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Diversity and principal component analysis of world veg *Amaranthus* collections

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Abstract

Amaranthus (*Amaranthus sp* L.) species have high economic and nutritional value, only limited information is available on intra- and interspecific genetic diversity and relationships among the species. The World Veg *Amaranthus* collection, comprises of more than 800 accessions of 19 species plus accessions with insecure species attribution. Morphological characterization of the 50 accessions based on the AVRDC-GRSU characterization Record sheet to characterize the phenotypic data. Diversity analysis and grouping of *Amaranthus* species by NJ tree. The principal component analysis (PCA) indicated the most of the 45.7% of the variation were more explained by ten principal components PC₁, PC₂, PC₃, PC₄, PC₅, PC₆, PC₇, PC₈, PC₉, PC₁₀. From this major 22% of the variation were explained by PC₁ and PC₂. The present study focused on to confirm the presence of variability and diversity analysis in World Veg *Amaranthus* collections and this could be exploited in the genetic improvement, species identification by using markers, and germplasm conservation.

Keywords: *Amaranthus* accessions, principal component analysis, diversity analysis, morphological characterization

Introduction

Amaranthus is one the most popular and highly nutritious leafy vegetable. Its grain is an extremely nutritional pseudo-cereal with a higher amount of proteins when compared to true cereals. The health benefits credited include decreasing plasma cholesterol levels, exerting an antitumor activity, reducing blood glucose levels and anemia. It has the potential for significant impact on malnutrition (Maughan *et al.*, 2011) [1]. *Amaranthus* have excellent nutritional value because of their high content of essential micronutrients such as β -carotene, iron, calcium, vitamin C and folic acid (Priya *et al.* 2007) [2]. Grain *amaranthus* is considered traditional food as both leaf and seed consisted as a dual purpose in Africa and Asia (Das, 2016) [3]. *Amaranthus* species have high economic and nutritional value, only limited information is available on intra- and interspecific genetic diversity and relationships among the species. Information on genetic diversity and relationships within and among crop species and their wild relatives is essential to efficiently utilize plant germplasm (Anjali *et al.*, 2012) [4]. The World Veg *Amaranthus* collection comprises more than 800 accessions of 19 species plus accessions with insecure species attribution from the world veg. The greater genetic diversity with the use of molecular markers helps us to study the conservation of the species and for crop improvement (Palmer 2009) [5]. Keeping the above aspects under consideration, the current study was conducted to facilitate the characterization of the species classifications of World Veg *Amaranthus* gene bank accessions by diversity analysis and variation in the quantitative characters for species identification.

Materials and Methods

The research work was carried out at World Veg Centre, Taiwan which is situated at southern part of Taiwan of 24.7° N latitude and 121.5° E Longitude. The experiment methodology has been carried out with the nineteen species of fifty accessions of *Amaranthus* collected from AVRDC Gene bank, Taiwan. The species collection was mentioned in the Table 1.

The core collection of *Amaranthus* accessions from Gene bank collected from various parts of the world to study the diversity and variable analysis with the morphological data. The lines, codes/name and origin passport data are indicated in Table 2

The *Amaranthus* yield characters and morphological traits evaluation of 50 accessions were sown inside the glasshouse. The temperature in the glasshouse maintained in the range from 60° F - 70° F. *Amaranthus* seeds were sown in well trays and named the genotypes. Pricking out was done after two weeks of germination for selection of good plants. Maintain five plants for each accession to morphological diversity analysis.

Morphological characters were accessed based on the AVRDC- GRSU Characterization Record sheet (2008) [6]. descriptors. The quantitative characters such as growth habit, days to 50% flowering, seed characters and the qualitative characters such as stem, leaf, flower pigmentation and seed color all these characters observed during seedling, vegetative, flowering and seed stage.

Morphological analysis

Morphological analysis of the fifty accessions of quantitative and qualitative characters for identification of the correct species. PCA (Principal Component Analysis) eight quantitative characters of 10 components confirmed the presence of trait variability in the germplasm collection of Amaranthus by using Biplot method. Cluster analysis of the Amaranthus accessions based on NJ (Neighbor Joining) tree

Ntsys to estimate phylogenetic tree Saitou and Nei's (1987) [7].

Results

Morphological assessment of the fifty Amaranthus accessions of 17 species were characterized based on the AVRDC – GRSU characterization work sheet. The observations were started at 50% inflorescence stage. Five replications for each accession and the observations are recorded on the plant height, leaf color, terminal inflorescence stalk length, 1000 seed weight, seed color, inflorescence attitude is mentioned in the Table 3. The present results were in concurrence with the findings of Wu *et al.*, (2000) [8]. Who studied diversity among 229 genotypes from 20 Amaranthus species based on morphological characteristics and observed wide variability which was useful in cultivar improvement for agronomic traits like plant height, seed, leaf color and stem color.



Fig 1: AM320 leaf pigmentation

1 – Entire lamina purple or pink; 2 – Basal Area Pigmented; 3- Central spot; 6- Margin and vein pigmented; 8- Normal green



Fig 2: AM330 Leaf shape

1-lanceolate; 2- Elliptical; 4- Obovate; 6- Rhombic



Fig 3: AM 340 Leaf margins

1. Entire; 3. Undulate

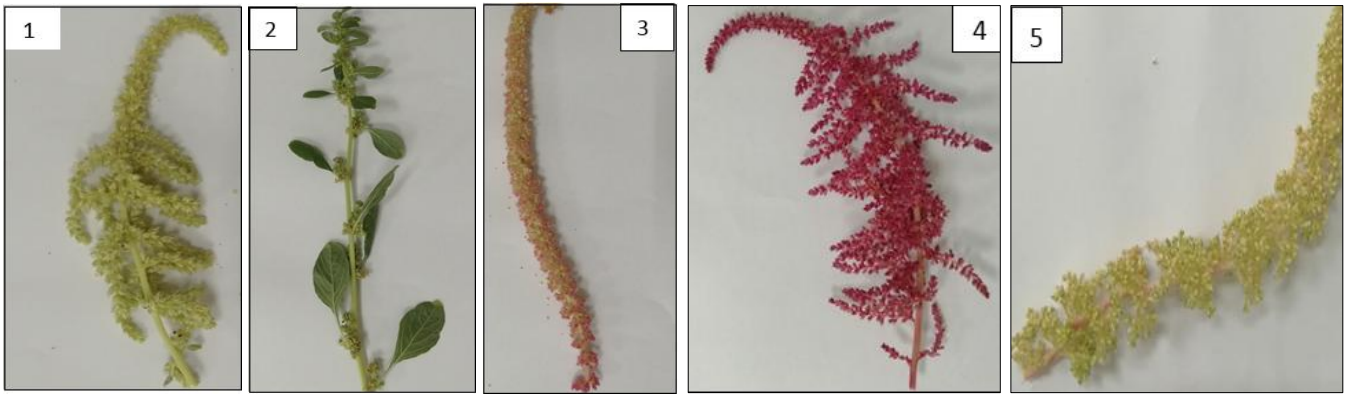


Fig 4: AM490 – Inflorescence color
1-yellow; 2-green; 3-pink; 4-red; X- mixture

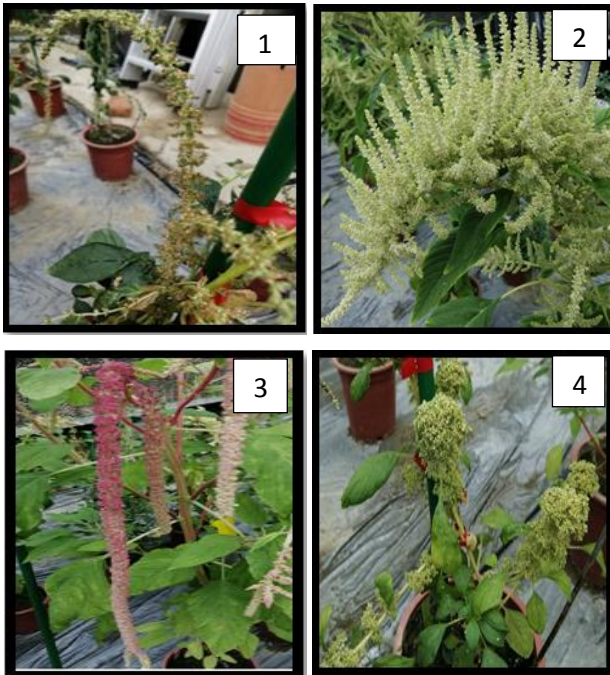


Fig 5: AM430- Terminal Inflorescence shape
1-spike; 2-panicle with short branches; 3-panicle with long branches;
4-club shaped at tips

PCA (Principal Component Analysis)

Principal Component Analysis performed based on fifty accessions of *Amaranthus* for eight quantitative characters for ten components. The ten-component had eigen value of 2.832, 2.381, 1.522, 1.431, 0.921, 0.738, 0.531, 0.423, 0.312, 0.281 respectively. These components had contributed 27.21percent, 20.32 percent, 19.21 percent, 12.31 percent, 10.12 percent, 8.21 percent, 5.15 percent, 3.21 percent, 2.53 percent, 1.83 percent of variance to the total variance. Together they

contributed 22.5percent of total variance. The eigen value, proportion of variance for each component was presented in table 4. The result shown that the eight characters were presented in screen plot with against Eigen value Fig 6.

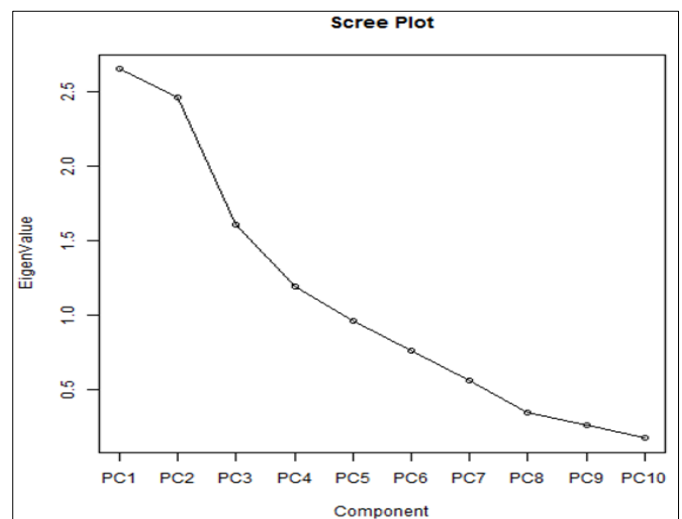


Fig 6: Screen plot for Principal Component Analysis

Diversity analysis

The phylogenetic relationship based on Neighbor Joining (NJ) method against the coefficient values shows most of the accessions grouped with other accessions belong to same species. The leaf *Amaranthus* *A. blitum*, *A. dubius*, *A. tricolor*, *A. viridis* are grouped together among the species but within the accessions its shows variation. The grain *Amaranthus* which includes *A. cruentus*, *A. hypochondriacus*, *A. caudatus* are grouped together in which *A. hybridus* lie between the *A. hypochondriacus* and *A. caudatus* as the ancestor as mentioned in Fig 7 (Markus *et al.*, 2016).

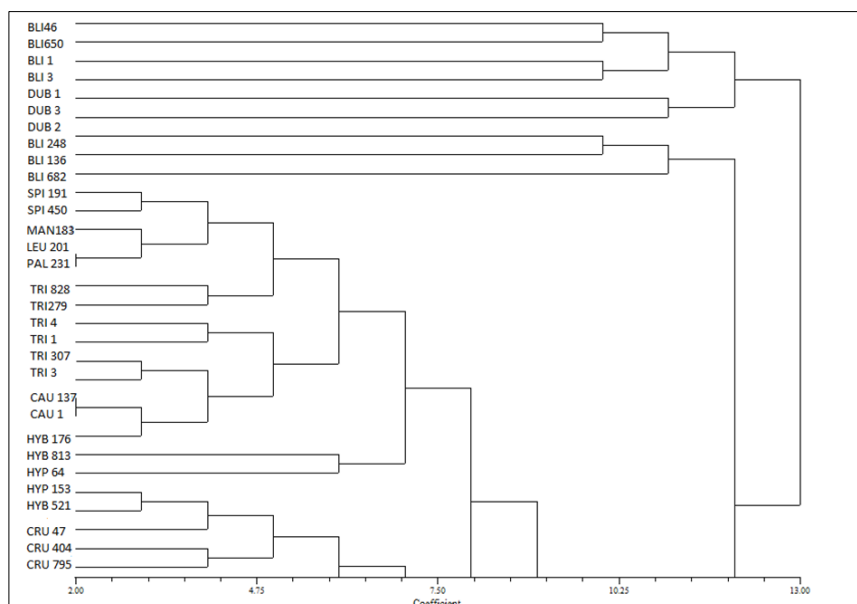


Fig 7: Neighbor joining tree for World Veg Amaranthus Collections

Conclusion

Morphological characterization of the fifty World Veg Amaranthus accession leads to correct identification of the species for future studies. Phylogenetic relationship of the accessions among and within the species it shows differentiation. The principal Component analysis traits plant height, days of emergence, terminal inflorescence length, lateral inflorescence length, leaf length, leaf width, days to 50% flowering were associated with principal component 1 (PC1). Days of emergence, leaf length, leaf width, lateral inflorescence, seed weight, days to 50% flowering were associated with principal component 2 (PC2) shows the highest variation in all quantitative characters indicated the most of the 45.7% of the variation were more explained by ten principal components PC₁, PC₂, PC₃, PC₄, PC₅, PC₆, PC₇, PC₈, PC₉, PC₁₀. From this major 22% of the variation were explained by PC₁ and PC₂. Characters with high variability are

expected to provide high level of gene transfer during breeding programs (Gana, 2006; Aliyu *et al.*, 2000) [9]. Therefore, high level of variability existing within the accessions and the characters will make for further improvement of the cultivars in breeding programs

Table 1: The Species collection from AVRC Gene bank

Leafy amaranthus		Grain Amaranthus
<i>A. spinosus</i>	<i>A. atropurpureus</i>	<i>A. cruentus</i>
<i>A. viridis</i>	<i>A. thunbergii</i>	<i>A. caudatus</i>
<i>A. graecizans</i>	<i>A. mantegazzianus</i>	<i>A. hypochondricus</i>
<i>A. blitum</i>	<i>A. leucocarpus</i>	
<i>A. dubis</i>	<i>A. retroflexus</i>	
<i>A. gracilis</i>	<i>A. spinosus</i>	
<i>A. tricolor</i>	<i>A. hybridus</i>	
<i>A. palmeri</i>		

Table 2: Passport data of Amaranth accessions from World Veg Centre, Taiwan

S. No	Accession no	Accessions	Species	Country
1	VI036227	BLI46	<i>A. blitoides</i>	Hungary
2	VI036228	CRU47	<i>A. cruentus</i>	Hungary
3	VI036225	GRA44	<i>A. graecizans</i>	Hungary
4	VI042947	HYP64	<i>A. hypochondricus</i>	Indonesia
5	VI044376	CRU127	<i>A. cruentus</i>	Ghana
6	VI044384	BLI136	<i>A. blitum</i>	India
7	VI044385	CAU137	<i>A. caudatus</i>	India
8	VI044397	HYP153	<i>A. hypochondricus</i>	India
9	VI044411	SPI166	<i>A. spinosus</i>	Puerto Rico
10	VI044405	BLI161	<i>A. blitum</i>	India
11	VI044419	HYB174	<i>A. hybridus</i>	USA
12	VI044421	HYB176	<i>A. hybridus</i>	USA
13	VI044435	ATR601	<i>A. atropurpureus</i>	Indonesia
14	VI044436	SPI191	<i>A. spinosus</i>	Thailand
15	VI044427	MAN183	<i>A. mantegazzianus</i>	USA
16	VI044432	VIR187	<i>A. viridis</i>	Indonesia
17	VI044441	HYP198	<i>A. hypochondricus</i>	Nigeria
18	VI044445	LEU201	<i>A. leucocarpus</i>	India
19	VI044460-A	HYP216	<i>A. hypochondricus</i>	USA
20	VI044465	GRA222	<i>A. graecizans</i>	India
21	VI044473	PAL231	<i>A. palmeri</i>	Senegal
22	VI046137	BLI248	<i>A. blitum</i>	Lao's people democratic Republic
23	VI047442	TRI279	<i>A. tricolor</i>	India
24	VI047666	TRI307	<i>A. tricolor</i>	Bangladesh

25	VI048310	RET377	<i>A. retroflexus</i>	Vietnam
26	VI048311	RET378	<i>A. retroflexus</i>	Vietnam
27	VI048391	RET379	<i>A. retroflexus</i>	Vietnam
28	VI049484	SPI450	<i>A. spinosus</i>	Thailand
29	VI050450	TRI828	<i>A. tricolor</i>	Unknown
30	VI050445	HYB475	<i>A. hybridus</i>	Unknown
31	VI050454	THU484	<i>A. thunbergii</i>	Unknown
32	VI050456	THU486	<i>A. thunbergii</i>	Unknown
33	VI050468	THU498	<i>A. thunbergii</i>	Unknown
34	VI051003	HYP526	<i>A. hypochondricus</i>	Kenya
35	VI050998	HYP521	<i>A. hypochondricus</i>	Cameroon
36	VI054569	GRA532	<i>A. gracilis</i>	Philippines
37	VI054578	GRA541	<i>A. gracilis</i>	Philippines
38	VI054797	VIR547	<i>A. viridis</i>	Lao people's democratic republic
39	VI056019	GRA612	<i>A. gracilis</i>	Cambodia
40	VI055959-A	VIR606	<i>A. viridis</i>	Lao people's democratic republic
41	VI057220	GRA642	<i>A. gracilis</i>	Cambodia
42	VI058952	BLI650	<i>A. blitum</i>	Unknown
43	VI058984	BLI682	<i>A. blitum</i>	Unknown
44	VI059043	HYB742	<i>A. hybridus</i>	Unknown
45	VI061505	CRU795	<i>A. cruentus</i>	Unknown
46	VI061517	CRU808	<i>A. cruentus</i>	Tanzania
47	VI061521	HYB813	<i>A. hybridus</i>	Madagascar
48	VI062429	CRU404	<i>A. cruentus</i>	Madagascar
49	VI064068-C	HYP211	<i>A. hypochondricus</i>	Tanzania
50	VI064156	HYP102	<i>A. hypochondricus</i>	Madagascar

Table 3: Morphological characterization of the *Amaranthus* accession samples based on the AVRDC – GRSU descriptors.

S.N O	Species	Corrected species	Plant height	Stem pigmentation	Spines in leaf axils	Leaf length	Leaf width	Leaf pubescence	Leaf pigmentation	Leaf shape	Leaf margin
1	BLI46	BLI	51.6	3	1	3.5	1.1	3	8	1	1
2	CRU47	CRU	69	2	1	7.7	4.2	7	8	1	1
3	GRA44	BLI	45.7	3	1	3.3	1.7	7	8	1	1
4	HYP64	HYP	117.4	3	1	16.6	7.1	7	9	1	1
5	CRU127	HYP	97.8	3	1	14.2	5.6	7	9	1	1
6	BLI136	BLI	89.6	X	1	5.1	2.7	3	8	4	1
7	CAU137	CAU	76.8	2	1	10.7	5.6	3	8	1	1
8	HYP153	HYP	45.2	2	1	6.7	3.5	3	1	1	1
9	SPI166	DUB	66.4	2	1	6.7	4.6	0	8	2	1
10	BLI161	BLI	73	2	1	3.8	2.1	0	8	4	1
11	HYB174	HYP	109.6	3	1	15.8	5.5	3	8	4	1
12	HYB176	HYB	30.4	1	1	5	3.3	7	9	4	1
13	ATR601	DUB	107	1	1	15	7.5	3	9	2	1
14	SPI191	SPI	122.7	2	2	7.1	3.2	3	9	1	3
15	MAN183	MAN	39.8	3	1	5.2	2.2	0	8	1	1
16	VIR187	VIR	91	1	1	6.8	4.5	0	8	6	1
17	HYP198	CRU	81.2	3	1	16.7	7.7	7	8	1	1
18	LEU201	LEU	57.6	3	1	9.5	3.8	0	8	1	1
19	HYP216	CAU	97	X	1	12.2	6.1	3	8	1	1
20	GRA222	BLI	71.2	2	1	3.6	2	0	6	4	1
21	PAL231	PAL	86.6	3	1	4.4	2.3	0	9	4	1
22	BLI248	BLI	59.2	3	1	5.7	3.5	0	8	4	1
23	TRI279	TRI	107.4	X	1	161.7	10.6	3	6	6	1
24	TRI307	TRI	101	2	1	14.6	10.3	3	2	6	1
25	RET377	HYB	112.7	x	1	12.9	6.8	3	2	1	1
26	RET378	HYB	111.7	1	1	9.7	4.6	0	9	1	1
27	RET379	TRI	91.5	2	1	15	9.3	3	6	6	1
28	SPI450	DUB	133.4	1	2	7.6	3.4	3	9	1	3
29	HYB475	TRI	54.3	x	1	8.1	3.9	0	8	2	1
30	TRI828	HYP	116	2	1	15.6	10.9	3	9	6	1
31	THU484	TRI	75.2	2	1	8.2	4.2	0	8	6	1
32	THU486	TRI	65.8	2	1	8.5	4.5	0	1	6	1
33	THU498	TRI	113.5	2	1	14.5	7.7	0	9	6	1
34	HYP526	HYP	89.6	3	1	10	4.6	0	8	1	1
35	HYP521	HYP	96.4	1	1	17.1	5.7	0	8	1	1
36	GRA532	VIR	88.8	42	1	6.9	5.5	0	8	6	1
37	GRA541	HYP	118	x	1	16.2	6.1	3	1	1	1
38	VIR547	VIR	72.4	2	1	5.3	3.9	0	8	6	1

39	GRA612	BLI	107	2	1	10.2	6.4	0	9	2	1
40	VIR606	BLI	55.8	1	1	5.1	3.2	0	8	2	1
41	GRA642	TRI	86	1	1	12	8.6	0	3	6	1
42	BLI650	BLI	104	x	1	13	6.2	0	2	2	1
43	BLI682	BLI	111.7	1	1	11.4	5.5	0	2	2	1
44	HYB742	DUB	77.6	x	1	8.1	5.7	0	9	6	1
45	CRU795	CRU	70.2	1	1	20.4	9.7	0	8	1	1
46	CRU808	CRU	113.2	1	1	21.1	9.4	0	8	1	1
47	HYB813	HYB	31.4	1	1	3.5	1.8	7	8	2	1
48	CRU404	CRU	132.2	2	1	19.9	9.1	0	3	1	1
49	HYP211	HYP	66	1	1	10	5.2	0	8	1	1
50	HYP102	CRU	89.6	2	1	20.7	9.6	0	3	1	1

S.NO	Species	Corrected species	Petiole pigmentation	Days to flowering (50%)	Terminal inflorescence stalk length	Terminal inflorescence laterals length	Terminal inflorescence shape	Terminal inflorescence attitude	Inflorescence color	Seed color	Seed weight
1	BLI46	BLI	1	33	0.3	1.4	2	1	2	5	1.4
2	CRU47	CRU	1	28	25.1	3.7	1	2	2	4	0.86
3	GRA44	BLI	5	28	0.4	1.3	2	1	2	5	0.68
4	HYP64	HYP	5	56	31.7	12.3	2	2	1	4	0.44
5	CRU127	HYP	5	46	31.7	10.9	2	2	1	4	0.46
6	BLI136	BLI	1	40	1.4	2.3	2	1	2	5	0.6
7	CAU137	CAU	1	46	40.7	3.5	3	2	3	2	0.39
8	HYP153	HYP	5	33	17.6	3.4	1	2	2	4	0.85
9	SPI166	DUB	3	35	15.4	2.9	1	2	2	5	0.48
10	BLI161	BLI	3	28	2.3	3.6	2	1	2	5	0.66
11	HYB174	HYP	3	51	27.8	10.5	2	2	1	4	0.44
12	HYB176	HYB	5	33	7.3	4.3	1	2	2	5	0.36
13	ATR601	DUB	1	26	17.4	3	1	2	2	5	0.54
14	SPI191	SPI	3	37	15.3	2.4	1	2	2	5	0.34
15	MAN183	MAN	5	28	10.8	7.7	4	1	1	1	1.12
16	VIR187	VIR	1	38	14.9	2.4	1	2	2	5	0.4
17	HYP198	CRU	5	46	22	7.5	2	2	1	4	0.52
18	LEU201	LEU	5	28	26	5.1	2	2	1	1	1.35
19	HYP216	CAU	5	47	33.8	4.3	3	2	3	2	0.36
20	GRA222	BLI	3	31	0.5	1.5	2	1	2	5	0.65
21	PAL231	PAL	5	31	1.7	2.7	2	1	2	5	0.62
22	BLI248	BLI	5	38	6.2	2.6	1	2	2	5	1
23	TRI279	TRI	4	55	10.2	4.8	1	1	2	4	1.4
24	TRI307	TRI	3	56	14	4.2	1	2	2	5	0.82
25	RET377	HYB	5	40	40.4	3.9	1	1	x	1	1.4
26	RET378	HYB	3	45	18.2	4.5	1	2	4	5	0.84
27	RET379	TRI	1	46	15.3	2.4	1	2	2	5	0.26
28	SPI450	DUB	1	28	23.2	3.4	1	2	2	1	1
29	HYB475	TRI	3	45	12.1	3.8	1	2	4	5	0.82
30	TRI828	HYP	3	38	17.3	4.2	1	2	2	5	0.74
31	THU484	TRI	3	38	11.4	3.6	1	2	4	5	1.2
32	THU486	TRI	3	62	10.7	4.8	1	2	2	4	1.4
33	THU498	TRI	1	40	30	3.9	1	2	2	4	1.12
34	HYP526	HYP	1	45	27.8	9.9	2	2	2	4	0.46
35	HYP521	HYP	x	38	12.1	2.2	1	2	2	5	0.4
36	GRA532	VIR	x	50	30.3	12.2	2	2	4	4	0.4
37	GRA541	HYP	3	37	10.3	2.1	1	1	x	5	0.4
38	VIR547	VIR	x	43	24.5	3.2	1	2	2	5	0.38
39	GRA612	BLI	5	28	7.1	2.7	1	1	2	5	1.1
40	VIR606	BLI	1	45	33.5	4.2	1	2	2	5	0.8
41	GRA642	TRI	5	33	26	1.9	1	2	3	4	0.32
42	BLI650	BLI	5	37	29.9	1.8	1	2	3	4	0.3
43	BLI682	BLI	1	37	16.8	2.5	1	2	2	5	0.37
44	HYB742	DUB	1	42	26	3.2	1	2	2	4	0.5
45	CRU795	CRU	1	45	28	3.4	1	2	2	4	0.8
46	CRU808	CRU	1	28	8	2.8	1	1	2	5	0.5
47	HYB813	HYB	x	47	39.3	8.1	1	2	x	1	1.12
48	CRU404	CRU	1	37	27	3.7	1	2	2	4	0.6
49	HYP211	HYP	x	47	36.9	8	1	2	x	1	1.12
50	HYP102	CRU	5	40	40.4	3.9	1	1	x	1	1.4

Table 4: Eigen value, proportion of variance for each component of Principal Component Analysis.

Characters	PC ₁	PC ₂	PC ₃	PC ₄	PC ₅	PC ₆	PC ₇	PC ₈	PC ₉	PC ₁₀
Days to emergence	-0.0889	-0.1029	0.3750	0.3967	-0.0504	0.1656	0.1014	-0.0930	0.0212	-0.0211
Plant height	0.2245	-0.0214	0.0721	-0.0843	0.5155	0.5186	0.1531	0.1326	0.1254	-0.0061
Leaf length	0.2044	0.1707	0.2837	0.3452	0.2128	-0.1383	-0.1611	-0.0867	-0.0315	-0.0215
Leaf width	0.1542	0.5741	0.0401	0.3139	-0.0948	0.0499	-0.1177	-0.0178	-0.0141	-0.0514
Days to 50% flowering	0.3130	0.0941	0.0032	-0.0165	0.0504	-0.0608	0.4060	0.0460	-0.0841	0.0621
Terminal Inflorescence length	0.2167	0.2534	-0.2309	0.1870	-0.1222	0.0853	-0.1824	-0.1824	-0.0674	0.1542
Lateral Inflorescence length	-0.1876	0.2436	-0.0719	-0.0685	0.2826	0.1081	0.4228	0.4228	0.0214	-0.0012
Seed weight	0.1656	0.0423	-0.2334	0.0791	0.1254	-0.984	0.2214	0.2154	0.0210	0.0058
Eigen value	2.832	2.381	1.522	1.431	0.921	0.738	0.531	0.423	0.312	0.281
Cumulative %	27.210	20.320	19.211	12.310	10.128	8.210	5.150	3.215	2.532	1.832

Discussion

Furthermore, studies on genetic diversity and markers are needed to differentiate the species and good quality germplasm maintenance is important for *Amaranthus* to avoid outcrossing. Since *Amaranthus* is the wide diversity crop and outcrossing leads to difficult identification of the species among and within the species.

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