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Full Length Article

Phenotypic and Isoenzymatic Variations in *Amaranthus* Species

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Abstract

This work was aimd at to estimate the amount of genetic variability within *Amaranthus* spp. accessions collected from different regions of the world using phenotypic and isoenzymatic markers. The analysis of the morphological characters showed that *A. cruentus* Ames 5310 from Mexico, Sonora had large seeds; A. *quitensis* PI511744 from Ecuador and *A. powellii* ssp. bouchonii Ames 5304 from Washington, USA were with the longest stem; and A. *hypochondriacus* PI337611 from Uganda, *A. hybridus* PI605351 from Greece and *A. cruentus* Ames 5310 from Mexico, Sonora were produced the largest leaf area. These accessions can be used for improving grains yield and plant vigor in amaranths. The correlations between the color of lower surface of cotyledonary leaves, color of plumule and color of stem were useful indices in the classification of vegetable amaranths whereas the correlation between seed size and each of stem diameter, color of veins, leaf area and length of blade indicated the potential for the improvement of these characters. The cluster analysis of the morphological and isoenzymatic data indicated that the environment had no influence on the distribution of the studied accessions. The principal component analysis defined the accessions that had high values for height of stem, seed size, stem diameter, leaf area and branching to be used in future hybridization work. The detection of allele C of the isozymes *α-Est*-1C, *α-Est*-2C, *α-Est*-3C and *Per*-1C in wild species only suggested its role in the adaption of the wild accessions to environmental stresses. Cluster analysis of the isozymes data confirmed the monophyletic origin of grains amaranths, being originated from *A. hybridus*. Results of this research showed great variations between accessions in morphological and isoenzymatic characters that breeders can use to improve the commercial cultivars of grains and leafy amaranths. © 2021 Friends Science Publishers

Keywords: *Amaranthus* spp.; Phenotypic markers; Isozymes markers; Pearson correlation; Correspondence analysis

Introduction

Amaranthus L. (Amaranthaceae) is a genus comprising of about 400 species; few of which are found worldwide (Rastogi and Shukla 2013). It comprised grains, vegetables, ornamental and weedy varieties (Andini *et al*. 2013; Adhikary and and Pratt 2015). *Amaranthus* species have different origins and centers of distribution. They are widely distributed in Africa, Australia, tropical America, Greece, India, China, Italy and Nepal (Akin-Idowu *et al*. 2016). They have a great amount of genetic variability with diversity in protein content, plant type, number of inflorescences, seed color, earliness, height of the plant, green matter and seed and yield (Jacobsen *et al.* 2000). They also resist heat and drought, adapts to adverse growing conditions, grows easily in agriculturally marginal lands and have no major disease problem (Casini and Rocca 2014; Thapa and Blair 2018).

Although, most of grain leafy and wild amaranths are self-pollinated, outcrossing is also possible (Ray and Roy 2009). The mean outcrossing rates under field conditions have been variously estimated at 3.5 to 34% for the grain species (Espitia-Rangel 1994). In *A. lividus* and *A. tricolor* as vegetable species outcrossing is substantially lower (Khoshoo and Pal 1972). Some wild *Amaranthus* species are dioecious and, therefore, must outcross (Sauer 1957).

Amaranth is underutilized but highly nutritious crop. It is rich in proteins, minerals, vitamin A and C (Repo-Carrasco *et al.* 2003). Leafy plants are consumed as vegetables at the pre-flowering stage when the protein concentration in the leaves reaches 25.3–32.9% and the levels of oxalates and nitrates are low (Shukla *et al*. 2010; Akin-Idowu *et al*. 2016). *Amaranthus* spp. can be used as commercial food coloring, as an alternative for the pigments from red beet (*Beta vulgaris* L.) plant (Cai and Corke 2000). *Amaranthus* spp. are recommended as a good food that have medicinal value for lactating mothers, young children and for patients who suffer from anemia, constipation, hemorrhage, fever or kidney complaints (Alegbejo 2013; Peter and Ganfhi 2017).

Knowledge of the amount and allocation of genetic diversity within a species is vital for selecting germplasm to be included in a breeding program and for helping in managing plant genetic resources (Yu *et al.* 2001).

Estimations of genetic variability are based on molecular, biochemical, cytological and morphological traits.

The morphological markers have been used by geneticists and evolutionists to describe genetic variation within and among populations of the same species (Oboh 2007; Zhang *et al.* 2007). They are based on the phenotypic traits of the plants, which are often susceptible to phenotypic plasticity. However, the effect of the environment can be overcome if the plants were grown under adjusted growth conditions (Govindaraj *et al.* 2015). The morphological markers are still having advantage, particularly for distinguishing the mature plants from their genetic contamination in the field, for example, flower/leaf color variants, bristled panicle and spiny seeds. A great variation in morphological and nutritional characters was observed among genotypes within the same species and among different *Amaranthus* species (Xiao *et al.* 2000; Sarker *et al.* 2015; Akin-Idowu *et al.* 2016).

Using isozymes markers, Chan and Sun (1997) observed 100% polymorphism at the interspecific level. They also observed high levels of inter-accessional genetic diversity within species and genetic uniformity within most accessions. On the contrary, Yudina *et al.* (2005) found low allozyme variation among various populations of the cultivated and weedy *Amaranthus* species. The objective of this work was to estimate the genetic variations in accessions of *Amaranthus* spp. using morphological and isoenzymatic characters. The data of this research may be useful in the understanding of the diversity of *Amaranthus* spp. accessions collected worldwide and also it may be used for the breeding purposes.

Materials and Methods

Plant materials

Twenty-four accessions of *Amaranthus* L. spp. represented nine species were used in this research. They were obtained as donation from United State Department of Agriculture (USDA), Agricultural Research Service (ARS). The origin and the accession number of the 24 accessions are recorded in Table 1.

Greenhouse planting

The studied accessions were grown in greenhouse located at the Department of Botany, College of Science, Tanta University, Tanta, Egypt, at 25–30°C. Seeds of the accessions were subjected to cold treatment for the first 24 h to improve germination, then germinated in plastic trays (5 x 5 holes; diameter, 5.0 cm; depth, 5.0 cm) containing ''Perlite'' (Carolina Perlite Company, USA) for three weeks. Five plants per accession from plants grown on ''Perlite'' were transplanted into pot containing bottoms as biological fertilizer mixed with silt in a 1:4 ratio respectively. The pots were arranged in completely randomized design, five pots for each accession.

Morphological characterization

Phenotypic traits were assessed five times per accession. Each time were assessed randomly on three tagged plants in a pot. Thus, a total of 15 observations were taken for each trait. The phenotypic traits recorded include stem habit, height of stem, diameter of stem, branching of stem, stipules, petiole color, color of vines, leaf shape, leaf blade shape, leaf apex width, leaf area, length of blade, spines at the apex of leaf, leaf apex notching, leaf base, shape of leaf margin, color of leaf margin and position of seed head. Slide caliper, from Digimatic Solar DC-S15 m, Mitutoyo, Japan, was used to measure stem diameter (mm); VH-analyzer image analysis software, version 2.20, Keyence Co., Ltd., Osaka, Japan, was used to determine the leaf area. Before leaf area measurement, the leaves were arranged on a white paper background and scanned on a GT-9800 F Scanner (EPSON, Tokyo, Japan). A 1-cm^2 color marker was used as the standard.

Protein extraction, electrophoresis and activity staining

To prepare isozymes crude extracts, 20 mg young leaves of 15 days old seedlings were macerated with 1 mL of extraction buffer consisted of 0.05M sodium phosphate buffer (pH 7.2), 20% v/v glycerol, 14 mM 2-mercaptoethanol and 0.05% v/v triton X-100 (Manchenko 1994). A clear supernatant was applied directly on 7% PAGE at 4°C in a Mini Protean III unit (BioRad, California, USA), under a constant current of 100 mA for 5 to 6 h, until the tracking dye had moved 5 to 7cm from the cathodal end. The gels were subjected to staining for acid phosphatase (*Acp*), alkaline phosphatase (*Alp*), α-Esterase (*α-Est*), β-Esterase (β-*Est*) and peroxidase (*Per*) isozymes following the protocols of Pasteur *et al*. (1988). Phosphorylase gels were incubated in 100 mL of 0.1 M sodium phosphate buffer (pH 5.1) at 37ºC for 3 to 5 h, then stained in 10 mM I_2 mixed with 14 mM KI, developing white bands on a dark blue background. At the bottom of phosphorylase gels, amylase isozymes appeared as chromatic or light brown bands. Catalase gels were stained in 1:1 mixture of solutions 2% potassium ferricyanide and 2% ferric chloride after incubation in a solution of 3% H_2O_2 for about 15 min, and then washed in water with agitation for a few minutes. After washing, catalase activity bands appeared in yellow color on background with a blue-green color. The gels of α and ß-esterases were incubated in 100 mL staining solution consisted of 0.05 M phosphate buffer (pH 7.2) containing 1% α or β naphthyl acetate for α and ß-esterases respectively and 50 mg Fast Blue RR at 37°C for 15 min until brown colored bands appeared. The stained gels were immediately photographed and stored in 3% acetic acid. At least 5 and generally 10 plants per accession were examined for isozymes patterns.

Data analysis

The statistical analyses of the phenotypic traits and isozymes data were performed using the software package "PAST", Version 4.02, Natural History Museum, University of Oslo, 1999–2020 which included descriptive statistics, Pearson correlation and multivariate analyses (principal components analysis and cluster analysis). Pearson correlation analysis was performed to explain the relationships among the investigated traits. The isozymes bands at a particular locus in each accession were scored as "0" for presence and "1' for absence, then transformed into a binary character matrix. Phenotypic and isoenzymatic data were analyzed using Ward's method of cluster analysis to group amaranths accessions.

Results

Morphological analysis

Qualitative character: The resultes of the assessement of gentic diversity in qualitative morphological characters of *Amaranthus* spp. showed that two alleles were responsible on controling stipules, color of vines, leaf shape, spines at apex of leaves, shape of leaf margin and color of leaf margin (Table 2). The stipules were present in 4.17% of assessed accessions and absent in 95.83%. The color of veins was found to be green in 87.5% and purple in 12.5%, whereas the color of leaf margin was found to be green in 62.5% and purple in 37.5%. The leaf shape was found to be either V-shaped (70.83%) or Egg-shaped (29.17%). The spines at apex of leaves were found to be absent in 37.5% and present in 62.5%, whereas the shape of leaf margin was entire in 58.3% and emarginated in 41.7%. The other assessed qualitative characters were controlled by more than two alleles.

Pearson ccorrelations: The results of the association between the assessed traits of the accessions of *Amaranthus* spp. are presented Table 3. Color of lower surface of cotyledonary leaves was significantly correlated with color of plumule and color of stem. Size of seeds was significantly correlated with diameter of stem, color of veins, leaf area and length of blade. Leaf area was significantly correlated with seed size and stem diameter. Length of blade was correlated with seed size, stem habit, stem diameter and leaf area. Branching was significantly correlated with leaf apex width and stipules and negatively correlated with length of blade. Color of leaf margin was correlated with color of vines and leaf shape. Position of seed head was positively correlated with color of seeds, leaf apex notching, branching of stem, leaf apex width; and negatively correlated with stem habit, diameter of stem, leaf area and length of blade.

Diversity indices for morphological characters: The diversity indices were estimated for morphological characters (Table 4). Diversity indices ranged between 1.561 for branching of stem and 3.167 for stem habit and color of veins. In general, all the characters showed high diversity indices, with the exception of branching of stem, spines at apex of leaves, leaf apex notching and position of seed head.

Cluster analysis: The accessions were separated into two groups, named G1 and G2 at genetic distance of 16.5 (Fig. 1). Majority of the accessions of *A. hypochondriacus* and *A. caudatus* were clustered in G2. The accessions of the other species were separated in G1 with *A. hypochondriacus* PI 540446 and *A. caudatus* PI 619264. At genetic distance 7, the accessions were separated into 7 clusters named C1, C2, C3, C4, C5, C6, C7. The accessions of *A. hybridus* were distributed in C1 and C5. The two accessions of *A.powellii* ssp.bouchonii were separated in C3 with *A. palmeria* PI 604557 Mexico, Puebla. The other accessions of *A. palmeria* were collected in C4 with *A. spinosus.* The two accessions of *A. cruentus* were clusstered in C1 and C2 which were grouped at genetic distance 9.

Principal component analysis: The principal component analysis grouped the accessions over the quadrants based on the traits of their morphological characters (Fig. 2 and 3).The accessions in **t**he top right quadrat (*A. palmeria* PI 604557 Mexico, Puebla, *A. hypochondriacus* PI 540446 Pakistan, *A. cruentus* Ames 5310 Mexico, Sonora, *A. cruentus*PI 628793 Zaire, Shaba, *A. hybridus* Ames 21188 South Africa, *A. hybridus* PI 605351 Greece) were closely associated with the traits of color of plumule, height of stem after 2 months, stipules, petiole color, leaf blade shape, leaf base, color of margin, margin shape. The right bottom quadrate consisted of the accessions (A. *hypochondriacus* except *A. hypochondriacus* PI 540446 Pakistan, *A. caudatus*, *A. quitensis* and *A. retroflexus*) of related traits of size of seeds, color of upper surface of cotyledonary leaves, color of stem, diameter of stem, color of vines, leaf shape, leaf area, length of blade, spines at apex of leaves, leaf apex notching. The accessions in the top left quadrate (*A. hybridus* PI 636181 USA, Delaware, *A. hybridus* Ames 23369 Brazil, Goias, *A. hybridus* Ames 26852 Portugal, Coimbra, *A. palmeria* PI 607455 USA, Kansas, *A. palmeria* PI 607461 USA, Kansas and *A. powellii* ssp.) were closely related in the terms of color of seeds, radical length, color of lower surface of cotyledonary leaves, leaf apex width, position of seed head. The bottom left quadrate clustered *A. spinosus* PI619234 from Indonesia, Sumatra with the characters of plumule length, height of stem, branching of stem.

Isozymes analysis

Loci and alleles scored: Enzyme electrophoresis resulted in clear staining for four enzymes encoded by 18 polymorphic putative loci (Table 5). A total of 40 alleles were observed across the 18 loci. Average allele frequency was ranged between 0.008 in α-Est-2C and 0.86 in α-Est-5A. The frequency of *Alp*-1A, *α-Est*-1B, *α-Est*-2C and *Per*-1C were

Table 1: The accession number and the origin of the studied accessions of *Amaranthus* species

Code	Species	Accession Number	Origin	Code	Species	Accession Number	Origin
A	*A. hypochondriacus*	PI 274279	India, Himachal Pradesh	M	*A. hybridus*	PI 636181	USA, Delaware
B	*A. hypochondriacus*	PI 337611	Uganda	N	*A. hybridus*	Ames 23369	Brazil, Goias
C	*A. hypochondriacus*	PI 477917	Mexico	O	*A. hybridus*	Ames 26852	Portugal, Coimbra
D	*A. hypochondriacus*	PI 540446	Pakistan	P	*A. palmeria*	PI 604557	Mexico, Puebla
E	*A. caudatus*	PI 166045	India	Q	*A. palmeria*	PI 607455	USA, Kansas
F	*A. caudatus*	PI 619264	Nepal	R	*A. palmeria*	PI 607461	USA, Kansas
G	*A. caudatus*	PI 553073	USA, new jersey	S	*A. palmeria*	PI 632235	USA, Arizona
H	*A. caudatus*	PI 511679	Argentina	T	*A. quitensis*	PI 511744	Ecuador
I	*A. cruentus*	Ames 5310	Mexico, Sonora	U	*A. retroflexus*	PI 572263	USA, Iowa
J	*A. cruentus*	PI 628793	Zaire, Shaba	V	*A. spinosus*	PI619234	Indonesia, Sumatra
K	*A. hybridus*	Ames 21188	South Africa	W	*A. powelliissp.bouchonii*	Ames 5304	USA, Washington
L	*A. hybridus*	PI 605351	Greece	X	*A. powelliissp.bouchonii*	PI 572262	France

Table 2: Qualitative traits noted on 24 accessions of *Amaranthus* species

Character	Trait	% age
Stipules (Spiny)	Absent	95.83
	Present	4.17
Color of veins	Green	87.5
	Purple	12.5
Leaf shape	V-shape	70.83
	Egg-shape	29.17
Spines at apex of leaves	Absent	37.5
	Present	62.5
Shape of leaf margin	Entire	58.3
	Emarginated	41.7
Color of leaf margin	Green	62.5
	Purple	37.5

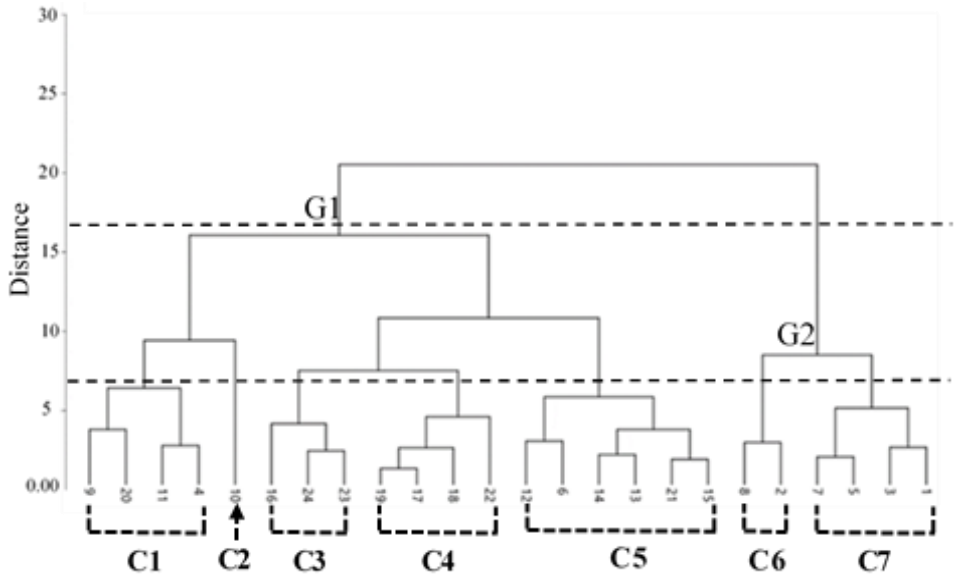

Fig. 1: UPGMA dendrogram of 24 accessions of *Amaranthus* species based on phenotypic characteristics

0.008, 0.09 and 0.008 respectively. The mean frequency of allele was 0.38. The average allele frequency of accessions ranged between o.43 in *A. powellii* ssp. Bouchonii, accession PI 572262, from France and 0.73 in *A. cruentus*, accession Ames 5310 from Sonora, Mexico. The allele's α-Est-1C, α-Est-2C, α-Est-3C and Per-1C were detected in wild species only, whereas the allele *Alp*-1C was present in cultivated and wild species. The average of allele's frequency in the cultivated accessions (0.577) and wild accessions (0.559) was approximately the same. The highest mean number of alleles per locus was noted in *A. caudatus* PI 511679 from Argentina, *A. cruentus* Ames 5310 from Sonora- Mexico, *A. palmeria* PI 604557 from Puebla-Mexico, and *A. palmeria* PI 607461 form Kansas - USA.

Table 3: Pearson correlation coefficient among 26 traits of *Amaranthus* species

Code	Character	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
A.	Size of seeds	1.0																									
B.	Color of seeds	-0.4	1.0																								
C.	Radical L.	-0.1	0.3	1.0																							
D.	Color of upper surface of cot. Leaves	0.1	0.1	-0.1	1.0																						
E.	Color of lower surface of cot. Leaves	-0.1	0.4	-0.1	0.4	1.0																					
F.	Color of plumule	0.1	0.3	-0.2	0.4	0.9	1.0																				
G.	Plumule length	-0.3	-0.1	0.5	-0.1	0.1	-0.2	1.0																			
H.	Color of stem	0.4	0.1	-0.1	0.3	0.6	0. 7	0.1	1.0																		
I.	Stem habit	0.3	-0.1	-0.1	0.1	-0.1	0.1	-0.3	0.1	1.0																	
J.	Height of stem after 2 month	0.2	-0.1	0.1	-0.1	-0.2	-0.1	0.2	0.2	-0.1	1.0																
K.	Diameter of stem	0.7	-0.5	-0.2	0.1	-0.4	-0.1	-0.4	0.1	0.3	0.4	1.0															
L.	Branching of stem	-0.4	0.1	0.3	-0.1	0.1	-0.1	0.3	-0.1	-0.9	-0.1	-0.4	1.0														
M.	Stipules	-0.5	0.2	0.1	0.1	0.1	-0.1	0.1	-0.3	-0.4	-0.4	-0.3	0.7	1.0													
N.	Petiole color	0.3	0.1	-0.1	0.1	0.1	0.4	-0.1	0.5	0.4	0.1	0.4	-0.4	-0.2	1.0												
O.	Color of vines	0.5	0.1	0.2	0.2	-0.2	0.1	-0.2	0.3	0.2	0.31	0.5	-0.2	-0.1	0.7	1.0											
P.	Leaf shape	-0.1	-0.3	-0.2	0.2	-0.3	-0.3	0.2	-0.1	0.3	-0.1	0.1	-0.3	-0.1	0.1	-0.2	1.0										
Q.	Leaf blade shape	-0.1	0.2	0.2	-0.4	-0.4	-0.4	-0.2	-0.2	-0.1	0.2	0.2	0.1	-0.1	0.1	0.3	-0.3	1.0									
R.	1) Leaf apex width	-0.5	0.6	0.2	0.1	0.2	0.1	0.1	-0.2	-0.4	-0.1	-0.5	0.5	0.5	-0.3	-0.1	-0.3	-0.1	1.0								
S.	Leaf area	0.6	-0.3	-0.1	0.5	-0.1	0.1	-0.4	0.2	0.4	-0.2	0.6	-0.4	-0.3	0.3	0.3	0.3	-0.2	-0.4	1.0							
T.	1) Length of blade	0.7	-0.4	-0.2	0.4	-0.2	0.1	-0.4	0.2	0.5	0.1	0.7	-0.5	-0.3	0.4	0.4	0.2	-0.2	-0.4	0.9	1.0						
U.	Spines at apex of leaves	-0.1	-0.4	-0.1	-0.1	-0.1	-0.1	0.1	-0.1	0.2	0.1	0.2	-0.1	0.1	-0.1	-0.2	0.3	-0.3	-0.3	-0.2	-0.1	1.0					
V.	Leaf apex notching	-0.6	0.5	0.2	0.2	0.1	0.1	-0.1	-0.3	-0.2	0.1	-0.3	0.3	0.6	-0.2	-0.1	-0.2	-0.1	0.7	-0.3	-0.2	-0.1	1.0				
W.	Leaf base	-0.1	0.1	0.1	0.1	-0.1	-0.1	0.1	-0.1	0.1	-0.1	0.1	-0.1	-0.1	0.1	-0.2	0.4	-0.1	0.1	0.2	0.1	-5.4	0.1	1.0			
X.	Shape of leaf margin	0.2	-0.1	-0.5	-0.1	-0.2	0.1	-0.5	-0.1	-0.2	0.1	0.4	0.2	0.2	0.1	0.2	-0.1	0.2	0.1	0.1	0.1	-0.1	0.2	0.1	1.0		
Y.	Color of leaf margin	0.2	0.2	0.1	-0.2	0.1	0.2	0.1	0.3	0.4	0.1	0.1	-0.4	-0.2	0.8	0.5	-0.1	0.3	-0.3	-0.1	-0.1	0.1	-0.3	-0.1	-0.1	1.0	
Z.	Position of seed head	-0.4	0.6	0.4	-0.1	0.1	-0.1	0.3	-0.2	-0.5	0.1	-0.6	0.5	0.3	-0. 4	-0.2	-0.3	-0.1	0.8	-0.5	-0.5	-0.4	0.5	0.2	0.1	-0.2	1.0

Table 4: Estimates of diversity indices for morphological characters among *Amaranthus* species

Code	Charactes	Diversity index (H^I)	Code	Charactes	Diversity index (H^I)
A	Size of seeds	3.144	N	Petiole color	3.091
B	Color of seeds	3.08	O	Color of veins	3.167
C	Radical L.	3.105	P	Leaf shape	3.121
D	Color of upper surface of cot. Leaves	3.113	Q	Leaf blade shape	3.075
E	Color of lower surface of cot. Leaves	3.119	R	2) Leaf apex width	3.096
F	Color of plumule	3.122	S	Leaf area	3.125
G	Plumule length	3.12	T	2) Length of blade	3.09
H	Color of stem	3.142	U	Spines at apex of leaves	2.708
I	Stem habit	3.167	V	Leaf apex notching	2.967
J	Height of stem	3.149	W	Leaf base	3.086
K	Diameter of stem	3.11	X	Shape of leaf margin	3.119
L	Branching of stem	1.561	Y	Color of leaf margin	3.118
M	Stipules	0	Z	Position of seed head	2.598

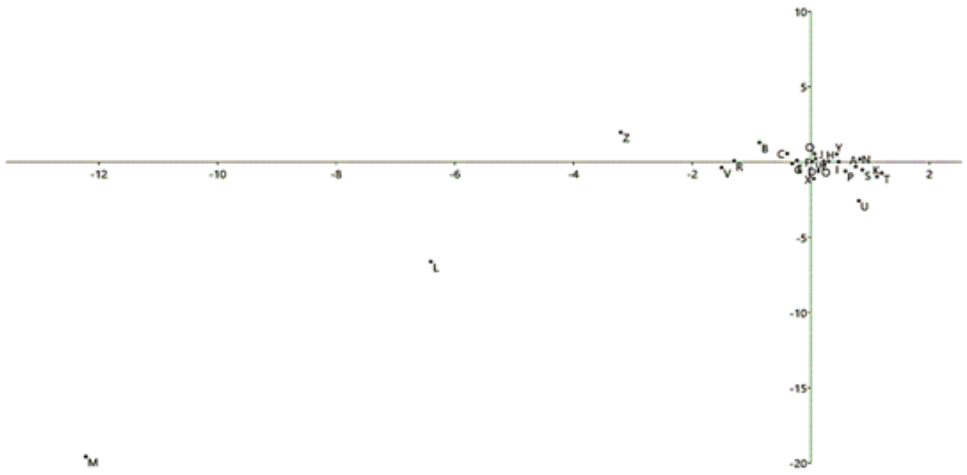

Fig. 2: Principal component analysis loading plot of 26 phenotypic traits of 24 *Amaranthus* accessions. The accession numbers were shown in Table 1

Pearson correlations: Based on Pearson correlation analysis, accessions of *A. hypochondriacus* were correlated with values of 0.4 or more. The accessions of *A. caudatus* were significantly correlated, except *A. caudatus* PI 511679 from Argentina which was correlated with *A. hybridus* PI 605351Greece and *A. hybridus* PI 636181 USA, Delaware. *A. palmeria*, *A. quitensis*, *A. retroflexus* and *A. spinosus* were significantly correlated. *A. hybridus* PI 636181 USA, Delaware was highly correlated with *A. hybridus* Ames 23369 from Goias, Brazil, at genetic diversity 0.7. *A. hybridus* Ames 21188 from South Africa was highly correlated with *A. hybridus* PI 605351 from Greece and *A. hybridus* PI 636181 from Delaware, USA. *A. palmeria* PI 632235 from Arizona, USA was correlated with accessions *of A. hypochondriacus* and accessions *A. caudatus* with exception of *A. caudatus* PI 511679 from Argentina.

Cluster analysis: Cluster dendrograsm based on isozymes data divided the accessions into two groups: G1 and G2 at genetic distance 10.2. G2 contained the accessions of *A. hypochondriacus* and the Asian accessions of *A. caudatus* (PI166045 from India and PI619264 from Nepal). The other species were separated in G2. At genetic distance 4.8, the accessions were grouped into 5 clusters named C1, C2, C3, C4 and C5. C1 inclded the American accessions of *A. caudatus.* The accessions of *A. hybridus* were distributed in C1, C3 and C4. *A. spinosus* and *A.powellii* ssp.bouchonii were separated in C3. *A. quitensis* and *A. retroflexus* were grouped in C2. The accessions of *A. palmeria* were distrbuted in C1, C2 and C4. The accessions of *A. cruentus* were separted in C2 (*A. cruentus* Ames 5310 from Sonora, Mexico,) and C3 (*A. cruentus* PI628793 from Shaba, Zaire).

Discussion

Amaranthus spp. have a great amount of genetic diversity which can be used in management of *Amaranthus* germplasm and in designing breeding programs for improving the characters that favorable for farmers and households.

The resultes of the assessement of genetic diversity in stipules (spiny), color of veins, leaf shape, spines at apex of leaves, shape of leaf margin and color of leaf margin showed that these characters were controlled by two alleles. Gottlieb (1984) and Dasriani *et al*. (2020) reported that a significant proportion of the differences in plant structure and shape is governed by one or two gene loci; and the variation in the quantitative characters are usually governed by many genes. Although the color of the seeds has six traits of yellow pale, yellow pale with purple margin, dark yellow, faint purple with yellow margin, brown and black; 50% of accessions was with black color and 20.8% was with brown one. This suggested that multiple alleles of a single gene were controlling the inheritance of seed color and dominance of the black allele over brown allele over other alleles, as has been reported in testa of lentil (Bakhsh *et al.* 2013). The seed size of *Amaranthus* spp. was small, intermediate and big. The accessions with intermediate seeds represented 50%, whereas the accessions with small seeds represented 41.7% and the accessions of big seeds were 4.16 %. The accession with big seeds was *A. cruentus* Ames 5310 from Mexico, Sonora. The accessions with the biggest stem height were *A. quitensis* PI511744, Ecuador and *A. powellii* ssp. bouchonii Ames 5304, USA, Washington. The accessions with largest leaf area were *A. hypochondriacus* PI337611 from Uganda, *A. hybridus* PI605351 from Greece and *A. cruentus* Ames 5310 from Mexico, Sonora. Accessions with large and intermediate seed size can be used for improving seeds yield of grains amaranths; and the accessions with large leaf area can be used for improving plant vigor in leafy amaranths.

The significant correlation between the color of lower surface of cotyledonary leaves, color of plumule and color of stem were useful indices in the classification of vegetable amaranths (Akin-Idowu *et al.* 2016). The significant correlation between seed size and each of stem diameter, color of veins, leaf area and length of blade indicated the potentiality of the improvement of these characters through selection to enhance seed yield and plant vigor. The significant correlation between leaf area and plant height in Leafy and grain amaranths in South Africa and South West Nigeria respectively was also reported by Akin-Idowu *et al.* (2016) and Gerrano *et al.* (2015) respectively. A negative correlation between branching, plant height, stem habit and stem diameter is an indication that most of the tall plants had few number of branches, narrow stem diameter and erect stem. A positive correlation between and among characters indicated that selection and improving of the primary characters of interest would have a positive effect on the secondary traits in the breeding program.

Cluster dendrogram based on morphological characters separated the studied accessions into two groups: G2 includes cultivated accessions and G1 includes wild and cultivated accessions. Furthermore, the studied accessions were not separated on the basis of geographical regions. In this respect Hadian *et al*. (2008) reported little geographic cohesiveness in the distribution of genetic diversity among accessions of *Amaranthus*; and did not agree with the work of Akin-Akin-Idowu *et al*. (2016) who showed some extent of geographic cohesiveness in their study on grain *Amaranthus* spp, in South West Nigeria. The discrepancy between our data and the data of Akin-Idowu *et al*. (2016) could be due to that their study was carried out on local taxa and on grains amaranths only. The grouping of *A. palmeria* PI604557 from Puebla, Mexico, with *A. powellii ssp. Bouchonii* indicated that they may be closely related in terms of phenotypic characteristics.

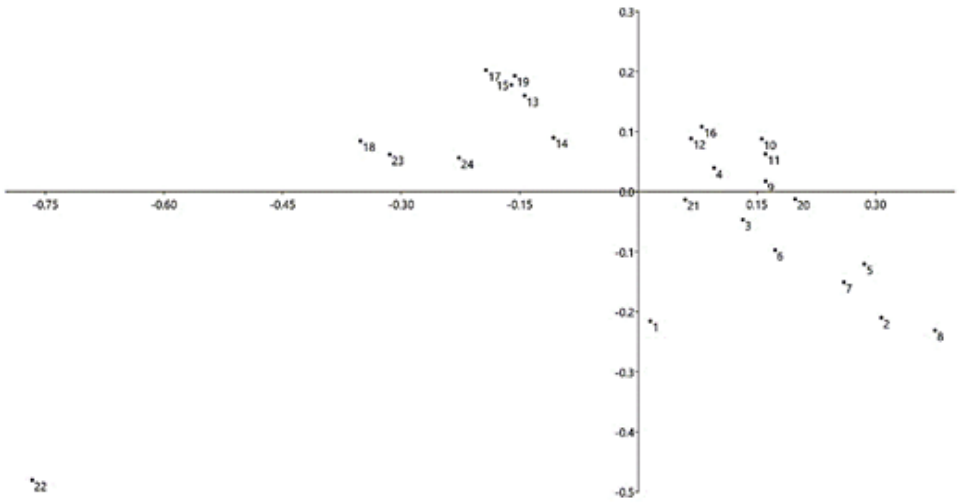

Fig. 3: Principal component analysis score plot of the first and second principal components. The accession numbers were shown in Table 1

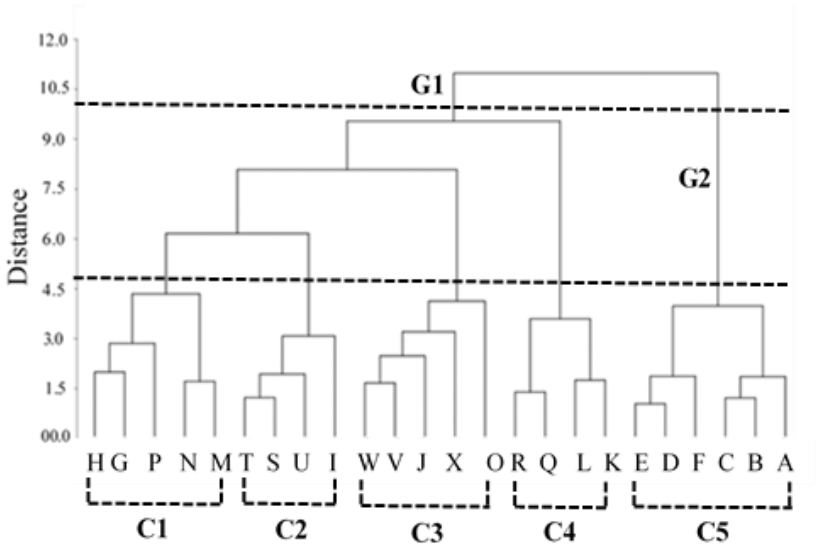

Fig. 4: UPGMA clustering of 24 accessions of *Amaranthus* species based on isozymes data

The principal component analysis grouped the accessions over the quadrants based on the traits of their morphological characters. The accessions in the tope right quadrate had high values for height of stem; the accessions in the right bottom quadrate having high values for size of seeds, diameter of stem, leaf area; the accessions in the bottom left quadrate having high values for branching. These accessions could serve as promising sources of genes for these characters in future hybridization work.

Although, most of the species of *Amaranthus* are predominantly self-pollinated, the frequency of the 18 putative loci observed herein was greater than it is expected for this type of breeding system. Such an allele frequency in *Amaranthus* spp. could be due to the reason that some species can cross freely as in the progenitor species of the cultivated species (Sauer 1957; 1967; 1972) or can cross with difficulty giving few fertile seeds as in the species of the secondary gene pool of *Amaranthus* (Jain *et al.* 1982). Higher allelic frequencies could also be interpreted on the basis of the wide range of morphological diversity in accessions of *Amaranthus* spp. and on the wide geographical distance between the locations of the collected accessions (Palomino and Ruby 1991). Although, 18 loci were polymorphic, the extent of polymorphism varied from 43% in *A. powellii* ssp. bouchonii PI572262 from France to 73% in *A. cruentus* Ames 5310 from Sonora, Mexico. High isozymes variation in the present work agreed with data of Hauptli and Jain (1984) and Chan and Sun (1997) and contradicted the works of Jain *et al.* (1980) and Iudina *et al*. (2005) who found that allozyme variation in the cultivated and weedy *Amaranthus* spp. was low; many of them were monomorphic for the enzymes examined. The frequency of

Table 5: Allelic frequencies for 18 isozyme loci in 24 accessions of *Amaranthus* species

Allele No.	Loci	Allele Code	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Mean Frequency of allele	Probability
1	*Acp-1*	A	0.8	0.8	0.8	1	0.8	0.8	0.6	0	1	0.9	0.2	0.2	0	1	0	0.8	1	1	1	0.8	1	1	0.8	0.8	0.71	0*
2	*Acp-1*	B	0.2	0.2	0.2	0	0.2	0.2	0.4	1	0	0.1	0.8	0.8	1	0	1	0.2	0	0	0	0.2	0	0	0.2	0.2	0.29	
3	*Acp-2*	A	0.5	0.5	0.5	0.1	0.1	0	0	0.2	0.5	0.8	1	0	1	1	1	0	1	0.4	1	0.8	0.8	1	0.8	0.8	0.58	0*
4	*Acp-2*	B	0.5	0.5	0.5	0.9	0.9	1	1	0.8	0.5	0.2	0	1	0	0	0	1	0	0.6	0	0.2	0.2	0	0.2	0.2	0.43	
5	*Alp-1*	A	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0.1	0	0	0	0	0	0	0	0	0	0	0.008	0.445
6	*Alp-1*	B	0.5	0.5	0.5	0.6	0.2	0.2	0	0.5	0.5	0	0.5	0.5	0.5	0.5	0	0.5	1	1	0	0.5	0.5	0.5	1	1	0.48	
7	*Alp-1*	C	0.5	0.5	0.5	0.4	0.8	0.8	1	0.5	0.5	1	0.5	0.4	0.5	0.4	1	0.5	0	0	1	0.5	0.5	0.5	0	0	0.51	
8	*Alp-2*	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0.13	
9	*α-Est-1*	A	1	1	1	1	1	1	1	0	0	0.3	0	0	1	1	1	1	1	1	1	0	0	0.5	0.4	0	0.63	0*
10	*α-Est-1*	B	0	0	0	0	0	0	0	0	1	0.4	0	0	0	0	0	0	0	0	0	0	0	0.5	0.2	0	0.09	
11	*α-Est-1*	C	0	0	0	0	0	0	0	0	0	0.3	1	1	0	0	0	0	0	0	0	0	0	0	0.2	1	0.15	
12	*α-Est-2*	A	1	1	1	1	1	1	0.8	0.5	0	0.3	0	0	0	0.4	0	0	0	0	0.5	0.5	0.5	0	0.2	0.5	0.43	0*
13	*α-Est-2*	B	0	0	0	0	0	0	0.2	0.5	1	0.6	1	1	1	0.6	1	1	1	1	0.5	0.5	0.5	1	0.7	0.5	0.57	
14	*α-Est-2*	C	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0.008	
15	*α-Est-3*	A	0.5	0	0.6	0.6	0.3	1	0	0	0	0.4	1	0.8	1	0	0.4	0.8	0.4	0	0.2	0	0	0	0.5	0	0.35	0.000086*
16	*α-Est-3*	B	0.5	1	0.4	0.4	0.7	0	0	0	0	0.1	0	0.2	0	0.5	0.3	0.2	0.6	1	0.8	1	0.6	1	0	0	0.39	
17	*α-Est-3*	C	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0.5	0.3	0	0	0	0	0	0.4	0	0.5	1	0.13	
18	*α-Est-4*	A	0	0	0.4	0.8	0.6	0	0	1	1	0	1	0.6	1	0.5	0.38	0	1	0	0.5	0.75	0.5	0.5	0	0.5	0.46	0.1343
19	*α-Est-4*	B	0	0	0.6	0.2	0.4	1	0	0	0	0	0	0.4	0	0.5	0.68	0	0	0	0.5	0.25	0.5	0.5	1	0.5	0.30	
20	*α-Est-5*	A	1	1	1	1	1	1	0.4	1	0.2	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	0.86	0*
21	*α-Est-5*	B	0	0	0	0	0	0	0.6	0	0.8	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0.10	
22	*β-Est-1*	A	0.4	0.2	0	0.4	0.2	0	1	1	0.6	0.5	0	0	1	1	0	1	0	0	0.38	0	0.5	0.7	1	1	0.45	0*
23	*β-Est-1*	B	0.6	0.8	1	0.6	0.8	1	0	0	0.4	0.5	1	1	0	0	0	0	1	1	0.63	1	0.5	0.3	0	0	0.51	
24	*β-Est-2*	A	1	1	1	0.4	0.2	0	0.5	0.5	0.25	1	0.6	0	1	0.8	0.6	1	0	0	0.5	0.5	0.5	1	1	1	0.60	0*
25	*β-Est-2*	B	0	0	0	0.6	0.8	1	0.5	0.5	0.75	0	0.4	1	0	0.2	0.4	0	0	0	0.5	0.5	0.5	0	0	0	0.32	
26	*β-Est-3*	A	1	1	1	0.4	0.2	0	0	1	0	0.5	0.5	0.7	1	1	1	0	0	0	0	0	0	0.5	0.17	0	0.46	0*
27	*β-Est-3*	B	0	0	0	0.6	0.8	1	0	0	0	0.5	0.5	0.3	0	0	0	0	0	0	0	0	0	0.5	0.83	1	0.25	
28	*β-Est-4*	A	0	0	0	0.5	0.5	0.88	1	0	0	0.3	1	0.63	1	0.5	0.63	0	1	1	0.5	0.5	0	0.9	1	1	0.54	0.689
29	*β-Est-4*	B	0	0	0	0.5	0.5	0.13	0	0	0	0.7	0	0.38	0	0.5	0.38	0	0	0	0.5	0.5	1	0.1	0	0	0.22	
30	*β-Est-5*	A	0	0.8	0.4	0.6	0.13	0	0	0	1	0.7	0.2	0.5	1	1	1	1	0.2	0	0.5	0	1	0.5	0.7	0	0.47	0.00002*
31	*β-Est-5*	B	1	0.2	0.6	0.4	0.88	1	0	0	0	0.3	0.8	0.5	0	0	0	0	0.8	1	0.5	0	0	0.5	0.3	0	0.37	
32	*Per-1*	A	0.2	1	0	1	1	0.8	0.2	0	0	1	0	0.4	0	0.5	1	0.2	0	0	0.3	0.4	0	0.5	0.5	1	0.42	0*
33	*Per-1*	B	0.8	0	0	0	0	0.2	0.8	1	0	0	0	0.6	1	0.5	0	0.8	0	1	0.7	0.6	0.8	0.1	0	0	0.37	
34	*Per-1*	C	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0.2	0.4	0.5	0	0.09	
35	*Per-2*	A	0.1	0.8	0.8	0.1	0	0.2	0.2	0.4	0.2	0.8	0	0.7	0	0	1	1	1	1	0.1	0	0	0.5	1	1	0.45	0*
36	*Per-2*	B	0.9	0.2	0.2	0.9	1	0.8	0.8	0.6	0.8	0.2	1	0.3	1	1	0	0	0	0	0.9	1	1	0.5	0	0	0.55	
37	*Per-3*	A	0	0	0	1	1	1	1	0	1	0	0	0.5	0	0	1	1	0.9	0.5	1	1	1	1	0.8	0.4	0.59	0*
38	*Per-3*	B	0	0	0	0	0	0	0	0	0	0	1	0.5	1	1	0	0	0.1	0.5	0	0	0	0	0.2	0.6	0.20	
39	*Per-4*	A	1	1	1	0	0.6	1	0.4	0.2	0.5	1	0	0	1	1	1	0.2	0.3	0.5	1	1	1	0.8	1	0.8	0.68	0*
40	*Per-4*	B	0	0	0	0	0.4	0	0.6	0.8	0.5	0	1	1	0	0	0	0.8	0.7	0.5	0	0	0	0.2	0	0.2	0.28	
Mean number of alleles/ locus			0.55	0.5	0.45	0.53	0.63	0.55	0.6	0.7	0.73	0.53	0.53	0.5	0.53	0.65	0.68	0.7	0.53	0.7	0.45	0.63	0.55	0.5	0.45	0.43	0.38^{**}	$^{0.07}$

**Average of the mean frequency of alleles

* Significant at $p< 0.05$

Alp-1A, *α-Est*-1B, *α-Est*-2C and *Per*-1C were 0.008, 0.09 and 0.008 respectively. The allele had this percentage of frequency known as rare alleles. The presence of this allele could be due to deleterious mutations or may be due to evolutionary relics (Sammour *et al*. 2019). The detection of rare allele in combination with high allelic frequency of other loci led to the conclusion that the studied accessions had obvious genetic differentiation. The allele C of the isozymes *α-Est*-1C, *α-Est*-2C, *α-Est*-3C and *Per*-1C were detected in wild species only, whereas the allele C of *Alp*-1C was present in cultivated and wild species. The frequency of *Alp*-1C in cultivated species (0.65) was higher than in the wild species (0.41). The disappearance of the Allele C of *α-Est* and *Per*-1C in the cultivated species could be due: (1) the effect of genetic drift of the multiple breeding of these species and (2) these alleles could play a role in the adaptation of the wild accessions to environmental stresses. Therefore, the transfer of the genes controlling these alleles to the cultivated species could make them tolerant to environmental stresses.

Based on Pearson correlation analysis, *A. hypochondriacus* and *A. caudatus* were the most closely related pair in grain *Amaranthus* spp. These results agreed with the findings of Gupta and Gudu (1991) and Chan and Sun (1997). The close relationship between *A. caudatus* on one hand, and A. *hybridus* and *A. hypochondriacus* on the other hand supported the results of Akin-Idowu *et al*. (2016) in their study on grain amaranth using RAPD analysis. Cluster analysis of the isozymes data showed that at least one of the accessions of *A. hybridus* was correlated with accessions of grains amaranths. This supported the monophyletic hypothesis of Sauer (1967) that suggested that *A. hybridus* is most likely the common ancestor of the grain amaranths.

Conclusion

This research work gave a comprehensive insight into the genetic diversity among accessions of cultivated and wild Amaranth collected from different geographical regions

using phenotypic and isoenzymatic markers. The results obtained indicated a considerable genetic diversity among *Amaranthus* accessions. This diversity is not connected to geographical distribution of the studied accessions, and useful in management of genetic resources and favorable breeding programs. However, there is a desperate need for extensive future molecular studies to affirm the genetic diversity between *Amaranthus* accessions from different regions of the world.

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Author Contributions

Reda H. Sammour: Conceptualization, methodology, investigation, software, formal analysis, writing - review and editing; Mohammed Mira: Methodology, investigation, software, formal analysis, writing - original draft; Safa A. Radwan: Methodology, validation, supervision, writing – original draft.

Conflicts of Interest

All authors declare no conflict of interest

Data Availability

Data presented in this study will be available on a fair request to the corresponding author

Ethics Approval

Not applicable in this paper

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