC and the CC by T-test and F-test, respectively, and mean and variance difference percentages, coincidence, and variable rate were calculated according to Hu et al. (2000). In addition, the percentage of categories of qualitative data found in the WC and maintained in the CC was calculated.

2.3 | Evaluation of agronomic and nutritional traits

The CC was evaluated over two seasons (warm and cool seasons) in the field at the WorldVeg Research Station in Arusha, Tanzania, and in the hot and rainy season at WorldVeg headquarters (HQ) in Taiwan (Shanhua, Tainan). In addition, a drought tolerance prescreening was done in the summer season in a screenhouse at WorldVeg HQ. Temperature data were received from weather stations at WorldVeg Tanzania and WorldVeg HQ, and during a gap in the records of the weather station at WorldVeg Tanzania, from worldweatheronline.com (Supplemental Table S2). Improved amaranth cultivars included in the core set were used as checks together with local cultivars as available (Supplemental Table S1). The agronomic evaluation in Africa and Asia applied local management conditions. In Africa, young leaves were harvested, whereas in Asia, after pinching the plants to stimulate side-shoot growth, young shoots were harvested. Under Asian conditions, harvesting was started 16 d after transplanting vs. 30

to 35 d after transplanting under African conditions. The harvesting intervals in Taiwan were shorter (about 7 vs. 14 d) and a sixth harvest was performed during the cool season in Taiwan (Supplemental Table S2). Due to phytosanitary and logistical reasons, not all accessions of the core set were available for the evaluations in Tanzania and Taiwan. The overlapping set across all trials consisted of 64 entries of the 76-accession CC (Supplemental Table S1).

The seed for the warm season field trial in Arusha, Tanzania (3.37˚ south, 36.8˚ east, elevation 1,235 m), was sown on 4 Nov. 2019 and seedlings were transplanted on 27 Dec. The cool season trial was sown on 15 May 2020 and transplanted on 15 June. Planting was done in a randomized complete block design with three replications in plots of 24 plants arranged in two rows with 60 cm between rows and 30 cm between plants. The area planted and harvested per plot comprised 6.48 m^2. Five harvests at 11- to 16-d intervals were performed (Supplemental Table S2). The data collected during harvest comprised number of leaves harvested per plot at each harvest, leaf yield per plot (g) at each harvest, leaf length and width (cm) from 10 randomly picked leaves from 10 random plants at first and last harvest, and number of branches per 10 random plants per plot at maturity stage. For nutrient analysis, at least 300 g of leaves were randomly sampled from the material harvested from multiple plants, weighed, and oven-dried. Weight was taken after harvest and after drying. The dried samples were shipped to WorldVeg HQ for nutrient analysis.

The yield trial during the warm season at WorldVeg HQ in Taiwan (23.12˚ north, 120.3˚ east, elevation 12 m) was performed under field conditions in a randomized complete block design with two replications. The commercial cultivars AM#53 and AM#74 and some WorldVeg genebank accessions of interest for breeding were used as additional checks (Supplemental Table S1). The seed was sown on 21 May 2019 in seedling trays in the greenhouse and seedlings were transplanted to the field on 15 July into double rows in 1.5- by 3.5-m plots, with 14 plants per plot. The plants were pinched on 29 July. Five harvests at intervals of six to nine days (Supplemental Table S2) were performed with a sickle. For comparisons of the yield with those of the other trials, the lower number of plants per plot (10 vs. 20) was taken into consideration.

The CC set tested during the warm season was also evaluated under spring (cool season) conditions at WorldVeg HQ, using the same field layout as the warm season trial. Instead of 14 plants per accession, 24 plants per plot were grown, using the same spacing as the warm season trial. Commercial cultivars AM#53 and AM#74 and WorldVeg breeding materials were used as checks (Supplemental Table S1). The seed was sown on 11 Feb. 2020, seedlings were transplanted on 5 Mar., the plants were pinched on 17 Mar., and six harvests were performed (Supplemental Table S2). Material from the second harvest was used for mineral and oxalate analysis.

For the dry down experiment, the CC set plus commercial cultivars AM#53 and AM#74 were planted in plastic trays with a soil depth of 23 cm in a randomized complete block design with six plants per tray and four replicated plants per accession in a screenhouse on 22 July 2019 at WorldVeg HQ. Irrigation was stopped in the drought treatment 21 d after after sowing (12 Aug. 2019). Wilting was scored on a scale from 0 (no wilting) to 6 (plant was dried out) 6, 9, and 12 d after withholding irrigation.

Amaranth leaves sampled from the warm and cool season trials at both locations were measured for mineral and antinutrient content (oxalate). For measuring oxalate content, 100 mg of dried leaf powder was defatted three times in 1.8 ml of hexane under shaking for 1 h. The suspensions were centrifuged at 13,000 rpm for 5 min, the supernatant was discarded, the pellets were rinsed and then suspended in 10 ml of distilled water, and 1.5 ml of the suspension was centrifuged at 13,000 rpm for 5 min and filtered through a membrane with 0.45-µm pore size. Twenty µl of the filtered extract was separated on a high performance liquid chromatography (HPLC; Waters Alliance 2695) using an ICSep ICE-ORH-801 organic acid column (0.65 by 30 cm, Transgenomic) and oxalate was quantified in comparison to a commercial standard using a photo array detector (Waters). The running conditions were set at 35 ˚C using isocratic HPLC with 0.01 N H_2SO_4 at 0.8 ml min^{-1}. Calcium, Fe, and Zn analysis was performed by ICP-OES. For this purpose, 0.2 g of dried sample powder was loaded into digestion tubes, 5 ml of H_2SO_4 was added and on the following day the samples were heated to 300 ˚C for 2 h, removed from the digestion tubes, and cooled down to 150 ˚C. Then 2 ml of 30% (v/v) H_2O_2 was added, and the samples were incubated in the digester at 300 ˚C for 1 h, cooled to 40 ˚C, filled up to 50 ml with distilled water, and the elements were determined on an 8000 ICP-OES (PerkinElmer).

3 | RESULTS

3.1 | Core collection

Genotyping by sequencing resulted in 76,420 SNP markers for 803 accessions of 13 species. Filtering the SNP sites for maximum missing data of ≤50% and a minimum allele frequency of ≥0.25% resulted in 27,540 polymorphic markers for the total genebank collection of 13 species. These markers were used to perform a diversity analysis on 281 accessions of the four *Amaranthus* species mainly used for breeding-improved vegetable and dual use cultivars in Africa: *A. cruentus*, *A. hypochondriacus*, *A. caudatus*, and *A. dubius*, to generate a CC of 70 accessions representing a maximum of the genetic diversity for this group. The CC consisted of a similar proportion of *A. hypochondriacus* and *A. cruentus* than the whole collection but contained a larger proportion of

TABLE 1 Quality assessment of the core collection (CC) based on quantitative phenotypic characteristics

Descriptor		Am110	Am220	Am240	Am250	Am290	Am300	Am400	Am410	Am420	Am460	Am540
t-test		.00**	.91	.02*	.11	.23	.60	.74	.54	.43	.92	.85
Mean	WC	2.80	127.89	42.38	22.84	16.77	9.37	46.59	27.20	16.41	14.56	0.42
	CC	2.52	128.82	33.07	18.95	17.66	9.19	47.43	26.18	15.22	14.73	0.43
Range	WC	12	280.8	137.5	112	33.8	26.55	117	68.61	42.3	38.8	1.36
	CC	5	264.6	107.4	73.3	20.7	12.8	85	48	39.4	38.3	0.99
F-test		.00**	.82	.13	.07	.37	.02*	.81	.01**	.92	.68	.33
CV	WC	.51	.48	.78	.83	.36	.34	.41	.56	.71	.61	.74
	CC	.37	.46	.86	.83	.31	.28	.39	.44	.75	.63	.65

Note. CV, coefficient of variation; WC, whole collection.
*Significant at the .05 probability level.
**Significant at the .01 probability level.

A. caudatus (16 vs. 8%) and a smaller proportion of *A. dubius* (18 vs. 29%), reflecting the intraspecific diversity of the germplasm. The CC was completed with important cultivars and selections, resulting in a CC of 76 accessions. The representativeness of this CC was verified by analyzing population genetic parameters, and on the phenotypic level, by comparing the trait diversity of 11 quantitative and 22 qualitative descriptors in the WC and the CC.

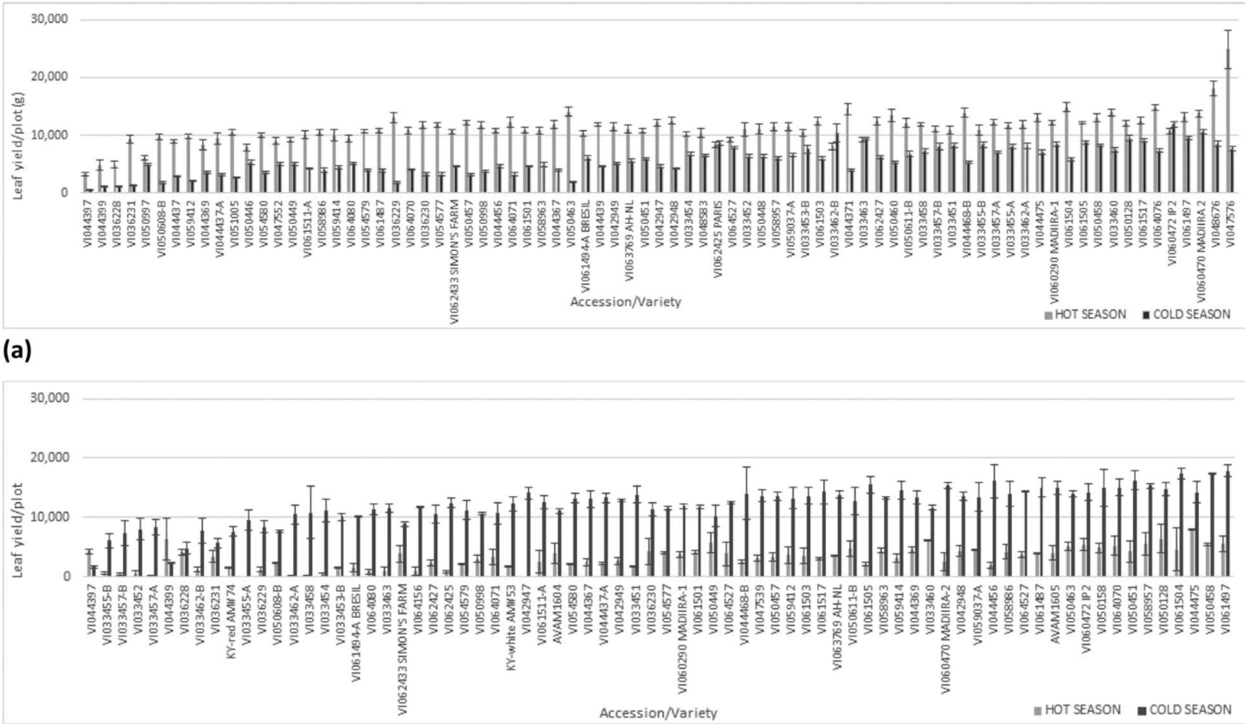
Conservation of the allelic diversity in the core collection was expected, as the method to generate the CC was based on selecting accessions representing the genetic diversity in the WC. Population genetic analysis confirmed that the reduction of the collection size from 281 accessions to a CC comprising about 30% of the WC resulted in similar Shannon's Diversity Index (WC: .22, CC: .25) and Nei's expected heterozygosity (WC: .15, CC: .14), suggesting the genetic diversity of the WC was well maintained in the CC.

A major indicator for the quality of a CC is the representation of the phenotypic diversity of the WC. The difference of the phenotypic means between the WC and CC was insignificant ($p = .93$). Values were significantly different between WC and CC for only two (Am110 - days to germination and Am240 - mean length of basal lateral branches) out of the 11 quantitative traits (Table 1). The coincidence and variable rates amounted to 73 and 93% and were in the acceptable range for core collections (Hu et al., 2000). According to F-test, there was a significant decrease of variance for three quantitative traits in the CC: number of days to germination (Am110), leaf width (Am300), and terminal inflorescence stalk length (Am410; Table 1). Accessions with a longer germination period (>6 d), narrower and wider leaves (<3.9 cm and >16.7 cm), as well as longer terminal inflorescence stalk (>49 cm) were not represented in the CC. Variation in plant height (Am220), terminal lateral inflorescence length (Am420), and length of lateral inflorescence (Am460) were particularly well maintained in the CC. From the 22 qualitative descriptors in a total of 93 categories describing the form and color of plant organs, 84 categories were noted in the WC of the four amaranth species, and 67 (80%) of these categories were maintained in the CC. Categories absent in the CC concerned very slow germination rate and the presence of spines in the leaf axils. There was less diversity of terminal inflorescence shapes and leaf, petiole, stem, and seed pigmentation in the CC than in the WC.

Compared with the WC and CC, the seven cultivars and selections included in the CC, on average, had a shorter germination period (Am110), shorter mean length of basal lateral branches (Am240), and shorter axillary inflorescences (Am460) than the mean of the CC, but were on average higher (Am220), had larger leaves (Am290), were flowering later (Am400), had longer terminal inflorescences (Am420), and a larger 1,000 seed weight (Supplemental Table S1). The averages of the top lateral branch length (Am250), leaf width (Am300), and terminal inflorescence stalk length were in the range of the averages of the CC. For traits with higher means in cultivars, CC accessions with even larger trait values than the greatest value in the cultivars were available, except for larger leaves (Am290). However, there were accessions with still larger leaves available in the WC (Supplemental Table S1).

3.2 | Agronomic evaluation of the CC

The CC was evaluated for yield in the warm and cool seasons in Arusha, Tanzania, and in Shanhua, Taiwan. The trials were done under local cultivation conditions and local harvesting methods were applied. The warm season trial in Africa was not affected by pests and diseases, whereas in the cool season, about one third of the plots were very mildly affected, 4% of the plots were mildly affected by the insect pest *Hymenia recurvalis*, and one fifth of the plots were very mildly affected by an unidentified fungal disease. No diseases or pests were recorded in the trials in Taiwan. The differences of the minimum, maximum, and average temperatures between the warm and cool season trials in Tanzania were



(b)

FIGURE 1 Leaf yield per plot (20 plants) in Arusha, Tanzania (a) and World Vegetable Center headquarters in Taiwan (b). The highest yields were obtained in Tanzania during the warm season and in Taiwan during the cool season

3, 5.6, and 2.9 ˚C, respectively. In Taiwan, the differences between the minimum, maximum, and average temperatures in the cool and warm season trials were 7.8, 4.8, and 6.3 ˚C (Supplemental Table S2). Among all trials in this study, the cool season trial in Taiwan and the warm season trial in Tanzania showed the most similar temperature profiles. The minimum temperatures in the cool season trial in Taiwan were on average 0.9 ˚C warmer than in the warm season trial in Tanzania, whereas the maximum and average temperatures were on average 2.2 and 0.7 ˚C higher in the warm season in Tanzania than in the cool season in Taiwan, respectively.

Leaf yield varied among entries, seasons, and locations, but was significantly positively correlated between all trials except for the warm season trial in Taiwan (Figure 1a and 1b; Table 2). Largest yields were achieved in the cool season in Taiwan and in the warm season in Tanzania, whereas during the warm season in Taiwan the yields were lowest. Average yield reduction of the germplasm set in the Taiwanese warm season was 73% compared with the cool season. In Tanzania, the yield reduction in the cool season compared with the warm season was 48%. The largest yields across environments and seasons were obtained with accession VI044475 (*A. dubius*), selection IP-2 AVAM1602 (VI060472. *A. cruentus*), VI061497 (*A. dubius*), and VI050128 (*A. hypochondriacus*). Cultivar Madiira 2 (VI060470, *A. cruentus*) was under the best performers and ranked sixth for yield across all trials. The best performers in Tanzania in terms of leaf yield over the warm and cool seasons were cultivar Madiira 2 (VI060470), accession VI061497, and selection IP-2 AVAM1602 (VI060472). Also in Taiwan, when considering both seasons, accession VI061497 was among the best performers, together with VI050458 and VI044475, whereas Madiira 2 ranked eighth for combined yield of both seasons. Under the hot conditions of the warm season trial in Taiwan, accessions VI044475 and VI050128 yielded best, with about 50% of the yield of the cool season trial. Under cold conditions of the cool season trial in Tanzania, selection IP2 AVAM1602 (VI060472) yielded even more than in the warm season, and cultivar Madiira 2 (VI060470) had only 22% yield loss

TABLE 2 Correlations between yields at Harvests 1–5 (H1–H5) with leaf length and width

Season	H1	H2	H3	H4	H5
Arusha warm season					
Leaf length	.13*	.1	.16*	.35**	.58**
Leaf width	.19*	.12	.16*	.39**	.52**
Arusha cool season					
Leaf length	−.01	.41**	.19**	.23**	.12
Leaf width	.01	.32**	.08	.047	.09

*Significant at the .05 probability level.
**Significant at the .01 probability level.

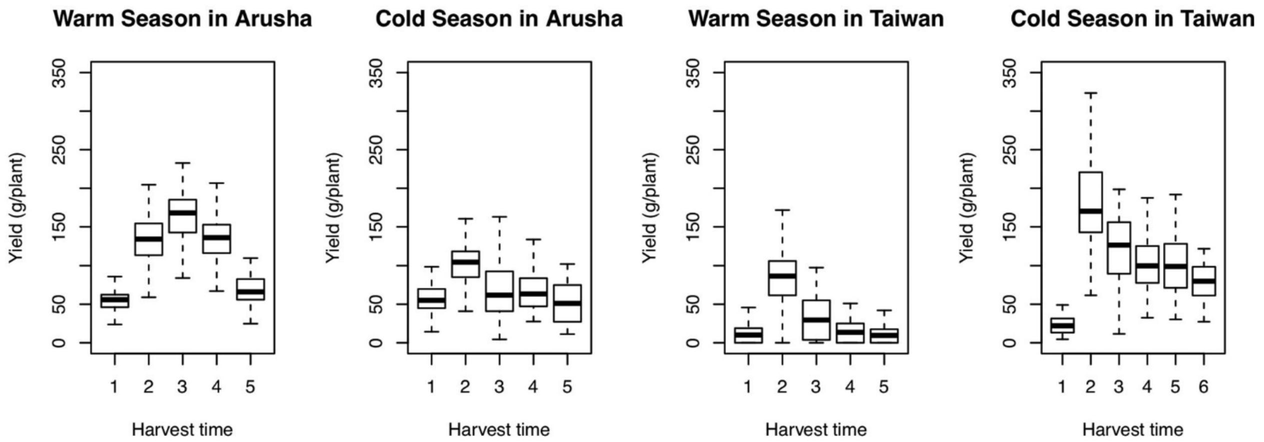


FIGURE 2 Average yields per harvest in the warm and the cool seasons in Tanzania (Arusha) and Taiwan in g plant^{-1}. Harvesting time refers to the first to the sixth harvest (Supplemental Table S2)

compared with the warm season trial. Local Taiwan cultivars KY-white and KY-red had low yields in both the warm and cool season trials in Taiwan (Figure 1b).

Yields of subsequent harvests in Tanzania increased over time during the warm season and only dropped at the fifth harvest (Figure 2). During the cool season in Tanzania, the yields per harvest were lower than in the warm season, and for many accessions no harvest could be obtained at the fifth harvest. In Taiwan, all harvests during the warm season were much smaller than in the cool season. In the cool season of Taiwan, a sixth harvest was possible increasing the yields per plant per season (Figure 2).

In Tanzania, during the warm season, correlation between yield and leaf length and width increased at later harvests (Table 2). In the cool season trial, yield of the second harvest was strongly correlated to leaf length and width; in subsequent harvests, this correlation decreased. In Harvests 3 and 4, only leaf length remained significantly correlated with yield, indicating other factors than leaf morphology contributed yield in later harvests of the cool season trial. There was no significant association detectable between insect pest or disease incidence with yield. In the trials in Taiwan, the available data on leaf size were not sufficient to allow determination of correlations with yields.

3.3 | Evaluation of dry matter, oxalate, and mineral content in the CC

Dry matter, as well as mineral concentrations (Ca, Fe, and Zn) and oxalate were measured in edible plant parts harvested during the yield trials in Tanzania and Taiwan. The variation found in the CC for dry matter content was 1.5-fold at each site and season, and for oxalate and Ca about two-fold. For Fe, the variation in the germplasm set was the highest in the warm season in Tanzania and the lowest in the warm season in Taiwan. Overall, average Fe contents were higher in the trials in Tanzania than in Taiwan. Average Zn contents were similar across regions and seasons, but the variation in the germplasm was the highest in the cool season in Tanzania (Table 3). The highest Zn concentrations were found in cultivar Simon's Farm (VI062433) across all environments. Ca contents were on average higher in the cool than in the warm season at both locations. The average concentration of oxalate was the lowest during the warm season in Taiwan, but significantly higher during the cool season ($p < .01$), whereas in Arusha the oxalate concentration difference across seasons remained insignificant. Correlations between yield and oxalate accumulation were significant in the cool season trials in Tanzania and Taiwan, where high yielding accessions showed lower oxalate accumulation. No significant association between oxalate and mineral accumulation was found except for the cool season trial in Taiwan, in which oxalate levels were correlated with Ca and Zn levels. Association between Ca and Fe contents were significant across seasons and environments (Table 4). Figure 3 illustrates the differences in yield and concentrations of oxalate, Ca, Fe, and Zn among the trials in the cool and warm seasons in Taiwan and Tanzania. Overall, the analysis suggests that in terms of yield, the accessions behave quite similarly in the warm season in Tanzania and the cool season in Taiwan ($r = .62, p < .01$, Figure 3, Supplemental Table S3). The response of accessions in terms of oxalate and Fe accumulation is similar across seasons and environments except for Tanzania in the warm season, and for Ca across the cool seasons at both locations.

3.4 | Variation in drought tolerance

A pretest assessing wilting of CC accessions upon withholding irrigation revealed variation among the germplasm (Figure 4). No significant correlation between morphological

TABLE 3 Minimum (Min.), average, maximum (Max.), and coefficient of variation (CV) values for yield, dry matter (DM), oxalate (Ox), and calcium (Ca), iron (Fe), and zinc (Zn) in edible plant parts harvested from the amaranth core collection grown in Arusha, Tanzania, and Shanhua, Taiwan

Variable	Yield	DM	Ox	Ca	Fe	Zn	Yield	DM	Ox	Ca	Fe	Zn
	g plot^{-1}	%		mg 100 g^{-1} fresh weight			g plot^{-1}	%		mg 100 g^{-1} fresh weight		
	Shanhua, Taiwan, warm season						**Shanhua, Taiwan, cool season**					
Min.	130	10.1	180.8	226.7	2.8	0.4	1,670	9.8	250.4	362.6	2.1	0.3
Max.	7,966	15.0	382.1	653.8	5.8	1.4	17,820	16.0	618.4	773.3	7.7	1.9
Average	3,247	12.8	261.6	402.9	4.0	0.7	11,965	13.1	375.2	529.2	4.0	0.6
SD	1,816	1.0	45.1	89.4	0.7	0.2	3,323	1.4	66.2	93.4	1.0	0.3
CV	55.9	7.9	17.2	22.2	17.6	27.5	27.8	10.6	17.6	17.6	25.8	44.2
	Arusha, Tanzania, warm season						**Arusha, Tanzania, cool season**					
Min.	3,213	10.8	272.7	356.7	5.9	0.4	530	11.7	300.5	364.5	5.9	0.3
Max.	14,873	15.9	515.0	766.0	26.4	1.6	11,879	18.9	555.2	847.3	15.2	2.4
Average	10,956	13.5	386.4	477.2	9.8	0.6	5,648	13.8	401.5	580.2	9.0	0.6
SD	2,056	0.9	56.1	75.9	3.6	0.2	2,601	1.4	56.9	111.9	2.0	0.4
CV	18.8	6.9	14.5	15.9	37.1	30.5	46.0	10.0	14.2	19.3	22.5	61.5

TABLE 4 Correlations (r) between yield, dry matter (DM), and nutritional parameters such as oxalate (Ox), calcium (Ca), iron (Fe), and zinc (Zn) during the warm and the cool season in Tanzania and Taiwan

Variable	DM	Ox	Ca	Fe	Zn	DM	Ox	Ca	Fe	Zn
	Tanzania warm season (n = 78)					**Tanzania cool season (n = 78)**				
Yield	.11	.01	−.13	−.19	.01	−.23	−.36**	−.60**	−.21	−.08
DM		.10	.56**	−.03	.34**		.31**	.57**	.45**	.20
Ox			.05	.06	.01			.18	−.19	.22
Ca				.25*	.07				.53**	.18
Fe					.17					.13
	Taiwan warm season (n = 69)					**Taiwan cool season (n = 69)**				
Yield	−.03	.14	.37**	.04	.49	−.12	−.34**	−.22	.08	−.33**
DM		−.12	.64**	.59**	.33**		.16	.65**	.35**	.00
Ox			−.05	.02	−.03			.31*	−.09	.24*
Ca				.69**	.61**				.25*	.28*
Fe					.32**					−.20

*Significant at the .05 probability level.
**Significant at the .01 probability level.

features such as plant height, leaf length or width, and wilting under drought could be detected, indicating traits other than leaf morphology contributed to reduced wilting. However, wilting scores on Days 6 and 9 were positively correlated with yield ($r = .31$, $p < .05$), indicating high yielding accessions wilted earlier than low yielding accessions. On Day 12 after withholding irrigation, no association between wilting under drought and yield under normal conditions was detected. The most drought susceptible accessions belonged to *A. dubius*, and the most tolerant to *A. caudatus* and *A. cruentus*. Wilting scores of the amaranth cultivars ranged around the wilting score average of the germplasm panel, except Madiira 2 which showed relatively low wilting under drought stress. Likewise, the local checks AM#53 and AM#74 were relatively tolerant to drought.

4 | DISCUSSION

Traditional vegetables such as amaranth have the potential to contribute essential nutrients to diets and generate income for farmers (Mwadzingeni et al., 2021), but productivity is constrained due to a lack of access to quality seed (Ndinya et al., 2020). A few improved amaranth cultivars were

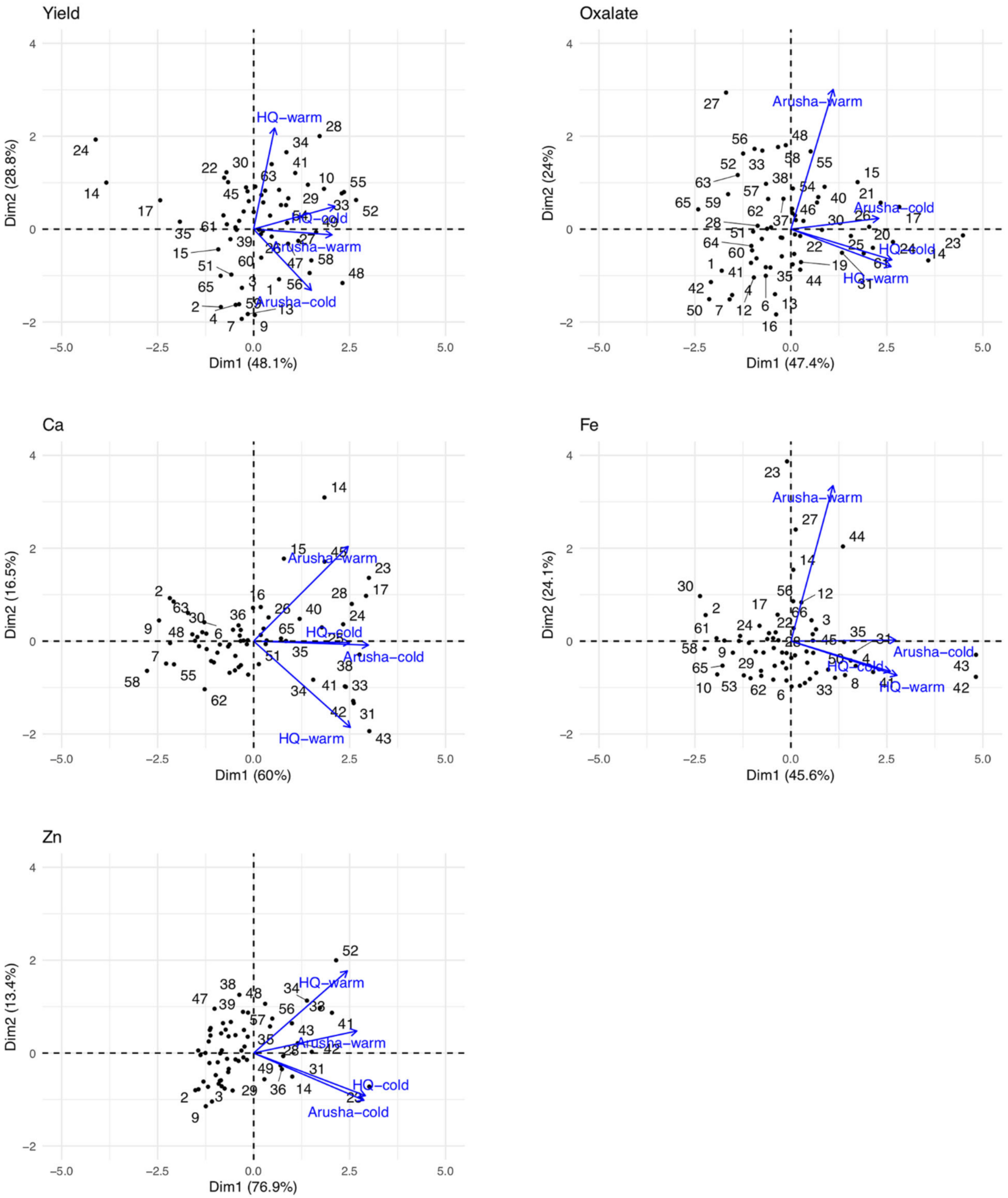


FIGURE 3 Principal component analysis of yield (a) and concentrations of (b) oxalate. Calcium (Ca; c), iron (Fe; d), and zinc (Zn; e) in the core collection (CC) accessions across environments World Vegetable Center (WorldVeg) headquarters (HQ) and WorldVeg Tanzania (Arusha). The CC accessions are numbered according to Supplemental Table S1

introduced and were well adopted by farmers, but more cultivars, especially those with tolerance to biotic stress, are required (Ochieng et al., 2019). Developing improved cultivars with farmer-requested traits is important to meet the contemporary challenges of improving human nutrition and agricultural sustainability (Sequeros et al., 2021). A crucial requirement for developing improved cultivars is access to genetic variation of traits for breeding. Genebanks worldwide conserve the diversity of crops and crop wild relatives and serve as a source of germplasm and traits for crop

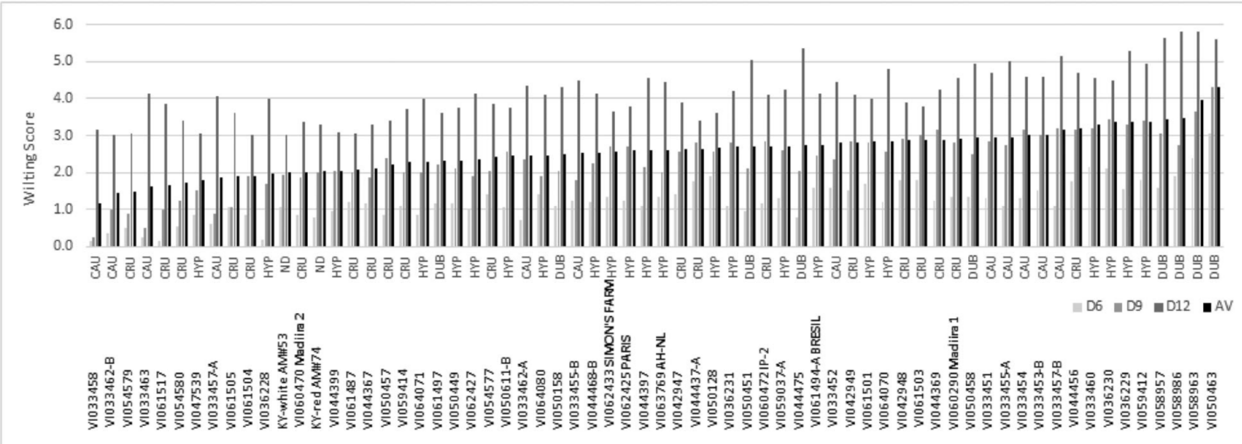


FIGURE 4 Drought tolerance pretest. Wilting was scored from 0 (no wilting) to 6 (plant was dried out). CAU, *A. caudatus*; CRU, *A. cruentus*; DUB, *A. dubius*; HYP, *A. hypochondriacus*; ND, not determined; D6, D9, and D12: Day 6, 9, 12 after withholding irrigation; AV, average of D6, D9, and D12

improvement (Ebert, 2020). Screening large collections for traits of interest can be laborious, especially when multilocation evaluation of the material for multiple traits is involved. Core collections represent a large portion of the diversity present in large collections. These relatively small germplasm sets can be screened for traits of interest at lower cost (van Hintum et al., 2000). Access to diversity at lower cost is particularly important for small breeding programs with limited budgets. A CC was developed for amaranth, a crop species with extraordinary potential to diversify agricultural production and contribute a highly nutritious grain and a nutritious leafy vegetable to human diets. Although the size of the CC was minimized for easy screening, the population genetic parameters and the conservation of a large part of the trait variation present in the WC indicate the CC represents a large portion of the diversity of the WorldVeg germplasm collection of the four species mostly used for breeding amaranth cultivars for Africa: *A. cruentus*, *A. hypochondriacus*, *A. caudatus*, and *A. dubius*. For all analyzed traits except for leaf size, the CC provides variation beyond the one found in current cultivars. Current breeding aims for vegetable amaranth with high leaf yields, delayed flowering, tolerance to drought and heat stress, good taste, and high nutrient content (Adeniji & Aloyce, 2013; Joshi et al., 2018). Variation has been found in the CC for most of these traits. Accessions with higher yields, tolerance to drought, and higher nutrient content than current cultivars have been identified, suggesting that further improvement of cultivars using the CC as a source for new traits should be possible. However, taste was not evaluated in the CC. Previous research indicated *A. dubius* cultivars are often preferred for the taste of their leaves (Ochieng et al., 2019), whereas other research found no association between specific species and sensory properties, as cultivars of the same species had partly showed contradictory sensory properties (Hiscock et al., 2018). In South Africa, mild taste was preferred (Mncwango et al., 2021), but more organoleptic analyses are required to identify preferred tastes of vegetable amaranths across regions and consumer groups.

Amaranth is rich in minerals like macro- and microelements, such as nitrogen (N), P, K, Ca, Mg, Fe, manganese (Mn), copper (Cu), sodium (Na), and Zn (Chakrabarty et al., 2018; Sarkar et al., 2016); high quality protein with essential amino acids (Sakar et al., 2014); and vitamins (Sarkar et al., 2015). One breeding target could be to improve the nutrient content of the grain and the leaves even further. However, the grain also can contain variable amounts of antinutrients such as phytate, saponins, tannins, oxalates, and small amounts of protease inhibitors, and the leaves contain oxalate and small amounts of saponins and nitrates (Aderibigbe et al., 2020). The effects of oxalates and phytates in inhibiting nutrient uptake have raised concerns when promoting consumption of leafy vegetables. Processing can lower antinutrient contents of amaranth (Babatunde & Gbadamosi, 2017; Njoki, 2015) and cooking methods have been developed to lower the effect of antinutrients and enhance nutrient uptake (Nyonje et al., 2021). The antinutrient concentration also could be lowered by breeding, but this could lead to a reduction of antimicrobial or insecticidal activities in the plant (Soetan, 2008) and result in cultivars more susceptible to insect pests and diseases.

Oxalate and Ca concentrations in amaranth were correlated in the cool season in Taiwan. A correlation between Ca and oxalate would not be surprising, as a part of the Ca in the cell is present in the form of Ca oxalate crystals. Oxalate has been reported to be synthesized from glycolate, and to a lesser extent from L-ascorbic acid in spinach (*Spinacia oleracea*) leaves (Fujii et al., 1993). Calcium oxalate crystal formation in plants has been attributed various roles, including tissue Ca regulation, defense against herbivory, and metal detoxification (Franceschi & Loewus, 1995; He et al., 2013). Tooulakou et al. (2016), Nakata (2012), and Webb (1999;

2019) hypothesized Ca oxalate crystals function as carbon stores that can be activated under drought conditions, when stomata are closed. Tooulakou et al. (2016) demonstrated that partial stomatal closure during the day is accompanied by a gradual decrease of the Ca oxalate crystal volume which is recovered during the night, confirming that Ca oxalate crystals are indeed a dynamic system. The reduction of the Ca oxalate crystal amounts during "alarm photosynthesis," when stomata are closed, has been shown in amaranth (*A. hybridus*), but also in C3 plants and in the CAM plant *Portulacaria afra*, suggesting Ca oxalate may be used as a carbon store in many plant families. Amaranth, as a C4 species, reduces photorespiration by taking up CO_2 in the darkness when transpiration rates are low, and stores it as malate which can be transported to the bundle sheet cells during the day where photosynthesis is taking place. This minimizes interference from oxygen, thereby avoiding carbon loss through photorespiration. Accumulation of Ca oxalate crystals may be an additional mechanism to allow for photosynthesis while stomata remain closed, resulting in a better adaptation of the plant to stressful conditions. Taking into consideration the relatively low variation of oxalate content among amaranth genotypes and the putative importance of oxalate in stress tolerance, it may be favorable to consider preparation methods that minimize the antinutritive effects of Ca oxalate rather than select cultivars with low oxalate concentrations. Interestingly, on average the lowest oxalate levels were found during a dry period of the warm season trial at WorldVeg HQ, where transpirational demand likely was the highest compared with the other trials. Additional research is required to verify diurnal changes of oxalate concentration in vegetable amaranth, which could lead to the introduction of harvesting methods to minimize the oxalate content in the produce.

The agronomic evaluation in Tanzania and in Taiwan over two seasons showed the germplasm panel performs the best at an average temperature around 21 ˚C with minimum temperatures around 15˚C and a maximum around 30 ˚C. Reduction of the temperature of on average 3 ˚C leads to yield losses of in average 50% whereas a rise of the average temperatures by 6 ˚C and of the minimal temperatures by 7 to 8 ˚C causes yield losses of more than 70% on average. The warm season in Tanzania and the cool season in Taiwan were the most appropriate growing seasons for the amaranth germplasm panel. Some accessions like VI044475 and VI050128 produce acceptable yield of around 50% of their yield potential under optimal conditions, also under hot conditions in Taiwan, whereas in Tanzania selection IP-2 AVAM1602 (VI061472) and cultivar Madiira 2 (VI060470) had greater or nearly as much vegetable yield during the cool season as in the warm season. No reliable precipitation data were available for the field trials in Tanzania, but data from a weather station in the region indicates that on most days of the warm season trial was at least a little rain, whereas during the cool season trial, most days were without precipitation, except for the last 2 wk, where a few rainy days were recorded. The relatively warm weather combined with light rain in the warm season apparently generated overall better conditions for amaranth growth than the dry and cooler climate of the cool season trial in Tanzania. The insect pest and disease incidence in the field during the cool season was very low and did not significantly damage the plants. The greatest differences in yield between the warm and the cool season appeared in the later harvests, and many accessions did not yield at the fourth and fifth harvest.

The demand for amaranth is rising, generating opportunities for farmers to market additional grain and vegetable amaranth. For example, in Kenya maize (*Zea mays*) flour is fortified with milled amaranth seed to increase protein, Fe, and Zn content (Kamotho et al., 2017) which increases demand for amaranth grain. Amaranth grain is also promoted as functional food (Martinez-Lopez et al., 2020), used as a no-gluten alternative to cereals in bakeries (Gebreil et al., 2020), for beverages (Manassero et al., 2020) including beer (Yang & Gao, 2020), and to extract a high quality oil for cosmetics (Huang et al., 2009) and food applications (Becker, 2018), to mention just a few current uses. Vegetable amaranth is increasingly popular in Asia and Africa, but vegetable amaranth marketing faces limitations in Africa that restrict the economic benefits for farmers (Dizyee et al., 2020). Dual-use cultivars for harvesting leaves multiple times during the growing season and for a final grain harvest could contribute to satisfy the rising demand for amaranth grain and leaf while allowing farmers to generate income with two products instead of one (Dinssa et al., 2018). Through good choice of harvesting intensity, cultivar, and environment, the grain yield penalty due to leaf harvest can be minimized (Hoidal et al., 2019). Amaranth cultivars varied in grain yield in response to defoliation. Entries PARIS (AVAM1606) and BRESIL (AVAM1607) produced high grain yields only if plants were not defoliated; entries IP-5-Sel, SIMON'S FARM (AVAM1601) and Madiira 2 yielded ample leaves but relatively little grain; and entries such as AH-TL-Sel, AH-NL (AVAM1603), TZSMN102-Sel, and 'Mchicha' yielded moderate amounts of grain and leaf (Dinssa et al., 2018). We suggest using the CC to screen for genotypes with vigorous early leaf growth, late flowering, and strong seed set as candidates for dual use cultivars.

As C4 plants, amaranth can efficiently tolerate abiotic stresses such as heat (Hwang et al., 2018) and drought (Sarkar & Oba, 2018a). In addition, amaranth is relatively tolerant to salinity stress (Sarkar & Oba, 2018b). Both drought and salinity stress cause oxidative damage (Sarkar & Oba, 2018c), and the presence of both antioxidant enzymes and nonenzymatic antioxidants may contribute to the detoxification of reactive oxygen species (Sarkar & Oba, 2020). Moreover, the high drought and salt tolerance of amaranth is associated with a low basal stomatal conductance due to a low number of stomata and low degree of stomata aperture, supported by the C4 metabolism, which contributes to avoid water loss under osmotic stress (Estrada et al., 2021). The relative osmotic stress tolerance makes amaranth an option for horticultural production in dry environments, and makes it suitable for use in various other environments and production systems, including urban agriculture. Nevertheless, the number of cultivars for grain and vegetable production available for farmers is limited, which warrants investment in breeding.

Multilocation trials demonstrated that amaranth leaf yields are strongly affected by the environment. Both trial locations were located in the tropics with small day length variation over the year, suggesting variation in day length had only a minor effect on yield, if at all. But the yield comparisons in the present study between sites in Tanzania and Taiwan were not only influenced by climatic conditions. The cultivation methods were also different. Taiwan farmers prefer harvesting from younger plants and harvest young shoots in shorter intervals, whereas farmers in Tanzania harvest leaves at about 2-wk intervals. Therefore, the evaluation of the accessions not only accounted for different environments and seasons, but also for different local cultivation practices. The WorldVeg developed improved cultivar Madiira 2 stood out in the warm and cool season trials in Tanzania and in the cool season trial for relatively high yield, ranking near the top among all accessions. Wilting under drought was delayed in this cultivar, but the mineral content of this line was not above average. As yield and Fe and Ca content are not associated during the seasons when amaranth provides high yields, an increase of Ca and Fe should be possible without negatively affecting yield.

The average Fe amounts of 100-g amaranth leaves harvested in the trials in Tanzania exceed the recommended daily Fe intake for women at reproductive age of 14.8 mg (NHS, 2021). In Taiwan, the Fe levels in amaranth leaves were half as high, probably due to differences in Fe concentration and/or availability in the soil. For Ca, 100-g fresh amaranth leaves contain on average half of the daily recommended dose of 800 mg. The content of Zn in amaranth leaves on average of 0.6 mg per 100 g fresh weight is too low to provide a significant contribution to the 9.5 mg required by humans per day. The proportion of the Fe and Ca in amaranth leaves, which is actually bioavailable for humans, depends on multiple factors including the presence of antinutrients and the preparation method (Yang & Tsou, 2006). High contribution of environmental conditions to mineral content suggests that breeding for high mineral content is likely less effective. Variable micronutrient bioavailability likely can be addressed by improved preparation methods (Nyonje et al., 2021). Overall, the high mineral content, especially for Fe and Ca, makes amaranth leaves particularly useful for populations affected by micronutrient deficiencies.

5 | CONCLUSION

A CC representing the genetic and phenotypic diversity of amaranth species with great importance in Africa held by the WorldVeg genebank was produced with the intention to facilitate access to the biodiversity of this crop for breeding-improved vegetable and dual use amaranth cultivars. Analysis of available descriptor data of the CC and WC corroborated that the CC represents a large part of the diversity of the WC. The CC was completed by including cultivars and selected lines. Evaluation of the core collection over two seasons in two environments identified accessions and cultivars in the CC with high and stable yields. The most stressful environment in which the CC was tested in this study was the warm season in Taiwan, where hot temperatures limit crop productivity; nevertheless, several genebank accessions were identified that yielded under these stressful conditions. Likewise, amaranth lines yielding relatively well in both the cool and warm season in Tanzania were identified. Yield and content of Fe and Ca were not associated, suggesting improvement of both traits is possible without yield or nutrient penalty. The CC, but also all accessions of the WC, are made available by WorldVeg to the public under a standard material transfer agreement for breeding, research and training for food and agriculture purposes (https://avrdc.org/our-work/managing-germplasm/).

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AUTHOR CONTRIBUTIONS

Roland Schafleitner: Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Writing – original draft. Ya-ping Lin: Data curation; Investigation; Methodology; Software; Validation; Visualization. Sognigbé N'Danikou: Data curation, Investigation. Fekadu Fufa Dinssa: Conceptualization; Data curation; Investigation; Methodology. Richard Finkers: Investigation; Software. Ruth Minja: Investigation. Mary Abukutsa-Onyango: Investigation.

Winnie Nyonje: Investigation. Chen-yu Lin: Investigation. Tien-hor Wu: Data curation; Investigation. Jeremiah Phanuel Sigalla: Investigation. Maarten van Zonneveld: Investigation. Yun-yin Hsiao: Investigation. Sanjeet Kumar: Investigation. Wan-jen Wu: Investigation. Hsin-I Wang: Investigation. Shou Lin: Investigation. Ray-yu Yang: Investigation.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

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