

Supplementary Materials

Photosynthetic phenomics of field and greenhouse grown amaranths vs. sensory and species delimits

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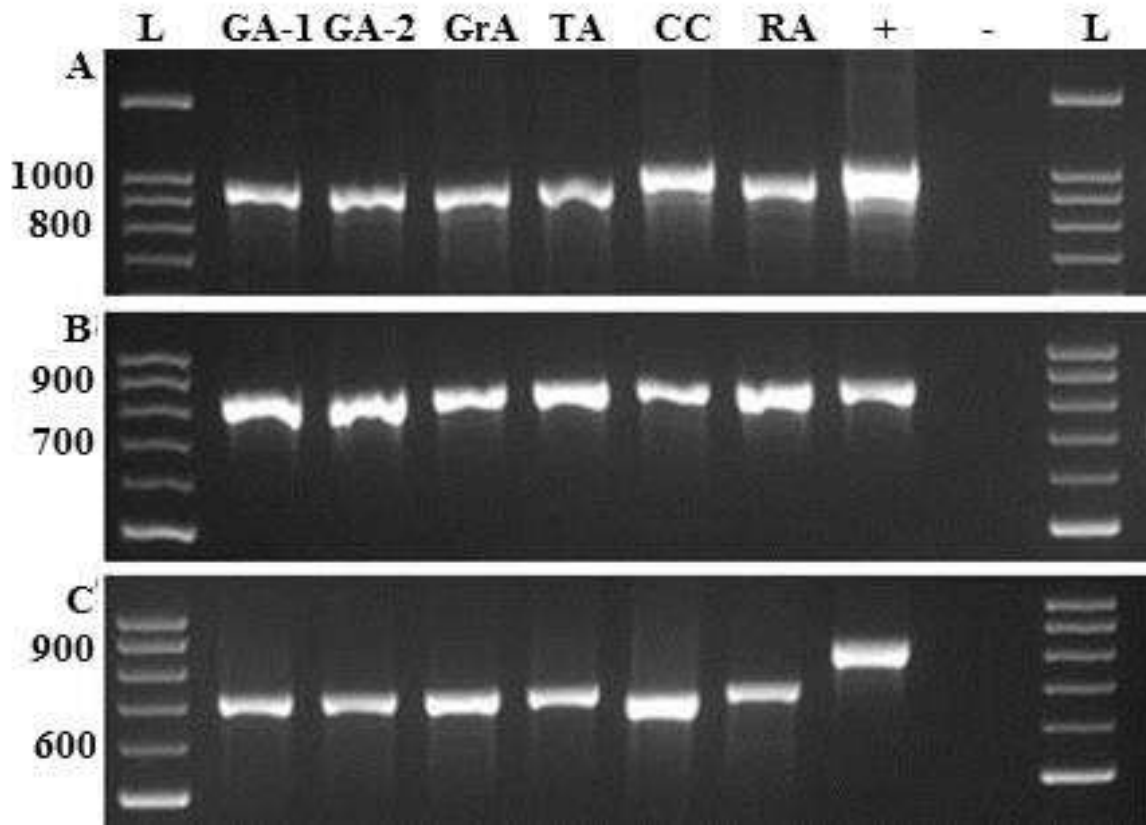


Figure S1. PCR bands obtained for the three plant DNA barcoding markers used. A: atpB- rbcL; B: matK-trnT; C: ITS 1-4. L: 100bp DNA Ladder; GA-1: Giant Amaranthus-1 [*A. cruentus*]; GA-2: Giant Amaranthus-2 [*A. cruentus*]; GrA: Green Amaranthus [*A. viridis*]; TA: Thorny Amaranthus [*A. spinosus*]; CC: Cockscomb [*Celocia cristata*]; RA: Red Amaranthus [*A. tricolor*], + : Positive control [rice DNA]; - : Negative control [no template in PCR]. The sizes of the two key bands are shown for each marker.

S1 Table. Sampling locations

Botanical name	Common name	Abbreviation	Location and GPS Coordinates
<i>A. hybridus</i> subsp. <i>cruentus</i> L.	Giant Amaranthus-1	GA-1	<i>Peradeniya</i> (N 7° 16' 11.51"; E 80° 35' 37.80")
<i>A. hybridus</i> subsp. <i>cruentus</i> L.	Giant Amaranthus-2	GA-2	<i>Balalla</i> (N 7° 48' 25.53"; E 80° 15' 15.37")
<i>A. viridis</i> L.	Green Amaranthus	GrA	<i>Nawalapitiya</i> (N 7° 2' 40.92"; E 80° 30' 57.82")
<i>A. spinosus</i> L.	Thorny Amaranthus	TA	<i>Gampola</i> (N 7° 10' 23.75"; E 80° 33' 37.84")
<i>A. tricolor</i> L.	Red Amaranthus	RA	<i>Weligalla</i> (N 7° 11' 19.96"; E 80° 35' 29.05")
<i>C. cristata</i> L.	Cockscomb	CC	<i>Gampola</i> (N 7° 10' 23.75"; E 80° 33' 37.84")

S2 Table. The details of the DNA barcoding markers and PCR

Marker	Sequence (5'→3')	PCR Profile										References
		Initial denaturation		Denaturation		Primer Annealing		Initial extension		Final extension		
		T ^x	Time	T	Time	T	Time	T	Time	T	Time	
<i>atpB-rbcL spacer</i>	PF: GAAGTAGTAGGATTGATTCTC	94	4 min	94	30 sec	45	30 sec	72	2 min	72	5 min	[1]
	PR: TACAGTTGTCCATGTACCAG											
<i>matK-trnT spacer</i>	PF: GCATAAATATAYTCCYGAARATAAGTGG	95	1.5 min	95	30 sec	48	1 min	68	2 mins	68	20 mins	[2]
	PR: TGGGTTGCTAACTCAATGG											
<i>ITS1-4</i>	PF: TCCGTAGGTGAACCTTGCGG	95	3 min	95	1 min	55	1 min	72	1.5 min	72	4 min	[3]
	PR: TCCTCCGCTTATTGATATGC											

^xTemperatures (T) are given in °C.

References

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S3 Table. The DNA sequences used for the phylogenetic analysis

Species	Families	Location	Voucher number	Genbank accession number			References	
				<i>ITS</i>	<i>atpB-rbcL</i>	<i>matK-trnT</i>		
<i>Oxybaphus nyctagineus</i>	Nyctaginaceae	Beijing, Haidian	13524	KY9688946	Not assessed	Not assessed	Xu <i>et al.</i> , (2017)	
<i>Chenopodium glaucum</i>	Chenopodiaceae	Jiangsu, Jiangyin	13506	KY9688937	Not assessed	Not assessed		
<i>Dysphantha pumilio</i>	Chenopodiaceae	Jiangsu, Jiangyin	13316	KY9688827	Not assessed	Not assessed		
<i>Alternanthera philoxeroides</i>	Amaranthaceae	Hunan, Changsha	201509177	KY9688879	Not assessed	Not assessed		
<i>Alternanthera philoxeroides</i>	Amaranthaceae	Hunan, Changsha	201509153	KY9688872	Not assessed	Not assessed		
<i>Amaranthus blitum</i>	Amaranthaceae	Hunan, Changsha	201509155	KY9688874	Not assessed	Not assessed		
<i>Amaranthus blitum</i>	Amaranthaceae	Liaoning, Dalian	2015054	KY9688859	Not assessed	Not assessed		
<i>Amaranthus cruentus</i>	Amaranthaceae	Hunan, Zhuzhou	201509205	KY9688887	Not assessed	Not assessed		
<i>Amaranthus hybridus</i>	Amaranthaceae	Hunan, Leiyang	201509228	KY9688895	Not assessed	Not assessed		
<i>Amaranthus hybridus</i>	Amaranthaceae	Jiangsu, Zhenjiang	13369	KY9688907	Not assessed	Not assessed		
<i>Amaranthus hybridus</i>	Amaranthaceae	Jiangsu, Jiangyin	13489	KY9688931	Not assessed	Not assessed		
<i>Amaranthus palmeri</i>	Amaranthaceae	Hunan, Changsha	201509140	KY9688864	Not assessed	Not assessed		
<i>Amaranthus palmeri</i>	Amaranthaceae	Hunan, Changsha	201509141	KY9688865	Not assessed	Not assessed		
<i>Amaranthus spinosus</i>	Amaranthaceae	Hunan, Leiyang	201509229	KY9688896	Not assessed	Not assessed		
<i>Amaranthus tricolor</i>	Amaranthaceae	Hunan, Zhuzhou	201509212	KY9688890	Not assessed	Not assessed		
<i>Amaranthus tricolor</i>	Amaranthaceae	Hunan, Zhuzhou	201509213	KY9688891	Not assessed	Not assessed		
<i>Amaranthus viridis</i>	Amaranthaceae	Hunan, Changsha	201509139	KY9688863	Not assessed	Not assessed		
<i>Celosia argentea</i>	Amaranthaceae	Jiangsu, Taizhou	13398	KY9688912	Not assessed	Not assessed		
<i>Celosia argentea</i>	Amaranthaceae	Jiangsu, Yangzhou	13482	KY9688928	Not assessed	Not assessed		
<i>Celosia cristata</i>	Amaranthaceae	Jiangsu, Taizhou	13442	KY9688920	Not assessed	Not assessed		
<i>Celosia cristata</i>	Amaranthaceae	Beijing, Haidian	13519	KY9688942	Not assessed	Not assessed		
<i>Hydrocotyle vulgaris</i>	Umbelliferae	Yunnan, Kunming	201507071	KY9688850	Not assessed	Not assessed		
<i>Amaranthus tuberculatus</i>	Amaranthaceae	Liaoning, Dalian	2015052	KY9688858	Not assessed	Not assessed		
<i>Amaranthus tuberculatus</i>	Amaranthaceae	Jiangsu, Lianyungang	13461	KY9688925	Not assessed	Not assessed		
<i>Amaranthus tuberculatus</i>	Amaranthaceae	Jiangsu, Jiangyin	13495	KY9688934	Not assessed	Not assessed		
<i>Phytolacca octandra</i>	Phytolaccaceae	Jiangsu, Jiangyin	13305	KY9688823	Not assessed	Not assessed		
<i>Phytolacca octandra</i>	Phytolaccaceae	Jiangsu, Jiangyin	13311	KY9688825	Not assessed	Not assessed		
<i>Phytolacca thyrsoflora</i>	Phytolaccaceae	Yunnan, Kunming	201507058	KY9688845	Not assessed	Not assessed		
<i>Alternanthera philoxeroides</i>	Amaranthaceae	Hunan, border inspection	X12	KY9688963	Not assessed	Not assessed		
<i>Amaranthus spinosus</i>	Amaranthaceae	Jiangsu, border inspection	X13	KY9688964	Not assessed	Not assessed		
<i>Amaranthus viridis</i>	Amaranthaceae	Sri Lanka	DMB192	MN103755	MN104855	MN104861		This study
<i>Amaranthus tricolor</i>	Amaranthaceae	Sri Lanka	DMB193	MN103756	MN104856	MN104862		
<i>Amaranthus spinosus</i>	Amaranthaceae	Sri Lanka	DMB194	MN103757	MN104857	MN104863		
<i>Amaranthus hybridus</i> subsp. <i>Cruentus</i>	Amaranthaceae	Sri Lanka	DMB195	MN103758	MN104858	MN104864		
<i>Amaranthus hybridus</i> subsp. <i>Cruentus</i>	Amaranthaceae	Sri Lanka	DMB196	MN103759	MN104859	MN104865		
<i>Celosia cristata</i>	Amaranthaceae	Sri Lanka	DMB197	MN103760	MN104860	MN104866		

S4 Table. Morphological descriptions of *Amaranthus* spp. and cockscomb

Vernacular names	Botanical name	Botanical description
Giant Amaranthus-1 (GA-1), Mexican grain amaranth, <i>Yodha Thampala-1</i>	<i>A. hybridus</i> subsp. <i>cruentus</i>	Stems: greenish or reddish-purple, erect, stout, glabrous, cylindrical stems with ridges reaching up to about 150 cm – 180 cm in height. Leaves: greenish-purple, simple, long petiolated, broadly lanceolate to rhombic ovate shaped, length varies from 15 cm – 20 cm, width varies from 4 cm – 5 cm. Inflorescence: pink to maroon, large, complex inflorescence consisting of numerous agglomerated cymes arranged in axillary and terminal racemes and spikes growing up to about 40 cm in length with many lateral, perpendicular thin branches (Figure 1(a)). Flowers: reddish, sessile flowers arranged in a large complex inflorescence. Fruits: dry dehiscent fruit capsule and ovoid to ellipsoid in shape, compressed brownish-black seeds [1, 2].
Giant Amaranthus-2 (GA-2), Purple Amaranth, Prince-of-Wales feather, <i>Yodha Thampala-2</i>	<i>A. hybridus</i> subsp. <i>cruentus</i>	Stems: greenish, erect, cylindrical, stout and about 100 cm – 150 cm in height. Leaves: greenish, simple, long petiolated, entire margined, minutely emarginated leaf apex, broadly lanceolate to rhombic ovate shaped leaves which vary from 15 cm – 18 cm in length and 4 cm – 8 cm in width. Inflorescence: large, purple-greenish, complex inflorescence arranged into spikes at thick branches (Figure 1(b)). Flowers: small, greenish, flowers arranged in spikes. Fruits: dehiscent fruit capsules and ovoid in shape, ellipsoid brownish seeds [1, 2].
Green Amaranth (GrA), Slender amaranth, <i>Kola Thampala</i>	<i>A. viridis</i>	Stems: greenish, erect, slender, branched, glabrous, rounded stems with ridges which reach up to about 6 cm – 80 cm in height. Leaves: light greenish, simple, minutely emarginated leaf apex, obtuse base, entire to sinuate margined, deeply veined, long petiolated, ovate to lanceolate shaped, leaves varied from 7 cm – 9 cm in length and 4 cm – 5 cm in width. Inflorescence: greenish, slender, branched axillary to the terminal panicle spikes or in the lower part of the stem as dense axillary clusters, 2 cm – 12 cm in length and 2 mm – 5 mm in width (Figure 1(c)). Flowers: small, greenish male and female flowers arranged in a radial pattern along the spike/inflorescence. Fruits: wrinkled, indehiscent, small, and brownish fruits with glossy blackish, sub globose, slightly compressed, acute margined seeds [3, 4].
Thorny Amaranthus (TA), Spiny amaranth, Spiny pigweed, Prickly amaranth, <i>Katu Thampala</i>	<i>A. spinosus</i>	Stems: greenish or reddish-purple, erect or proximally ascending, heavily-branched, hard, obtusely angular, bushy, simple, stems growing up to about 60 cm – 150 cm in height, possess nodes comprised of oppositely arranged strong spines (modified bracts) of about 1.5 cm – 2.5 cm in length observed at the leaf axil. Leaves: dark greenish, simple, alternate, glabrous or sparse hairs below the main vein, long petiolated, entire margined, minutely emarginated leaf apex, elliptic to lanceolate-oblong shaped leaves. Inflorescence: greenish arranged into spikes at the thick branches (Figure 1(d)). Flowers: tiny greenish flowers; unisexual grouped in clusters in the axils of the leaves and branched terminal spikes. Fruits: dehiscent fruit capsules and ovoid in shaped fruits having 5 mm – 10 mm diameters with blackish, smooth, lens-shaped seeds having a thin margin [5-7].
Red amaranth (RA), <i>Rathu thampala</i> , Bireum, Tandaljo, Tandalja bhaji	<i>A. tricolor</i>	Stems: reddish to purplish, stout, much-branched, glabrous stems of about 100 cm – 125 cm in height. Leaves: greenish or purplish suffused, long petiolated, minutely emarginated apex, obtuse base, entire to sinuate margined, rhomboid-ovate or elliptic to lanceolate- oblong-shaped varied from 7 cm – 12 cm in length and 4 cm – 5 cm in width. Inflorescence: reddish inflorescence with axillary globose clusters of which upper clusters form terminal spikes, with male and female flowers intermixed (Figure 1(e)). Flowers: crimson flowers in globose clusters. Fruits: ovoid-shaped fruits 1.5 mm in length, and 1 mm – 1.5 mm in diameter with shiny brownish, smooth, lenticular seeds [3, 8].
Cockscomb (CC), <i>Kukulkaramal</i>	<i>C. cristata</i>	Stems: greenish, erect, herbaceous stems which grow up to about 30 cm – 100 cm in height. Leaves: light greenish with some maroon patches, simple, entire margined, rhombic ovate shaped, vary from 7 cm – 9 cm in length and 4 cm – 5 cm in width. Inflorescence: reddish dense undulating inflorescence that resembles the red color combs on the heads of roosters (Figure 1(f)). Flowers: brightly pinkish flowers which exhibited three parts; spikes, plumes, and crest. Fruits: thin, ovoid to almost globular 1.6 mm – 2.5 mm long fruit capsules and compressed, shiny blackish seeds [9, 10].

References of S4 Table

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S5 Table. Summary of the differences of photosynthetic parameters

	GA-1	GA-2	GrA	TA	RA	CC
	Field vs. GH	Field vs. GH	Field vs. GH	Field vs. GH	Field vs. GH	Field vs. GH
SPAD	S	H	H	L	H	L
LEF	S	H	L	S	S	H
ΦII	L	H	L	H	H	S
ΦNO	S	S	S	S	S	S
ΦNPQ	S	L	H	S	L	H

S: Significantly similar in field and GH conditions; H: Significantly higher in field compared to GH; L: Significantly lower in field compared to GH. (P<0.05)

S6 Table. Summary of PC analysis for photosynthetic parameters

PC loading status	PC1	PC2	PC3	PC4	PC5
Eigenvalue	2.42	1.54	0.79	0.17	0.07
Proportion of the variance explained	0.49	0.31	0.16	0.04	0.01
Cumulative variance explained	0.49	0.79	0.95	0.99	1.00

PC representation for each photosynthetic parameter					
SPAD	-0.48	0.36	0.47	0.64	0.13
LEF	-0.27	-0.59	-0.56	0.51	0.12
Φ II	-0.62	-0.08	-0.01	-0.27	-0.73
Φ NO	0.25	0.61	-0.55	0.33	-0.39
Φ NPQ	0.50	-0.38	0.41	0.40	-0.53