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Germplasm Characterization for Morphological Diversity in the Potential Futuristic Crop Amaranthus (*Amaranthus spp*.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study was carried out to characterize 209 Amaranth germplasm consisting of 124 grain amaranth and 85 vegetable amaranth accessions for various morphological traits in accordance with the DUS guidelines of PPVFRA, New Delhi. The genotypes were characterized for two seasons *viz*., *kharif* and summer, wherein same level of expression was observed for the traits. A total of 17 characters were scored, eight in grain amaranths and nine in vegetable amaranths related to vegetative morphology, inflorescence and seed traits. Distinct forms with respect to leaf colour, inflorescence colour, stem colour and other studied traits were observed among the genotypes. The majority of the grain amaranth accessions were green leaved with yellowish green stem, ridged stem surface having yellowish green, erect and dense inflorescence. Seed colour was predominantly creamish. Shannon-Weaver Diversity Index ranged from 1.778 (inflorescence colour) to 0.524 (presence of leaf blotch). Vegetable amaranth accessions had largely medium green leaf blade colour with green inflorescence and green stem. Further, most of the accessions

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were cutting types with black coloured seeds. Shannon-Weaver Diversity Index in vegetable amaranths varied from high for leaf blade colour (1.529) and stem colour (1.158) to low index for the trait presence of leaf blotch (0.190). Collection, morphological characterization and classification of germplasm helps in identifying distinct genotypes with contrasting characters, which would in turn facilitate to broaden the germplasm base and varietal identification.

Keywords: Germplasm; morphological characterization; PPVFRA; shannon-weaver diversity.

1. INTRODUCTION

Amaranthus (*Amaranthus* spp. L.) is considered as one of the ancient food crops of the world that had been disappeared for centuries, but rediscovered now as a promising crop for its remarkable nutritional value [1]. Genus *Amaranthus* belongs to the family Amaranthaceae and comprises of about 70 species that are distributed all over the world (Shukla et al., 2010; Thapa and Blair, 2018), about 40 of which are native to America [2]. Some species are cultivated for grain, some as leafy vegetables, as forage crops, ornamentals and few are wild species [3]. The most economically important species domesticated for grain production (2n=32, 34) are *Amaranthus hypochondriacus*, *A. cruentus* and *A. caudatus*. The major vegetable types are *Amaranthus tricolour* L., *A. dubius*, *A. blitum* and *A. viridis* [4,5]. The genus Amaranthus has received increased attention nowadays because of its nutritional properties of the grain and leaves and its ability to thrive well in diverse agro-geographic regions.

Morphological characterization of plant genetic resources for qualitative traits is a preliminary and basic requirement for generating vital information on useful traits, it is greatly implicated in pre-breeding and crop improvement programmes [6]. Morphological characters constitute an important set of well accepted descriptors for varietal characterization of crop species and help in establishing the distinctness of crop cultivars in Protection of Plant Variety (PPV) Systems [7,8]. As it is imperative to distinguish germplasm in plant breeding as well as for plant variety protection, these traits provide a simple way of quantifying genotypic variation and to establish distinctness of the genotypes. In addition, the morphological traits are selectively neutral [9], show simple inheritance (single/oligogenic), stable expression and are easily scorable. Hence, they serve as diagnostic descriptors of germplasm accessions and are helpful in developing ex-situ germplasm conservation strategies. These traits are useful in labelling, identification and easy retrieval of desired accessions from the germplasm database.

Amaranthus is the largest genus that includes an important group of plants exhibiting a great deal of morphological diversity. Several species of amaranthus distributed across the globe harbour remarkable diversity of landraces and cultivars. The close relationships that exist between the genotypes of different species and the wide adoptability of amaranths to different eco-geographic situations has resulted in tremendous diversity in morphological traits [10]. Amaranths, even though are monoecious [11] and self-pollinated [12], varying amounts of outcrossing [12] and frequent interspecific and intervarietal hybridization [13] has led to the creation of tremendous amount of variation in morphological traits among the genotypes. This make the genotype identification a long term challenge in amaranth. The amaranthus plants are characterized by small, green to reddish flowers arranged in dense clusters, stems and leaves that are green to deeply pigmented and dry, indehiscent, one-seeded fruit. The crop exhibits very high variability in leaf morphology, leaf colour, inflorescence colour and flowering pattern [14]. High phenotypic variability in the Amaranth germplasm was also envisaged in the earlier studies [10,15].

Recently, molecular markers in addition to physiological and biochemical markers are extensively used for the discrimination and grouping of the genotypes in different crop species. However, the morphological descriptors are important even today to obtain basic information on existing morphological variability in cultivated species as well as their wild relatives before the advanced plant breeding techniques are attempted [16]. Despite the merits of molecular and genetic markers, morphological descriptors are still important for exploring the genetic diversity, characterization and delineating the accessions for their possibility of incorporation into breeding programs [17]. Furthermore, the morphological descriptors are of great promise in identifying true hybrids in difficult to cross crops like amaranth. A green variety of *Amaranth* when crossed with a red variety, produce the hybrid with red foliage confirming its inheritance and facilitating the identification. Hauptli and Jain [18] estimated the outcrossing rate based on the red-green seedling colour trait.

Keeping these aspects in view, the present study was attempted for the characterization and quantification of the morphological diversity among the diverse amaranth germplasm lines collected from different environments using distinct morphological traits.

2. MATERIALS AND METHODS

Germplasm for the present study consisted of 209 amaranth accessions comprising 124 grain amaranth and 85 vegetable amaranth accessions. Grain amaranth germplasm belonging to the species *Amaranthus hypochondriacus, A. cruentus*, *A. caudatus and A. hybridus* were used which consisted of 98 accessions collected from National Bureau of Plant Genetic Resources (NBPGR), Regional Station, Phagli, Shimla (Himachal Pradesh) and 22 collected from regions across Gujarat and Karnataka and four released varieties namely Suvarna, GA-2, KBGA-1 and CO-4. Vegetable amaranth accessions belonging to three different species *viz., Amaranthus tricolor, A. viridis, A. dubius* that comprised of 62 accessions collected from the National Bureau of Plant Genetic Resources (NBPGR), Regional Station, Vellanikkara (Kerala), India; 15 accessions from different eco-geographical regions of India and eight released varieties.

Experiments for the morphological characterization of grain amaranth germplasm were carried out at the Botany garden, Department of Genetics and Plant Breeding, Agriculture College, Dharwad, which is situated at $15^0$25’ North latitude and $70^0$25’ East longitude, with an altitude of 678 meters above mean sea level and this falls under transitional Zone VIII. Whereas, morphological characterization of vegetable amaranth germplasm was conducted at Kittur Rani Channamma College of Horticulture, Arabhavi, located in the northern dry zone *i.e.,* Zone III of Karnataka at $16^0$12' North latitude and $74^0$54' East longitude with an altitude of 612 meters above the mean sea level. Both grain and vegetable types were characterized in two separate sets of experiments using augmented block design [19] during the summer and *kharif* seasons. Grain amaranth genotypes were characterized for eight morphological traits in accordance with the PPVFR guidelines of grain amaranth during summer-17 and *kharif*-17 seasons. Similarly, 85 vegetable amaranth accessions were characterized for nine morphological traits as per PPVFR guidelines on vegetable amaranthus during summer-18 and *kharif*-18 seasons.

Observations were recorded based on the visual assessment by a single observation on a group of plants or parts of plants for all the morphological descriptors studied both in grain and vegetable types at prescribed growth stages as per DUS guidelines of Grain amaranth and Vegetable amaranth by Protection of Plant Varieties and Farmers’ Rights Authority (PPVFRA) New Delhi; Mahajan et al. [20]. In grain amaranth, leaf colour, presence of leaf blotch, inflorescence colour and inflorescence compactness were recorded at 50% flowering stage. Whereas inflorescence shape, stem colour and stem surface were observed at physiological maturity. In addition, seed colour was assessed on dry seeds at harvest time. Likewise, in vegetable amaranth, the morphological traits were assessed at various prescribed stages *viz.*, seedling anthocyanin coloration immediately after seedling emergence as the plant pigmentation is likely to change in the growing season; leaf blade colour, presence of leaf blotch, anthocyanin colouration of petiole, stem colour, anthocyanin colouration of stem base and plant type of harvesting when the leaves attained harvestable maturity; inflorescence colour at 50% of the flowering stage and seed colour at full seed maturity stage.

Further, the Shannon-Weaver diversity index (H), mathematical measure of composition and phenotypic diversity was also computed using the phenotypic frequencies of each class of the trait for all the germplasm lines [21,22]. The proportion of phenotypic class *i* relative to the total number of classes (p_i) is calculated, and then multiplied by the natural logarithm of this proportion (lnp_i).

Shannon diversity index (H)

$$H = \sum[(p_i) \times ln(p_i)]$$

Where, P_i = proportion of lines in the i^{th} class of an n-class character,

n = number of phenotypic classes for a character
In = natural logarithm.

$$\text{Evenness (E)} = H / H_{max}$$

H_{max} = In S = natural logarithm of the germplasm richness *i.e.,* maximum diversity possible.
Standardized H' were classified as low (0-0.33), intermediate (0.34-0.66), and high (>0.67) [21].

3. RESULTS AND DISCUSSION

Germplasm accessions were categorized based on the variability present for each of the studied distinct morphological traits under different sub-descriptors.

3.1 Morphological Characterization of Grain Amaranth

Variability for morphological characters and their frequency distribution in grain amaranth germplasm is shown in the Table 1 and their graphical representation is shown in Fig. 1. Two of the studied traits *viz*., presence of leaf blotch and stem surface had two variant forms whereas the other six traits were categorized into three or more than three classes showing clear distinctness among the accessions. A variety is said to be distinct from another variety, when it differs from that by at least more than one class [23].

Leaf colour (leaf pigmentation) varied widely among the grain amaranth genotypes, wherein majority of the accessions *i.e*., 87 out of 124 accessions showed green leaf colour (70.16%), followed by yellowish green accessions (12.90%), greenish pink (6.45%), greenish purple (5.65%) and least number of accessions had completely purple leaf colour (4.84%). Presence of red and green plant colour in *Amaranthus* was also indicated by Gueco et al. (2016). Another important germplasm distinguishing trait is the presence or absence of leaf blotch which was present in only 27 accessions (21.77%). Majority of the germplasm lines (78.23%) were without leaf blotch. The stem pigmentation is another noticeable characteristics in varietal identification. Stem colour was reported to be due to genetic control as well as the effect of prevailing light intensity and temperature during the crop growth. Accessions were grouped into three classes based on stem colour which was also reported by Gueco et al. [24]; Thapa and Blair, [8]; Shah et al. [25] and Jahan et al. [26]. Yellowish green stem (58.87%) was predominant and others included pink (21.77%) and red stem colour (19.35%). Most of the accessions (62.90%) possessed ridged stem surface.

The floral traits and seed colour are yet other useful traits for the characterization of grain amaranths. Based on the compactness of inflorescence, the genotypes were grouped into three distinct classes wherein, dense inflorescence was the most dominant one as it was found in 49.19% of the accessions. Genotypes with lax (33.87%) and intermediate (16.94%) inflorescence were also observed among the studied accessions. Inflorescence colour varied widely which ranged from dark purple to green to yellow, orange to mixed colours. Majority of the accessions had yellowish green colour (39.52%). However, several genotypes with purple, green, pink, orange and reddish green coloured inflorescence were also noticed [8,26]. Further, shape of the inflorescence, was predominantly erect (80.65%), while 14.52 % had drooping and 4.84 % had semi erect inflorescence shape. The terminal inflorescence of most of the accessions collected from different locations was dominantly dense and erect (inflorescence compactness and shape) as revealed by Jahan et al. [26]. Of the 124 grain amaranth accessions, creamish seed colour (61.29%), was predominant, whereas some accessions had yellow (15.32%), pink (16.13%), brown (4.03%) and black coloured seeds (3.23%).

Majority of the studied accessions had green leaves, yellowish green stem, ridged stem surface with yellowish green, erect and dense inflorescence. Most of the genotypes had cream coloured seeds. As is true with other qualitative traits, these monogenic/oligogenic traits can act as important morphological markers for grouping germplasm, purity assessment and identification of true F_1s. Gueco et al. [24] reported wide genetic diversity for qualitative traits among 18 amaranth germplasm collections. Akaneme and Ani [25]; Gerrano et al. [28]; Kamble and Gaikwad [29] also reported polymorphism for these traits among different amaranthus accessions.

Based on Shannon-Weaver Diversity Index (Table 2), six of the studied morphological characters were found to have high diversity index (>0.67), while two characters, presence of leaf blotch and inflorescence shape were less variant with intermediate diversity index. The

Table 1. Variability for morphological traits and their frequency distribution in grain amaranth germplasm

Sl. No.	Characteristic	Particulars / states	Descriptor code	Number of accessions	Proportion (%)
1	Leaf colour	Yellowish green	3	16	12.90
		Dark green/Green	3	87	70.16
		Greenish pink	7	8	6.45
		Greenish purple	7	7	5.65
		Purple	7	6	4.84
2	Presence of leaf blotch	Absence	1	97	78.23
		Presence	9	27	21.77
3	Stem colour	Yellowish green	3	73	58.87
		Pink	5	27	21.77
		Red	7	24	19.35
4	Stem surface	Smooth	1	46	37.10
		Ridged	9	78	62.90
5	Inflorescence colour	Light yellow	1	--	0.00
		Yellow	2	5	4.03
		Yellowish green	3	49	39.52
		Orange	4	1	0.81
		Pink	5	13	10.48
		Pinkish green	6	3	2.42
		Purple	7	23	18.55
		Red	8	9	7.26
		Reddish Green	9	1	0.81
		Green	10	16	12.90
		Others (Mottling)	99	4	3.23
6	Inflorescence compactness	Lax	3	42	33.87
		Intermediate	5	21	16.94
		Dense	7	61	49.19
7	Inflorescence shape	Erect	3	100	80.65
		Semi erect	5	6	4.84
		Drooping	7	18	14.52
8	Seed colour	Creamish	3	76	61.29
		Yellow	5	19	15.32
		Pink	7	20	16.13
		Brown	-	5	4.03
		Black	-	4	3.23

Table 2. Shannon-Weaver Diversity Index for morphological traits in 124 grain amaranthus germplasm

Sl.No.	Characteristic	Shannon index (H)	H_{max}= ln(n)	Evenness= H/H_{max}
1	Leaf colour	0.998	1.609	0.620
2	Presence of leaf blotch	0.524	0.693	0.756
3	Stem colour	0.962	1.099	0.875
4	Stem surface	0.659	0.693	0.951
5	Inflorescence colour	1.778	2.303	0.772
6	Inflorescence compactness	1.016	1.099	0.925
7	Inflorescence shape	0.600	1.099	0.546
8	Seed colour	1.122	1.609	0.697

highest Shannon diversity index was manifested by the trait inflorescence colour (1.778), followed by seed colour (1.122), inflorescence compactness (1.016) and leaf colour (0.998) which was also revealed in the earlier studies by Gueco et al. (2016). The lowest diversity index was observed for the trait presence of leaf blotch (0.524).

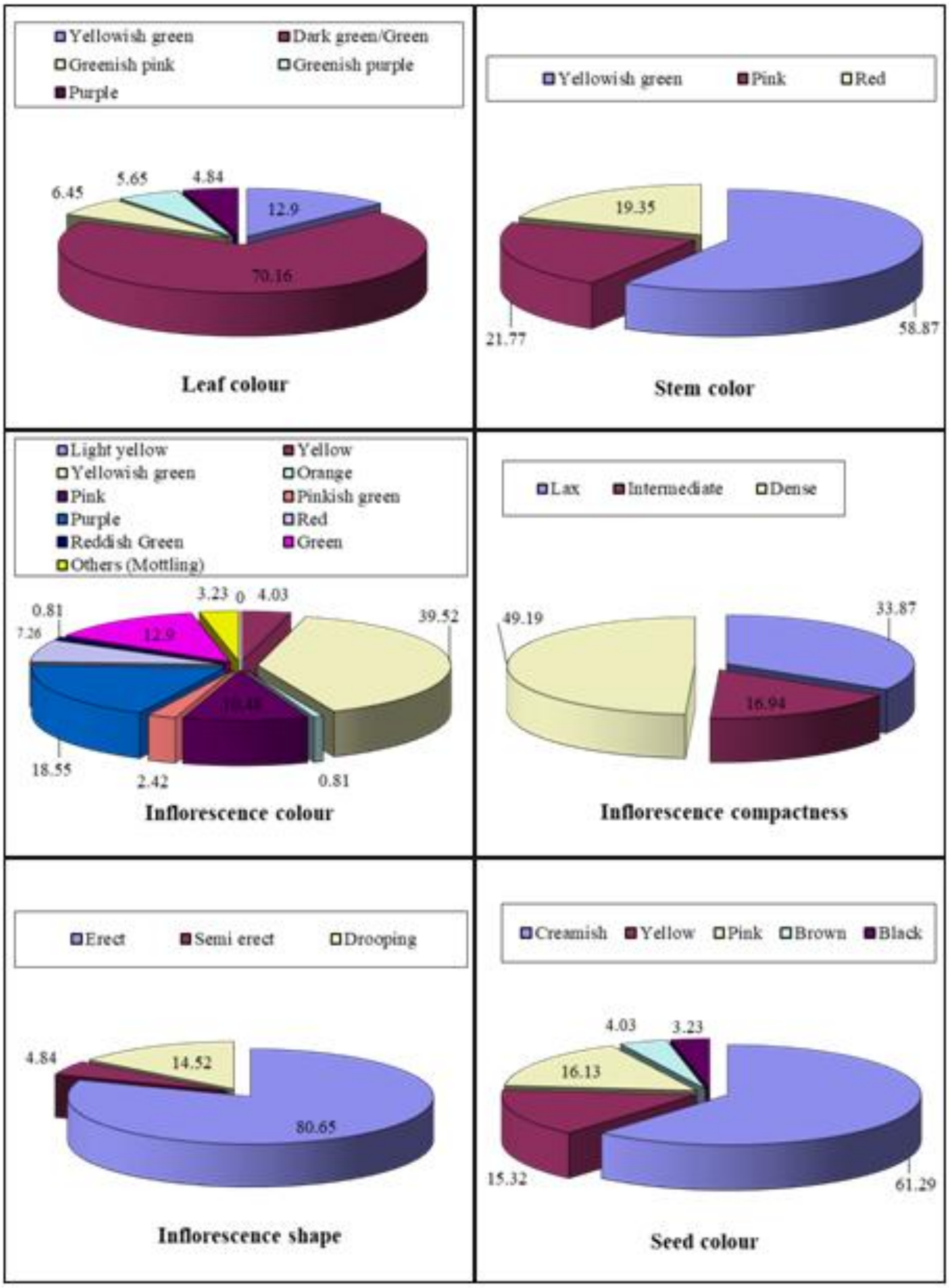

Fig. 1. Frequency distribution of morphological traits in grain amaranth germplasm

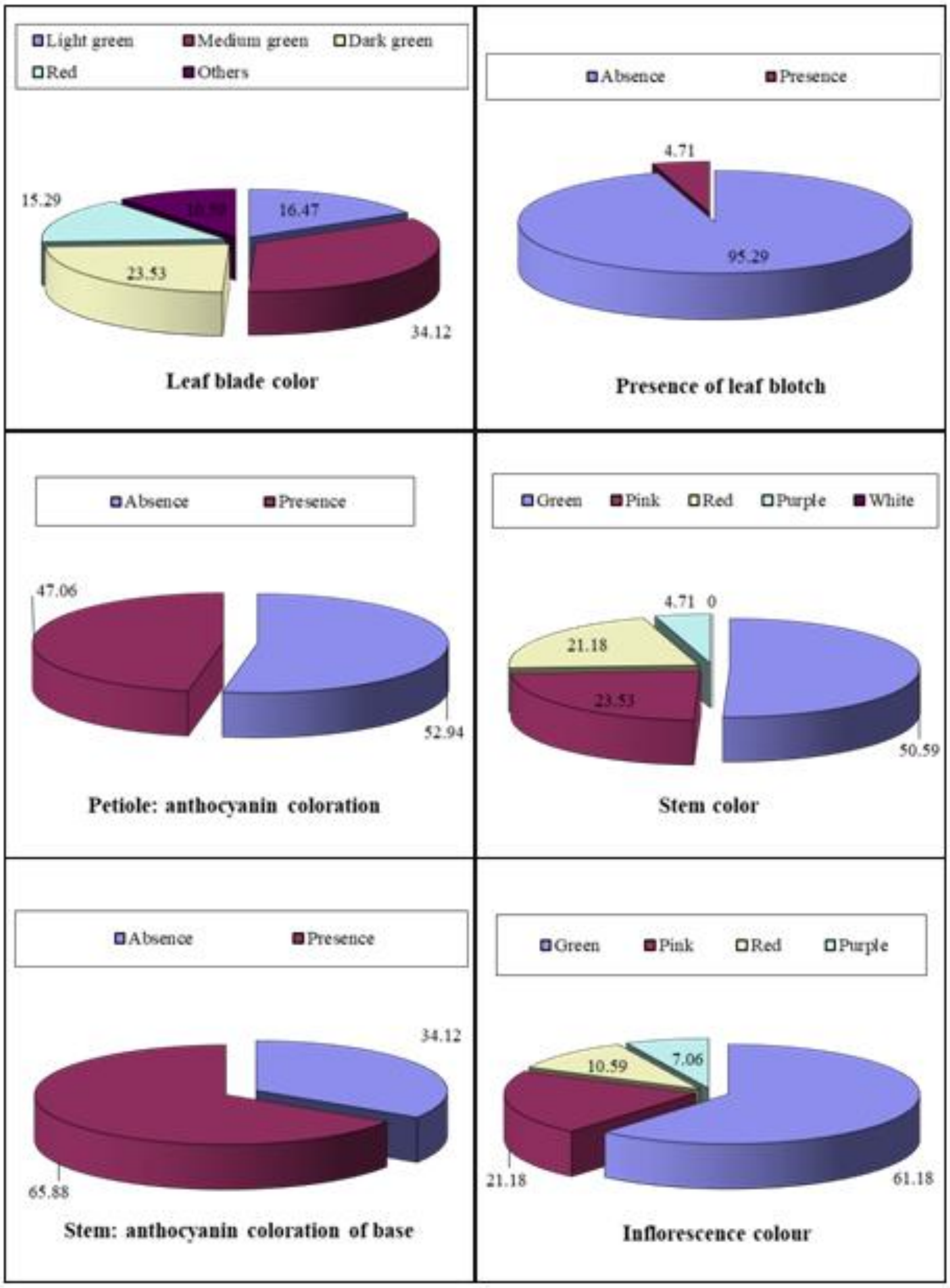

Fig. 2. Frequency distribution of morphological traits in vegetable amaranth germplasm

3.2 Morphological Characterization of Vegetable Amaranth

Variability for morphological traits and their frequency distribution in vegetable amaranth germplasm is presented in the Table 3 and graphically represented in the Fig. 2. The frequency distributions of the studied morphological traits indicated the presence of several morphs for these discontinuous variables, wherein the traits namely, leaf blade colour, stem colour and inflorescence colour were classified into more than two classes, and the remaining characters were dichotomous in nature with two distinct classes.

Table 3. Variability for morphological traits and their frequency distribution in vegetable amaranth germplasm (DUS guidelines of vegetable amaranth, PPVFRA, New Delhi)

Sl. No	Characteristic	Particulars / states	Descriptor code	Number of accessions	Proportion (%)
1	Seedling anthocyanin colouration	Absent	1	35	41.18
		Present	9	50	58.82
2	Leaf blade colour	Light green	1	14	16.47
		Medium green	2	29	34.12
		Dark green	3	20	23.53
		Red	4	13	15.29
		Others (Greenish Red, Green with red margins and veins)		9	10.59
3	Presence of leaf blotch	Absence	1	81	95.29
		Presence	9	4	4.71
4	Petiole: anthocyanin colouration	Absence	1	45	52.94
		Presence	9	40	47.06
5	Stem colour	Green	1	43	50.59
		Pink	2	20	23.53
		Red	3	18	21.18
		Purple	4	4	4.71
		white	5	0	0.00
6	Stem: anthocyanin colouration of base	Absence	1	29	34.12
		Presence	9	56	65.88
7	Plant type of harvesting	Pulling type	1	26	30.59
		Cutting type	2	59	69.41
8	Inflorescence colour	Green	1	52	61.18
		Pink	2	18	21.18
		Red	3	9	10.59
		Purple	4	6	7.06
9	Seed colour	Brown	1	5	5.88
		Black	2	80	94.12

Table 4. Shannon-Weaver Diversity Index for morphological traits in 85 vegetable amaranthus germplasm

Sl.No.	Characteristic	Shannon index (H)	H_{max}= ln(n)	Evenness= H/H_{max}
1	Seedling anthocyanin colouration	0.677	0.693	0.977
2	Leaf blade colour	1.529	1.609	0.950
3	Presence of leaf blotch	0.190	0.693	0.274
4	Petiole: anthocyanin colouration	0.691	0.693	0.998
5	Stem colour	1.158	1.386	0.835
6	Stem: anthocyanin colouration of base	0.642	0.693	0.926
7	Plant type of harvesting	0.616	0.693	0.888
8	Inflorescence colour	1.054	1.386	0.760
9	Seed colour	0.224	0.693	0.323

Table 5. Grain amaranth germplasm used for morphological characterization

Sl. No	Name of the accession	Sl. No	Name of the accession
Amaranthus hypochondriacus			
1	IC-26278	36	EC-321556
2	IC-35377	37	EC-322033
3	IC-35393	38	EC-323004
4	IC-35429	39	EC-328877
5	IC-35438	40	EC-328877-1
6	IC-35449	41	EC-328879
7	IC-35450	42	EC-328885
8	IC-35451	43	IC-519550
9	IC-35453	44	IC-551467
10	IC-35453-1	45	IC-467905
11	IC-35472	46	EC-223648
12	IC-279970	47	EC-359441
13	IC-279971	48	EC-359441-1
14	IC-311099	49	EC-323001
15	IC-311101	50	EC-387014
16	IC-311103	51	EC-198130
17	IC-313260		***Amaranthus caudatus***
18	IC-313266	52	IC-7915
19	IC-415290	53	IC-16636
20	IC-415462	54	IC-38155
21	IC-423408	55	IC-38165
22	IC-423410	56	IC-38181
23	IC-423508	57	IC-202290
24	IC-467883	58	IC-258248
25	IC-467884	59	IC-258252
26	IC-467888	60	IC-274446
27	IC-467890	61	IC-274448
28	IC-467899	62	EC-289375
29	IC-467900	63	EC-289376
30	IC-467901	64	EC-289378
31	IC-467909	65	EC-289380
32	IC-547506	66	EC-389416
33	EC-223672	67	EC-150189
34	IC-551496	68	IC-243177
35	IC-551502		

Contd...

Sl. No	Name of the accession	Sl. No	Name of the accession
	Amaranthus cruentus		***Amaranthus* spp**
69	EC-150193	98	EC-322364
70	EC-150191	99	EC-322994
71	EC-150192		**Collections**
72	EC-150194	100	GAC-144
73	EC-150195	101	GAC-145
74	EC-150196	102	GAC-146
75	EC-150197	103	GAC-147
76	EC-150198	104	GAC-148
77	EC-150199	105	GAC-149
78	EC-289395	106	GAC-150
79	EC-150200	107	GAC-151

Sl. No	Name of the accession	Sl. No	Name of the accession
	Amaranthus cruentus		***Amaranthus* spp**
80	EC-321562	108	GAC-152
81	EC-328889	109	GAC-153
82	IC-519543	110	GAC-154
83	IC-38408	111	GAC-155
84	EC-198120	112	GAC-156
85	EC-359417	113	GAC-157
86	EC-359417-1	114	GAC-158
87	EC-289412	115	GAC-159
88	EC-289408	116	GAC-160
89	EC-289408-1	117	GAC-161
90	EC-359418	118	GAC-162
91	EC-359420	119	GAC-163
92	AG-303	120	ACS-1
	A hybridus	121	CO-4
93	EC-351944	122	KBGA-1
94	EC-359421	123	Suvarna
95	EC-359422	124	GA-2
	A austrilus		
96	EC-359425		
97	EC-329425-1		

Table 6. Vegetable amaranth germplasm used for morphological characterization

Sl. No.	Accessions	Sl. No.	Accessions
	Amaranthus tricolour		
1	IC- 551506	45	IC- 536663
2	IC- 551494	46	IC- 469558
3	IC- 551494-1	47	IC- 553719
4	IC- 553744	48	IC- 551461
5	IC- 553731	49	IC- 469621
6	IC- 553737	50	IC- 469601
7	IC- 469651	51	IC- 553730
8	IC- 551486	52	IC- 536555
9	IC-536556	53	IC- 551462
10	IC- 536713		***Amaranthus viridis***
11	IC- 551483	54	IC-547515
12	IC- 551468	55	IC-547515-1
13	IC- 551459		***Amaranthus dubius***
14	IC- 551466	56	NIC-22578
15	IC- 551477	57	ICR- 1695
16	IC- 553743	58	ICR- 1695-1
17	IC - 551472	59	ICR- 1610
18	IC - 536714	60	ICR- 1616
19	IC- 550143	61	ICR- 1613
20	IC- 469694		**Local collections**
21	IC- 469645	62	VA-2
22	IC-522214	63	VA-3
23	IC- 536698	64	VA-4
24	IC- 551463	65	VA-5
25	IC- 541407	66	VA-6
26	IC - 551497	67	VA-7
27	IC- 469605	68	VA-9
28	IC- 469579	69	VA-10

Sl. No.	Accessions	Sl. No.	Accessions
	Amaranthus tricolour		
29	IC - 469646	70	VA-12
30	IC- 469658	71	VA-13
31	IC- 553745	72	VA-14
32	IC- 553749	73	VA-15
33	IC- 551506	74	VA-16
34	IC- 536729	75	VA-17
35	IC- 469528	76	Arka amaranthus
36	IC- 469722	77	Aruna
37	IC- 556649	78	Co-1
38	IC- 551473	79	Renushree
39	IC- 469652	80	Indam lal
40	IC- 551481	81	Arka Samraksha
41	IC- 551482	82	Arka Varna
42	IC- 551471	83	Arka Suguna
43	IC- 553720	84	Arka Arunima
44	IC- 550145	85	Pusa Kirti

Plate 1. Variation for inflorescence colour in Grain Amaranth

Table 7. Characterization of vegetable amaranth germplasm accessions for morphological traits

Sl. No	Accessions	Seedling: anthocyanin coloration of hypocotyls		Leaf blade colour		Leaf blotch		Petiole pigmentation		Type of harvesting		Stem colour		Anthocyanin coloration of stem base		Inflorescence colour		Seed colour
		P or A	Note	LG/MG/ DG/ Red/pu	Note	P/A	Note	P or A	Note	Pulling/ cutting	Note	G/pink/ R/Pu/ white	Note	P or A	Note	G/Pi/ R/Pu	Note	Brown or black
1	IC-551506	P	9	Red	4	A	1	P	9	Pulling	1	Pink	2	P	9	Pink	2	Black
2	IC-547515	P	9	LG	1	A	1	A	1	Pulling	1	Purple	4	P	9	Pinkish green	2	Black
3	IC- 547515-1	P	9	MG	2	A	1	P	9	Pulling	1	Purple	4	P	9	Pink	2	Black
4	IC-551494	P	9	LG	1	A	1	P	9	Pulling	1	Pink	2	P	9	Greenish pink	1	Brown
5	IC-551494-1	P	9	DG	3	A	1	P	9	Pulling	1	Pink	2	P	9	Pink	2	Brown
6	NIC-22578	A	1	DG	3	A	1	A	1	Pulling	1	Green	1	P	9	Green	1	Black
7	IC-553744	A	1	DG	3	A	1	A	1	Pulling	1	Green	1	A	1	Green	1	Black
8	ICR-1695	P	9	MG	2	A	1	A	1	Pulling	1	Green	1	P	9	Purple	4	Black
9	ICR-1695-1	P	9	DG	3	A	1	A	1	Pulling	1	Green	1	P	9	Green	1	Black
10	ICR-1610	P	9	MG+Pink margins	2	A	1	P	9	Pulling	1	Reddish green	3	P	9	Red	3	Black
11	IC-553731	P	9	LG	1	A	1	A	1	Cutting	2	Green	1	P	9	Green	1	Black
12	IC-553737	A	1	MG	2	A	1	A	1	Cutting	2	Green	1	A	1	Green	1	Black
13	IC-469651	P	9	MG	2	A	1	P	9	Cutting	2	Red	3	P	9	Greenish pink	2	Black
14	ICR-1616	P	9	MG	2	A	1	A	1	Pulling	1	Pink	2	P	9	Red	3	Black
15	IC-551486	P	9	DG+Red	3	A	1	P	9	Pulling	1	Red	3	P	9	Green	1	Black
16	ICR-1613	P	9	MG	2	A	1	A	1	Pulling	1	Green	1	P	9	Green	1	Black
17	IC-536556	A	1	MG	2	A	1	A	1	Pulling	1	Green	1	A	1	Green	1	Black
18	IC-536713	A	1	DG	3	A	1	A	1	Cutting	2	Green	1	A	1	Green	1	Black
19	IC-551483	P	9	DG	3	A	1	P	9	Pulling	1	Red	3	P	9	Purple	4	Black
20	IC-551468	A	1	MG	2	A	1	A	1	Cutting	2	Green	1	A	1	green	1	Black

Contd...

Sl. No	Accessions	Seedling: anthocyanin coloration of hypocotyls		Leaf blade colour		Leaf blotch		Petiole pigmentation		Type of harvesting		Stem colour		Anthocyanin coloration of stem base		Inflorescence colour		Seed colour
		P or A	Note	LG/MG/ DG/ Red/pu	Note	P/A	Note	P or A	Note	Pulling/ cutting	Note	G/pink/ R/Pu/ white	Note	P or A	Note	G/Pi/ R/Pu	Note	Brown or black
21	IC-551459	A	1	LG	1	A	1	A	1	Pulling	1	Green	1	A	1	Green	1	Black
22	IC-551466	A	1	DG	3	A	1	A	1	Cutting	2	Green	1	A	1	Green	1	Black
23	IC-551477	P	9	Red	4	A	1	P	9	Cutting	2	Red	3	P	9	Purple	4	Black
24	IC-553743	P	9	DG	3	A	1	P	9	Cutting	2	Red	3	P	9	Purple	4	Black
25	IC-551472	P	9	MG	2	A	1	P	9	Cutting	2	Red	3	P	9	Green	1	Black
26	IC-536714	A	1	MG	2	A	1	A	1	Cutting	2	Green	1	A	1	Green	1	Black
27	IC-550143	P	9	DG	3	A	1	A	1	Cutting	2	Green	1	P	9	Green	1	Black
28	IC-469694	P	9	Pinkish green	4	A	1	P	9	Cutting	2	Pinkish green	2	P	9	Pinkish green	2	Black
29	IC-469645	P	9	DG	3	A	1	A	1	Cutting	2	Pinkish green	2	P	9	Pinkish green	2	Black
30	Arka amaranthus	P	9	Red	4	A	1	P	9	Cutting	2	Red	3	P	9	Red	3	Black
31	IC-522214	P	9	Red	4	A	1	P	9	Cutting	2	Red	3	P	9	Red	3	Black
32	IC-536698	P	9	Green+ Pi -nk margins	3	A	1	P	9	Cutting	2	Red	3	P	9	Pink	2	Black
33	IC-551463	A	1	MG	2	A	1	A	1	Cutting	2	Green	1	A	1	Green	1	Black
34	IC-541407	A	1	MG	2	A	1	A	1	Cutting	2	Green	1	A	1	Green	1	Black
35	IC-551497	A	1	MG	2	A	1	A	1	Cutting	2	Green	1	A	1	Green	1	Black
36	IC-469605	A	1	MG	2	A	1	A	1	Cutting	2	Green	1	A	1	Green	1	Black
37	IC-469579	A	1	MG	2	A	1	A	1	Cutting	2	Green	1	P	9	Green	1	Black
38	IC-469646	A	1	LG	1	A	1	A	1	Cutting	2	Green	1	A	1	Green	1	Black
39	IC-469658	P	9	MG+Pink margins	2	A	1	P	9	Cutting	2	Pink	2	P	9	Pinkish green	2	Black
40	IC-553745	A	1	MG	2	A	1	P	9	Cutting	2	Light Pink	2	A	1	green	1	Black

Contd...

Sl. No	Accessions	Seedling: anthocyanin coloration of hypocotyls		Leaf blade colour		Leaf blotch		Petiole pigmentation		Type of harvesting		Stem colour		Anthocyanin coloration of stem base		Inflorescence colour		Seed colour
		P or A	**Note**	**LG/MG/ DG Red/pu**	**Note**	**P/A**	**Note**	**P or A**	**Note**	**Pulling/ cutting**	**Note**	**G/pink/ R/Pu/ white**	**Note**	**P or A**	**Note**	**G/Pi/ R/Pu**	**Note**	**Brown or black**
41	IC-553749	P	9	LG	1	A	1	P	9	Cutting	2	Pink	2	P	9	Pinkish green	2	Black
42	IC-551506	P	9	MG+ pink veins	2	A	1	P	9	Pulling	1	Pink	2	P	9	Green	1	Black
43	IC-536729	A	1	DG	3	A	1	A	1	Cutting	2	Light pink	2	P	9	Green	1	Black
44	IC-469528	A	1	MG	2	A	1	A	1	Cutting	2	Green	1	A	1	Green	1	Black
45	IC-469722	P	9	MG	2	A	1	A	1	Cutting	2	Green	1	P	9	Green	1	Brown
46	IC-556649	P	9	Greenish red	2	P	9	P	9	Cutting	2	Pink	2	P	9	Pinkish green	2	Black
47	IC-551473	A	1	LG	1	A	1	A	1	Cutting	2	Green	1	A	1	Green	1	Black
48	IC-469652	P	9	DG	3	A	1	P	9	Cutting	2	Pink	2	P	9	Pink	2	Black
49	IC-551481	A	1	DG	3	A	1	A	1	Cutting	2	Green	1	A	1	Green	1	Black
50	IC-551482	A	1	LG	1	A	1	A	1	Cutting	2	Green	1	A	1	Green	1	Black
51	IC-551471	A	1	LG	1	A	1	A	1	Cutting	2	Green	1	A	1	Green	1	Black
52	IC-553720	A	1	DG	3	A	1	A	1	Cutting	2	Green	1	A	1	Green	1	Black
53	IC-550145	P	9	Greenish red	2	A	1	P	9	Cutting	2	Bright pink	2	P	9	Pink	2	Black
54	IC-536663	P	9	Reddish green	4	A	1	P	9	Cutting	2	Pink	2	P	9	Pink	2	Black
55	IC-469558	P	9	Reddish green	4	P	1	P	9	Cutting	2	Red	3	P	9	Pink	2	Black
56	IC-553719	A	1	DG	3	A	1	A	1	Cutting	2	Green	1	A	1	Green	1	Black
57	IC-551461	P	9	DG	3	A	1	P	9	Cutting	2	Green	1	A	1	Green	1	Black
58	IC-469621	P	9	DG	3	A	1	P	9	Cutting	2	Light Pink	2	P	9	Green	1	Black
59	IC-469601	A	1	DG	3	A	1	A	1	Cutting	2	Green	1	A	1	Green	1	Black

Contd...

Sl. No	Accessions	Seedling: anthocyanin coloration of hypocotyls		Leaf blade colour		Leaf blotch		Petiole pigmentation		Type of harvesting		Stem colour		Anthocyanin coloration of stem base		Inflorescence colour		Seed colour
		P or A	**Note**	**LG/MG/ DG/ Red/pu**	**Note**	**P/A**	**Note**	**P or A**	**Note**	**Pulling/ cutting**	**Note**	**G/pink/ R/Pu/ white**	**Note**	**P or A**	**Note**	**G/Pi/ R/Pu**	**Note**	**Brown or black**
60	IC-553730	A	1	DG+ Blotch	3	P	9	A	1	Cutting	2	Pinkish green	2	A	1	Pink	2	Black
61	IC-536555	P	9	Reddish green	4	A	1	P	9	Cutting	2	Red	3	P	9	Red	3	Black
62	IC-551462	A	1	MG	2	A	1	A	1	Cutting	2	Pink	2	P	9	Green	1	Black
63	VA-2	A	1	MG	2	A	1	A	1	Pulling	1	Green	1	A	1	Green	1	Black
64	VA-3	P	9	LG+ Blotch	1	P	9	P	9	Cutting	2	Green	1	P	9	Green	1	Black
65	VA-4	A	1	MG	2	A	1	A	1	Pulling	1	Green	1	A	1	Green	1	Black
66	VA-5	P	9	LG+ Blotch	1	P	9	P	9	Cutting	2	Green	1	P	9	Green	1	Black
67	VA-6	P	9	MG	2	A	1	P	9	Cutting	2	Green	1	P	9	Green	1	Black
68	VA-7	P	9	Purplish green	4	A	1	P	9	Cutting	2	Red	3	P	9	Red	3	Black
69	VA-9	P	9	LG	1	A	1	P	9	Cutting	2	Light Pink	2	P	9	Green	1	Black
70	VA-10	A	1	MG	2	A	1	A	1	Pulling	1	Green	1	P	9	Green	1	Black
71	VA-12	A	1	LG	1	A	1	A	1	Cutting	2	Light Green	1	P	9	Green	1	Black
72	VA-13	P	9	Purple	4	A	1	P	9	Cutting	2	Purple	4	P	9	Purple	4	Brown
73	VA-14	P	9	Purple	4	A	1	P	9	Cutting	2	Purple	4	P	9	Purple	4	Brown
74	VA-15	P	9	MG	2	A	1	P	9	Pulling	1	Red	3	P	9	Green	1	Black
75	VA-16	A	1	MG	2	A	1	A	1	Cutting	2	Green	1	A	1	Green	1	Black
76	VA-17	A	1	MG	2	A	1	A	1	Cutting	2	Green	1	P	9	Gren	1	Black
77	Aruna	P	9	Red	4	A	1	P	9	Pulling	1	Red	3	P	9	Red	3	Black
78	CO-1	P	9	DG	3	A	1	A	1	Cutting	2	Green	1	P	9	Green	1	Black

Contd...

Sl. No	Accessions	Seedling: anthocyanin coloration of hypocotyls		Leaf blade colour		Leaf blotch		Petiole pigmentation		Type of harvesting		Stem colour		Anthocyanin coloration of stem base		Inflorescence colour		Seed colour
		P or A	**Note**	**LG/MG/ DG/ Red/pu**	**Note**	**P/A**	**Note**	**P or A**	**Note**	**Pulling/ cutting**	**Note**	**G/pink/ R/Pu/ white**	**Note**	**P or A**	**Note**	**G/Pi/ R/Pu**	**Note**	**Brown or black**
79	Renushree	P	9	LG + Pink margins	4	A	1	P	9	Pulling	1	Pink	2	P	9	Pink	2	Black
80	Indam lal	P	9	Red	4	A	1	P	9	Pulling	1	Red	3	P	9	Red	3	Black
81	Arka Samraksha	P	9	MG	2	A	1	A	1	Pulling	1	Green	1	P	9	Green	1	Black
82	Arka Varna	P	9	MG	2	A	1	P	9	Pulling	1	Red	3	P	9	Pinkish green	2	Black
83	Arka Suguna	A	1	LG	1	A	1	A	1	Cutting	2	Green	1	A	1	Green	1	Black
84	Arka Arunima	P	9	Red	4	A	1	P	9	Cutting	2	Red	3	P	9	Red	3	Black
85	Pusa Kirti	A	1	MG	2	A	1	A	1	Cutting	2	Green	1	A	1	Green	1	Black

Index

P	Present	LG	Light green
A	Absent	MG	Moderate green
Pu	Purple	DG	Dark green
R	Red	G	Green

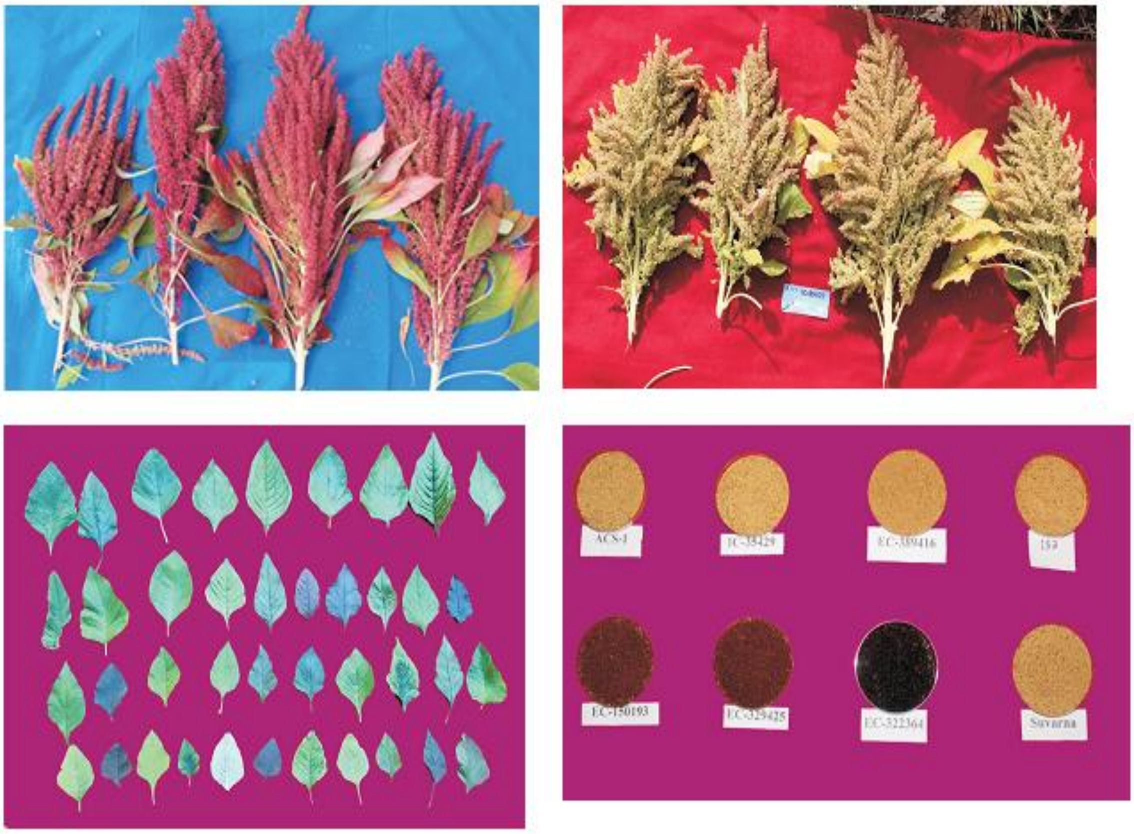

Plate 2. Inflorescence colour, leaf blade colour and seed colour variation in grain amaranthus

Seedling anthocyanin pigmentation was predominant in the accessions (58.82%), whereas the 41.18% of the accessions were greenish in colour. The most common distinguishing trait and the trait of consumer preference *i.e*., leaf blade colour (leaf pigmentation) exhibited high degree of polymorphism ranging from light green to dark green to red [26]. Accessions with medium green leaf blade colour (34.12%) were the major group, whereas red and others comprised of striped or mottled colouration (10.59%) were less frequent. Other genotypes had reddish green, pinkish green and yellowish green leaves. Andini et al*.* [30] also observed similar predominance for green leaf colour. The majority of the germplasm lines were without leaf blotch (95.29%). Further, the anthocyanin colouration of petiole was present in 47.06% of the accessions. Another most discriminating trait is stem colour which exhibited five distinct classes. Accessions with green stem colour were largest in number (50.59%). The stem base was green in 29 accessions. The presence of varied leaf colour and stem colour was also indicated in the earlier studies by Wu et al*.* [1]; Varalakshmi [31]; Andini et al*.* [30]; Akaneme and Ani [27] and Jahan et al*.* [28].

Plant type of harvesting is an important consideration for fresh green yield in vegetable amaranth wherein the accessions were grouped as either pulling type or cutting type. Multicut potentiality of cutting types make them highly advantageous to farmers and hence, preferred for higher yield levels. 59 accessions were categorized as cutting types (69.41%), while 26 lines (30.59%) were suited for pulling type of harvesting. Further, high degree of colour variation was documented for inflorescence colour which was organized into four classes. 52 accessions had green inflorescence colour (61.18%) whereas another 18 had pink (21.18%) and nine had red (10.59 %) inflorescence colour and the remaining number of accessions possessed purple coloured inflorescence (7.06 %). Inflorescence colour being a monogenically controlled trait with stable expression could be used as a morphological marker for maintaining the identity and purity of accessions besides the identification of true F_1s. Further, seed colour was less polymorphic as it documented only two colour classes *i.e*., black and brown, wherein most of the accessions were black seeded (94.12 %). Wu et al*.* [1], Akaneme and Ani [7] and Jahan et al*.* [26] also grouped the accessions as having black and brown seed colour.

Shannon-Weaver Diversity Index in vegetable amaranths (Table 4) revealed high diversity (>0.67) for leaf blade colour (1.529) followed by stem colour (1.158), inflorescence colour (1.054), petiole: anthocyanin colouration (0.691) and seedling anthocyanin colouration (0.677). Intermediate diversity was observed for stem: anthocyanin colouration of base and plant type of harvesting, whereas the other two traits presence of leaf blotch and seed colour had few variant forms revealing lower index. High Shannon-Weaver Diversity Index in amaranths was also indicated by Gueco et al. [24] for stem pigmentation, and leaf blade colour.

4. CONCLUSION

Morphological characterization of germplasm helps in identification of elite genotypes with unique characteristics as well as differentiation of the relative genotypes. It can be revealed from the study that the 124 grain amaranth and 85 vegetable amaranth germplasm could be effectively distinguished based on the morphological traits. However, it was very difficult to divide the accessions into morphotypes or to correlate the traits of particular species as these morphological traits were shared between the species. The most important discriminant morphological descriptors in grain amaranth were the inflorescence colour, leaf colour and stem colour as they displayed several distinct forms. Similarly, in vegetable amaranth, the three most distinguishing traits for varietal identification were leaf blade colour, stem colour, inflorescence colour as well as multicut potentiality. These traits for comparing the studied accessions for their distinctness have been considered by taking into account of their plasticity. Hence, these traits are transferable across the populations and would be equally efficient in comparing other varieties and germplasm accessions. The valuable information regarding the potential of the germplasm collections as well as to identify gaps for future collections as well as pre-breeding programs would be envisaged. Further, this study warranted the need for testing the amaranth genotypes for other distinct morphological traits and that the promising genotypes that exhibited distinct variation can be used in future breeding programme for the development of superior varieties in grain as well as vegetable amaranth. Key traits could be used to distinguish the germplasm in breeding and genetic resources conservation. It can be concluded that, both grain amaranth and vegetable germplasm accessions harboured substantial polymorphism and thus these traits can be used as diagnostic descriptors for grouping the amaranth accessions. Priority has to be given to descriptor states not found in the germplasm to enrich the genebank.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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