Qualitative morphological diversity of *Amaranthus* species

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Received 22 June 2017; received in revised form 28 June 2017; accepted 30 June 2017

Abstract

Morphological characterization of plant genetic resources generates important information for plant breeders useful for pre-breeding and breeding programmes of crops. *Amaranthus* is one of the underutilized and genetically potential orphan crops grown in tropical and sub-tropical regions of the world. It is a food security crop that can alleviate malnutrition and generate income for the rural communities in South Africa. A number of *Amaranthus* species have been collected from different regions in the world and conserved in the gene bank of the Agricultural Research Council, Pretoria, South Africa. The objective of the study was to assess the genetic diversity of these conserved *Amaranthus* species using qualitative morphological characters. Thirty two species of *Amaranthus* were evaluated for 16 qualitative morphological characters in the field using a randomized complete block design with three replications. The frequencies for each qualitative character were tabulated. The Shannon Weaver diversity index ($H^{'}$) was calculated and the result revealed a low to high diversity among the collection regions for the traits. The result of the study showed that the $H^{'}$ for all the species varied from 0.28 to 0.70, indicating the existence of a wide genetic diversity among species evaluated. The information obtained in this study could be used for the genetic improvement of *Amaranthus* species in South Africa for the development of cultivars.

Keywords: Amaranthus, Qualitative traits, Shannon Weaver diversity index

Introduction

Amaranthus species also known as *Amaranths* is one of the underutilized orphan crops grown in tropical and sub-tropical regions of the world. This genus belongs to the family Amaranthaceae that originates in South America (Janovska et al., 2012) and consist of approximately 70 species (Espitia-Rangel, 1994; Ebert et al., 2011) which are cultivated as leafy vegetables, grains and ornamental crops in different parts of the world. The grain types include *A. hypochondriacus*, *A. cruentus* and *A. caudutus,* while the leafy types include *A. viridis, A. spinosus, A. reftroflexus* and *A. hybridus* (Tony-Odigie et al., 2012). *Amaranthus* is grown in a wide range of agro-ecological locations and is found in most tropical and subtropical areas (Sauer, 1967; Katiyar et al., 2000; Schippers, 2000) in the world.

The cultivation of indigenous, traditional and indigenized plant species offers greater potential to improve food and nutritional security through crop breeding. Indigenous African Leafy Vegetables (ALVs) have the potential to play a major role in contributing to improved food and nutrition security of most low- and middle-class populations in

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Southern Africa. The genus *Amaranthus* is one of the neglected and underutilized plant species common in farming systems in the Southern African region. It includes several grain and leafy species widely distributed in South and Southern Africa, usually occurring as a weed in crop fields and eaten by various communities as spinach. The ALVs are important sources of vitamins and minerals in the rural communities. Some of the important *Amaranthus* species occurring in South Africa are *A. hybridus, A. thunbergii*, *A. spinosus*, *A. deflexus*, *A. hypochondriacus*, *A. viridus* and *A. greazicans* (Jansen van Rensburg et al., 2007). Although *Amaranthus* is an important food in the subsistence sectors in Southern Africa, little breeding work has been conducted to improve the nutritional value and yield (Gerrano et al., 2015). To supplement and complement the starchy staple meals, resource poor farmers mostly consume the cultivated species of Amaranthus and their wild relatives. A small amount is sold for income generation. It is generally regarded to be a good source of vitamins A and C, iron, calcium, potassium and essential amino acids, including lysine, and, thus, it is an important food source for people who are nutritionally vulnerable (Uusiku et al., 2010), hence the need for breeding research.

Figure 1. Qualitative morphological traits showing the diversity of geographical regions in the biplot

Qualitative plant characteristics such as leaf and stem colour, leaf shape, plant habit, flower colour, panicle colour and shape (Figure 2) can be used to classify a given genus into different species. Such classification is referred to as qualitative morphological characterisation. Morphological

Table 1. List of *Amaranthus* species used for the study

Species	Species	Origin
*A. caudatus*16	*A. caudatus*	USA[b]
A. caudatus 50613	*A. caudatus*	USA
A. cruentus PI477913	*A. cruentus*	USA
AMES22680	*A. cruentus*	USA
Arusha	*A. cruentus*	Tanzania
Green stem Imbuya	*Amaranthus* sp	SA[a], Kwa-Zulu Natal
Tanzania	*Amaranthus* sp	Tanzania
Botswana	*Amaranthus* sp	Botswana
W6927N	*A. viridus*	USA
Bosbok	*A. greazicans*	SA, Mpumalanga
ACAT seed fair	*Amaranthus* sp	SA, Kwa-Zulu Natal
Thohoyandou	*A. greazicans*	SA, Limpopo
Local 33	*Amaranthus* sp	SA
NL	*A.cruentus*	Tanzania
VukaniThepe	*A. greazicans*	SA, Limpopo
Amar	*A. cruentus*	Germany
Anna	*A. cruentus*	Germany
IP5	*Amaranthus* sp	Unknown
Red stem	*Amaranthus* sp	SA, Kwa-Zulu Natal
Appelsbosch	*A. greazicans*	SA, Kwa-Zulu Natal
A19	*A. tricolor*	SA
A550	*A. tricolor*	SA
A554	*A. tricolor*	SA
A5	*A. tricolor*	SA
A993	*A. tricolor*	SA
AC7	*A. tricolor*	SA
A. tricolor PI462129	*A. tricolor*	USA
A. tricolor	*A. tricolor*	USA
Arusha grain	*A. cruentus*	Tanzania
AM-fune	*A. cruentus*	Tanzania
AM-Gare	*A. cruentus*	Tanzania
Kobie	*A. cruentus*	Unknown

[a]SA=South Africa, [b]USA=United States of America

characterisation is utilised in crop breeding to estimate phenotypic variation and, therefore, genetic variation among germplasm. According to Adebola and Morakinyo (2006), it is essential to use morphological descriptors to obtain basic information on existing morphological variability in cultivated species and their wild relatives before the advanced plant breeding techniques are attempted in the genetic improvement of any species. Despite the merits of molecular and genetic markers, morphological descriptors in genetic

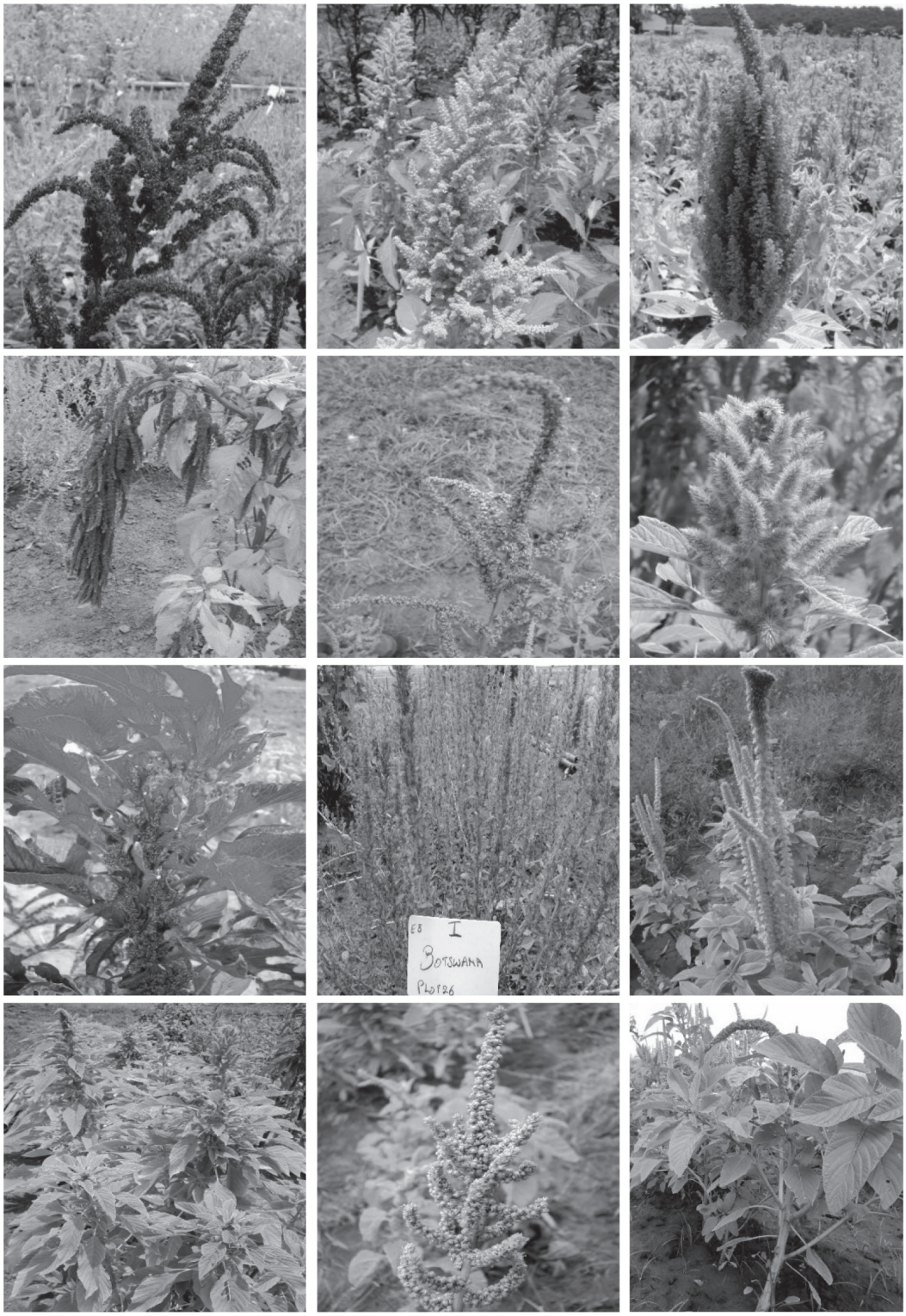

Figure 2. Different types of inflorescences of the *Amaranthus* species

Table 2. Character, descriptor and codes used for the characterization of *Amaranthus* species (IPGRI, 1999)

Qualitative markers	Descriptor and code
Growth habit	Erect (1) and prostrate (2)
Branching index (score if erect type)	No branches (1), Few branches (all near the base of the stem (2), Many branches (all near the base of the stem (3), and Branches all along the stem (4)
Stem pubescence	None (0), Low (3), and Conspicuous (7)
Stem pigmentation	Green (1), and Pink or purple (2)
Spines in leaf axils	Absent (1) and Present (2)
Leaf pubescence	None (0), Low (3), and Conspicuous (7)
Leaf margin	Entire (1), Crenate (2), Undulate (3) and Other (4)
Prominence of leaf vein	Smooth (1), Rugose (veins prominent) (2)
Leaf pigmentation	Entire lamina purple/pink (1), Basal area pigmented (2) Central spot (3), Two stripes (V shaped) (4), One stripe (V shaped) (5), Margin and vein pigmented (6), One pale green /chlorotic spot on normal green (7), Normal green (8), Dark green (9), Other (10)
Leaf shape	Lanceolate (1), Elliptical (2), Cuneate (3), Obovate (4), Ovatainate (5)
Petiole pigmentation	Green (1), Dark green (2), Purple (3), and Dark purple (4)
Terminal inflorescence shape	Spike (dense) (1), Panicle with short branches (2), Panicle with long branches (3), Club shape at tips (4), and Other (specify (5))
Terminal inflorescence attitude	Erect (1), Drooping (2)
Presence of axillary inflorescence	Absent (1) and Present (2)
Inflorescence density index	Lax (3), Intermediate (5), Dense (7)
Inflorescence colour	Yellow (1), Green (2), Pink (3), Red (4), and Other (specify (5))

diversity are still important when exploring the possibility of selecting germplasm for incorporation into breeding programmes (Sounigo et al., 1997). Thus, morphological characterisation is a preliminary and basic requirement for the exploitation of useful traits in plant breeding (Brandolini et al., 2000). The objective of this study was, therefore, to assess the genetic diversity of *Amaranthus* species using qualitative morphological traits and to identify the best parent(s) for the traits of interest that could be used in the *Amaranthus* breeding programme in South Africa.

Materials and Methods

Thirty-two *Amaranthus* species were used for the qualitative morphological study (Table 1). The seeds were sown in the seedling trays in a glasshouse. The seedlings were transplanted to the field after three weeks. No fertiliser was applied and the experimental plots were kept weed free for the duration of the study. The study was conducted at the Roodeplaat research farm (latitude 25°59'S; longitude 28°35'E; 1168 meter above sea level) of the Agricultural Research Council during the 2011-12 and 2012-13 cropping seasons. Each species was grown in three rows of 3 m in length, with 1 m spacing between rows and 0.3 m between plants. A randomized block design, with three replications, was used. The experimental farm received a total of 361.40 mm and 508.50 mm rainfall during the two growing seasons, respectively. The soil type was clay loam. *Amaranthus* descriptor (IPGRI, 1999) was used to categorize the species. The qualitative morphological markers, their descriptors and the codes used for characterization are listed in Table 2. The phenotypic frequency distributions of the phenomic markers were computed for all species. The Shannon Weaver diversity index (H') was used to analyse and interpret the phenotypic diversity for each of the markers using phenotypic frequencies. This index, as described by Perry and Mclntosh (1991), is given as: $H' = 1 - \sum_{i=1}^{n} P_i \log_e Pi$, where P_i is the

Table 3. Frequency distribution (%) of 16 qualitative morphological markers in Amaranthus species by location of collection

Location	Growth habit		Branching index (score if erect type)				Stem pubescence			Stem pigmentation		Spines in leaf axils		Leaf pubescence			Leaf margin				Prominence of leaf vein		Leaf pigmentation							
	1	2	1	2	3	4	0	3	7	1	2	1	2	0	3	7	1	2	3	4	1	2	1	2	3	4	5	6	7	8	9
Botswana	100	0	0	100	0	0	0	0	0	100	0	0	100	100	0	0	100	0	0	0	100	0	0	0	0	0	0	0	0	100	
Germany	50	50	0	50	0	50	0	0	0	50	50	0	100	0	0	0	0	0	100	0	50	50	0	0	50	0	0	0	0	50	0
South Africa	79	21	7	51	21	21	0	0	0	57	43	71	29	0	0	0	58	0	42	0	79	21	0	0	0	0	0	21	0	79	0
Tanzania	100	0	50	50	0	0	0	0	0	50	50	67	33	0	0	0	0	0	100	0	17	83	0	0	0	0	0	17	0	83	0
USA	71	29	14	43	0	43	0	0	0	43	57	71	29	0	0	0	14	0	86	0	86	14	14	0	0	14	0	29	0	43	0
Unknown	100	0	50	50	0	0	0	0	0	50	50	50	50	100	0	0	0	0	100	0	50	50	0	0	0	0	0	50	0	50	0

Location	Leaf shape				Petiole pigmentation				Terminal inflorescence shape					Terminal inflorescence attitude		Presence of axillary inflorescence			Inflorescence density index		Inflorescence colour				
	1	2	3	4	1	2	3	4	1	2	3	4	5	1	2	1	2	3	5	7	1	2	3	4	5
Botswana	0	100	0	0	100	0	0	0	0	100	0	0	0	100	0	0	100	0	0	100	0	100	0	0	0
Germany	0	100	0	0	100	0	0	0	100	0	0	0	0	100	0	0	100	0	0	100	0	100	0	0	0
South Africa	14	86	0	0	71	29	0	0	36	43	21	0	0	86	14	50	50	0	0	100	7	72	21	0	0
Tanzania	0	100	0	0	67	0	33	0	16	68	16	0	0	100	0	17	83	0	0	100	0	100	0	0	0
USA	0	100	0	0	71	0	29	0	43	43	14	0	0	86	14	29	71	0	0	100	0	71	29	0	0
Unknown	50	50	0	0	50	0	50	0	100	0	0	0	0	100	0	0	100	0	0	100	0	100	0	0	0

proportion of accessions in the i^{th} class of an n-class character and n is the number of phenotypic classes of traits. Each H^I value was divided by its maximum value ($\log_e n$) and normalised in order to keep the values between 0 and 1. By pooling these traits across the species, the additive properties of H^I were used to evaluate the genetic diversity of the qualitative traits between the *Amaranthus* species.

Results and Discussion

The frequency distribution of 16 qualitative morphological traits for the *Amaranthus* species is shown in Table 3. The species collected from Botswana, Tanzania and the unknown source were found to be most influenced by the erect growth habit (100%) over the other traits and this trait is considered as being the more dominant type. The species collected from Botswana were also categorized with the frequency distribution of having many branches (all near the base of the stem, green stem pigmentation, presence of spines in leaf axils, entire leaf margin, the smooth prominence of leaf vein, normal green leaf pigmentation, elliptic leaf shape, green petiole pigmentation, panicle with short branches, erect terminal inflorescence, presence of axillary inflorescence, and dense inflorescence density index). These species also had a green inflorescence. The species collected from Germany showed a dominant frequency distribution for the traits of spines in leaf axils (100%) with undulated leaf margins (100%). For all countries of collection, the traits with high frequency distribution was stem pubescence. The species collected from South Africa showed a high frequency distribution for the erect growth type without spines in leaf axils, smooth prominent leaf vein, normal green leaf pigmentation, elliptic leaf shape, green petiole pigmentation with erect terminal inflorescence, dense inflorescence density index, as well as green inflorescence, compared to the rest of the collection regions with no branching index. The high

Table 4. Estimates of diversity indices for qualitative morphological markers among *Amaranthus* species

Traits	Diversity index (H^I)
Growth habit	0.39
Branching index	0.70
Stem pigmentation	0.53
Spines in leaf axils	0.51
Leaf margin	0.48
Prominence of leaf veins	0.50
Leaf pigmentation	0.63
Leaf shape	0.28
Petiole pigmentation	0.47
Terminal inflorescence shape	0.70
Terminal inflorescence attitude	0.28
Presence of axillary inflorescence	0.49
Inflorescence colour	0.51
Average diversity index	0.50

Table 5. Shannon Weaver diversity indices for the 15 qualitative traits in *Amaranthus* species by source of collection

Geographical location	Qualitative Traits															
	GH	BI	SP	SPig	SLA	LM	PLV	LPig	LS	PPig	TIS	TIA	PAI	IDI	IC	Mean
Botswana	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Germany	0.53	0.00	0.00	0.00	0.00	0.00	0.87	0.87	0.00	0.00	0.87	0.87	0.87	0.00	0.87	0.35
South Africa	0.41	2.01	0.00	0.88	0.11	0.88	1.03	1.03	1.14	0.95	1.49	1.41	0.87	0.00	1.71	0.92
Tanzania	0.00	0.87	0.00	0.87	0.75	0.00	1.09	1.09	0.00	0.92	1.60	0.00	0.95	0.00	0.00	0.48
USA	0.47	0.87	0.00	0.88	0.95	1.18	0.44	1.95	0.00	0.95	1.54	1.14	0.95	0.00	0.95	0.79
Unknown	0.00	0.87	0.00	0.87	0.87	0.00	0.87	0.87	0.87	0.00	0.00	0.87	0.87	0.00	0.87	0.35

GH=growth habit; BI=branching index; SP=stem pubescence; SPig=stem pigmentation; SLA=spines in the axils; LM=leaf pigmentation; PLV=prominence of leaf veins; LPig=leaf pigmentation; LS=leaf shape; PPig=petiole pigmentation; TIS=terminal inflorescence shape; TIA=terminal inflorescence attitude; PAI=presence of axillary inflorescence; IDI=inflorescence density index; IC=inflorescence colour

phenotypic frequencies distribution for erect growth habit, elliptic leaf shape, green petiole pigmentation, erect terminal inflorescence, presence of axillary inflorescence, dense inflorescence index, as well as green inflorescence showed that these traits are the dominant qualitative traits for all regions of collections, indicating the existence of different species and the combinations of the species in these regions (Table 3). All species collected from different geographical locations had a dense inflorescence, as indicated in the Table 3.

The qualitative morphological diversity for individual qualitative traits over all *Amaranthus* species are shown in Table 4. The estimates of morphological qualitative diversity indices (H') for individual traits varied from 0.28 for leaf shape and terminal inflorescence to 0.70 for branching index and terminal inflorescence shape, with an overall mean diversity index of 0.49. The standardized Shannon and Weaver (1949) diversity index was classified as low (0-0.33), intermediate (0.34-0.66) and high (0.67-1). The leaf shape and terminal inflorescence had a low genetic diversity index and were monomorphic, while branching index and terminal inflorescence shape were polymorphic. Stem pigmentation, spines in leaf axils, leaf margin, prominence of leaf veins, leaf pigmentation, petiole pigmentation, presence of axillary inflorescence, and inflorescence colour showed an intermediate diversity index. The genetic diversity values for the qualitative traits revealed a wide genetic variability among the evaluated species. Thus, the diversity among *Amaranthus* species was successfully revealed by the recorded traits. Gerrano et al. (2014; 2015) also found the genetic variability among *Amaranthus* species using quantitative morphological markers. The high H' in the current study was mainly due to branching index and terminal inflorescence shape indicating that these two traits contributed to most of the genetic diversity among the species in this study. The Shannon index (H') increases with both the richness and the evenness of the species increase. Gueco et al. (2016) reported 0.67 diversity index in 18 amaranth germplasm collections in the Philippines, which is relatively similar to these results.

The relationship between the genetic diversity index for all the recorded traits and the geographical locations from where species were collected is shown in Table 5. The morphological qualitative markers were able to differentiate the species based on their geographical locations (Figure 1), indicating the existence of a wide genetic diversity among the species. The species also varied in their morphological traits and pattern due to the area of origin and adaptation. The genetic diversity index across the qualitative traits by region or locations varied from 0.00 to 0.92. The species that had the highest genetic diversity index were from South Africa and the USA (Figure 1).There was no genetic diversity index computed for Botswana since only one species was collected. The lowest values of genetic diversity index of 0.35, 0.35 and 0.48 were obtained for the species from Germany, Unknown Source and Tanzania, respectively. Among the qualitative traits, stem pubescence and inflorescence density index showed a very narrow genetic diversity in all geographical locations. No stem

Table 6. Classification of *Amaranthus* species based on qualitative morphological traits

Quantitative markers	Descriptors	Number in sample	% total
Growth habit	Erect	26	81.3
	prostrate	6	18.8
	Total	32	100
Branching index	No branches	4	12.5
	Few branches	16	50.0
	Many branches	5	15.6
	Branches all along the stem	7	12.5
	Total	32	100
Stem pigmentation	Green	17	53.1
	Pink or Purple	15	46.9
	Total	32	100
Spines in leaf axils	Absent	20	62.5
	present	12	37.5
	Total	32	100
Leaf margin	Entire	10	31.3
	Undulate	22	68.9
	Total	32	100
Prominence of leaf veins	Smooth	21	65.6
	Rugose (veins prominent)	11	34.4
	Total	32	100
	Central spot	2	6.3
Leaf pigmentation	Two strips (V shaped)	1	3.1
	Margin and vein pigmented	7	21.8
	Normal green	22	68.8
	Total	32	100
Leaf shape	Lanceolate	3	9.4
	Elliptical	29	90.6
	Total	32	100
Petiole pigmentation	Green	23	71.9
	Purple	9	28.1
	Total	32	100
Terminal inflorescence shape	spike (dense)	13	40.6
	Panicle with short branches	14	43.8
	Panicle with long branches	5	15.6
	Total	32	100
	Erect	29	90.6
Terminal inflorescence attitude	Drooping	3	9.4
	Total	32	100
Presence of axillary inflorescence	Absent	11	34.3
	Present	21	65.6
	Total	32	100
Inflorescence colour	Yellow	1	3.2
	Green	24	75.0
	Pink	7	21.9
	Total	32	100

pubescence was observed among all genotypes, whereas dense inflorescence index dominated compared to lax and intermediate types.

The morphological characters differed significantly in their frequency of distribution and the amount of variation in the genetic pool. The morphological qualitative descriptor showed that a total of 29 out of 32 (i.e. 90.6%) of the species evaluated had an elliptic leaf shape and drooping terminal inflorescence, followed by an erect growth habit and green petiole pigmentation (Table 6). In contrast, the V-shaped leaf pigmentation, yellow inflorescence and central spot leaf pigmentation represented 3.1, 3.2 and 6.3%, respectively, of the total number of species. For leaf traits, most species had normal green pigmentation, an elliptic shape, undulate margins, prominent smooth veins, and green petiole pigmentation.The terminal inflorescence of most of the species collected from different origins had a dominantly spike (dense) panicle with a short branch shape, as well as an erect attitude. In the present study, the two qualitative traits, namely, dense inflorescence index and stem pubescence, showed no variability among the species. Gueco et al. (2016) reported the diversity of 18 Amaranth germplasm collections in the Philippines and found a wide genetic variability among them for qualitative traits.

It is concluded that morphological traits can be used to characterize *Amaranthus* germplasm. The morphological qualitative characters used in the current study revealed wide genetic diversity among species within the ARC germplasm collection, which would help for the classification and conservation of genetic resources. There is a wide enough diversity in the ARC germplasm collection to support the selection of accessions for traits of interest in *Amaranthus* breeding programmes in South Africa.

Acknowledgement

The first author acknowledges the Agricultural Research Council, South Africa for the research fund.

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