Original Research Article

Germplasm Characterization for morphological diversity~~traits~~ in the potential futuristic crop Amaranthus (A*maranthus* spp.)

ABSTRACT

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. 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 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 and morphological characterization and classification of germplasm, helps in identifying distinct genotypes with contrasting characters, which will in turn facilitate the selection of genotypes with specific traits and to broaden the germplasm base.

Key words: Germplasm, DUS 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 it has been rediscovered as a promising crop for its remarkable nutritional value (Wu *et al*., 2000). Genus *Amaranthus* belongs to the family Amaranthaceae and comprises of about 70 species that are distributed all over the world (Shukla *et al*., 2010), with about 40 of which are native to America, while the remaining ones are native to the other continents (Costea *et al.* 2001). Some species are cultivated for grain, some as leafy vegetable, as forage crop, ornamentals and few are wild species (Brenner *et al*., 2000). The most economically important species domesticated for grain production (2n=32, 34) are *Amaranthus hypochondriacus*; *A. cruentus* and *A. caudatus*. The major

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vegetable types are *Amaranthus tricolour* L., *A. dubius*, *A. blitum* and *A. viridis* (Hazra *et al*. 2011). 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 (Brandolini *et al*., 2000). As qualitative traits are selectively neutral (Smith and Smith, 1992), show simple inheritance (single/oligogenic), stable expression and are easily scorable, these traits serve as diagnostic descriptors of germplasm accessions. These traits are useful in labelling, identification and easy retrieval of desired accessions from the germplasm database. The ability to distinguish germplasm is imperative for plant breeding as well as for plant variety protection. Morphological characters constitute an important set of well accepted descriptors for varietal characterization of crop species and help in establishing the distinctness, uniformity, and stability (DUS) of crop species in Protection of Plant Variety (PPV) Systems (Begum and Kumar, 2011).

Amaranthus is the largest genus that includes an important group of plants exhibiting a great deal of morphological diversity. The several species of amaranthus are distributed across the globe with an interesting diversity of landraces and cultivars. Frequent interspecific hybridization has led to the creation of tremendous amount of variation in morphological traits. 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 (Yadav *et al*., 2014). High phenotypic variability in the Amaranth germplasm was also envisaged in the earlier studies (Khurana *et al.*, 2013). Amaranths, even though are monoecious (Mosyakin and Robertson 2003) and self-pollinated (Murray 1940), varying amounts of outcrossing (Agong and Ayiecho 1991) and interspecific and intervarietal hybridization (Murray 1940; Rubaihayo 1994) have resulted in substantial variation in genotypes.

Even though, molecular, physiological and biochemical markers are being used extensively for the discrimination and grouping of the genotypes, ~~but the~~ morphological descriptors are being used~~ even today~~ to obtain basic information on existing morphological variability in cultivated species and their wild relatives before the advanced plant breeding techniques are attempted (Adebola and Morakinyo, 2006). Despite the merits of molecular and genetic markers, morphological descriptors are still important for exploring the genetic diversity, characterisation and delineating the accessions and the possibility of selecting germplasm for incorporation into breeding programs (Law *et al*., 2011). 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, produced the hybrid with red foliage confirming its inheritance and facilitating the identification (De Vries, 1980). Hauptli and Jain (1980) estimated the outcrossing rate based on the red-green seedling colour trait.

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Therefore, the aim of the study was to generate information that will result in the effective utilization of historical elite maize inbred lines. The objectives of the study were to characterise and quantify phenotypicgenetic diversity of founder lines using agronomical and DUS traits.

Keeping these aspects in view, the present study was attempted for evaluating the extent of genetic variability existing for different morphological characters in amaranth germplasm collected from different environments.

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 of 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 20 collected from regions across Gujarat and Karnataka and four released varieties namely Suvarna, GA-2, KBGA-1 and CO-4 and two local checks. Vegetable amaranth accessions belonging to three different species *viz., Amaranthus tricolor, A. viridis, A. dubius* that comprised of 62 accessions collected from 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.

Both grain and vegetable types were characterized in two separate sets of experiments using augmented block design (Federer, 1961) during summer and *kharif* seasons. Grain amaranth genotypes were characterized for eight qualitative morphologicalDUS 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 morphologicalDUS traits as per PPVFR guidelines on vegetable amaranthus during summer-18 and *kharif*-18.

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 (PPV&FRA) New Delhi; Mahajan *et al*. (2000). In grain amaranth, for leaf colour, presence of leaf blotch, inflorescence colour and inflorescence compactness, were recorded at 50% flowering stage. Whereas for 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 DUS 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.

Comment [mk1]: The background is about amaranth, how did you move on to maize inbred lines?

Comment [mk2]: morphological

Comment [mk3]: DUS is a testing criterion for candidate varieties, the requirement for distinctiveness is usually satisfied by contrasting morphological traits of candidates against existing varieties

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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 ((Shannon and Weaver, 1949; Spellerberg and Fedor, 2003). 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 ($\ln p_i$).

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.

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

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) (Shannon and Weaver, 1949)

Comment [mk4]: Write in equation format

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 DUS characters and their frequency distribution in grain amaranth germplasm is shown in the **Table 1** and their graphical representation is shown in **Figure 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 (UPOV, 2009).

Comment [mk5]: There is no need presenting both the figure and table since they convey the same information. I would rather you present bar graphs instead of pie-charts

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 Murray (1940). 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. Accessions were grouped into three classes based on stem colour. Yellowish green stem (58.87%) was predominant and others include 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 could be grouped into three

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distinct classes namely, dense inflorescence (49.19%), lax (33.87%) and intermediate (16.94%). Inflorescence colour is a prominent morphological marker for grouping grain amaranth germplasm. Inflorescence colour varied widely wherein most of the accessions had yellowish green colour (39.52%). However genotypes with puple, green, pink, orange and reddish green coloured inflorescence were also noticed. 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). Of the 124 grain amaranth accessions, creamish seed colour (61.29%), was predominant, whereas some had yellow (15.32%), pink (16.13%), brown (4.03%) and black coloured seeds (3.23%).

Most of the studied accessions had green leaves, yellowish green stem, ridged stem surface with yellowish green, erect and dense inflorescence. Leaf blotch was absent in large number of accessions. Majority 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.* (2016) reported wide genetic diversity for qualitative traits among 18 amaranth germplasm collections. Akaneme and Ani (2013); Gerrano *et al.* (2015); Kamble and Gaikwad (2017) also reported polymorphism for these traits among different amaranthus accessions.

Based on Shannon-Weaver Diversity Index (**Table 2),** six of the studied morphological ~~DUS~~ 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. 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). The lowest diversity index was observed for the trait presence of leaf blotch (0.524).

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 **figure 2**. The frequency distributions of the studied DUS traits indicated wide polymorphism 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.

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. 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*

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al. (2013) also observed similar predominance for green leaf colour. Majority of the germplasm lines were without leaf blotch (95.29%). Further, the anthocyanin colouration of petiole was absent in 52.94% 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. Presence of varied leaf colour and stem colour were also indicated in the earlier studies by Wu *et al.* (2004); Varalakshmi (2004); Andini *et al.* (2013) and Akaneme and Ani (2013).

Comment [mk6]: I would rather you report the proportion in which it was present

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 (any reference?) with stable expression could be used as a morphological marker for maintaining the identity and purity of accessions besides 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.* (2004), Akaneme and Ani (2013) 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.

4. CONCLUSION

Comment [mk7]: Please be more precise. For grain/leaf amaranth, which traits were most diverse and which which ones were least diverse? Were these traits stable across the two cultivation periods for all the accessions?
Mention those key traits that you recommend for adoption by breeding programs for germplasm characterization. For example, what is the take home home message about anthocyanin pigmentation and leaf blade color?

It can be concluded that, both grain amaranth and vegetable germplasm accessions harboured substantial polymorphism for the studied qualitative traits and thus these traits can be used as diagnostic descriptors for discriminating amaranth accessions. Morphological characterization of germplasm helps in identification of elite genotypes with unique characteristics as well as differentiation of the relative genotypes. The traits used for comparing the accessions for their DUS are also efficient in comparing other varieties and germplasm accessions as they have been considered by taking into the account the plasticity of morphological characteristics. This study will provide valuable information regarding the potential of the germplasm collections as well as to identify gaps for future collections as well as pre-breeding programs. Key traits on the basis of DUS characterization could be used to distinguish the germplasm in breeding and genetic resources conservation.

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Table 1: Variability for morphological traits and their frequency distribution in grain amaranth germplasm (DUS guidelines of grain amaranth, PPVFRA, New Delhi)

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

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		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

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	Absence	1	81	95.29

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	blotch	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

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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

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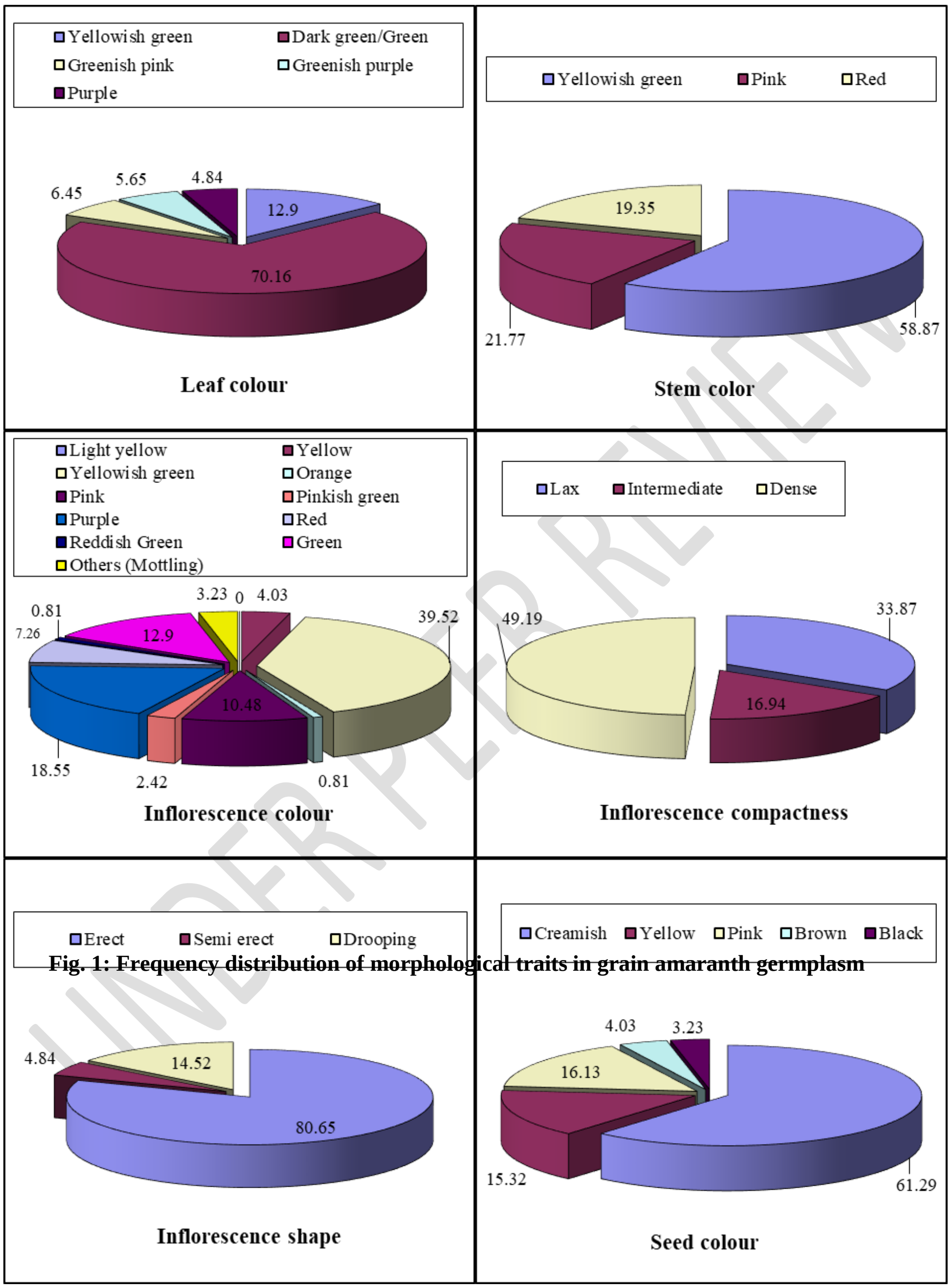

Fig. 1: Frequency distribution of morphological traits in grain amaranth germplasm

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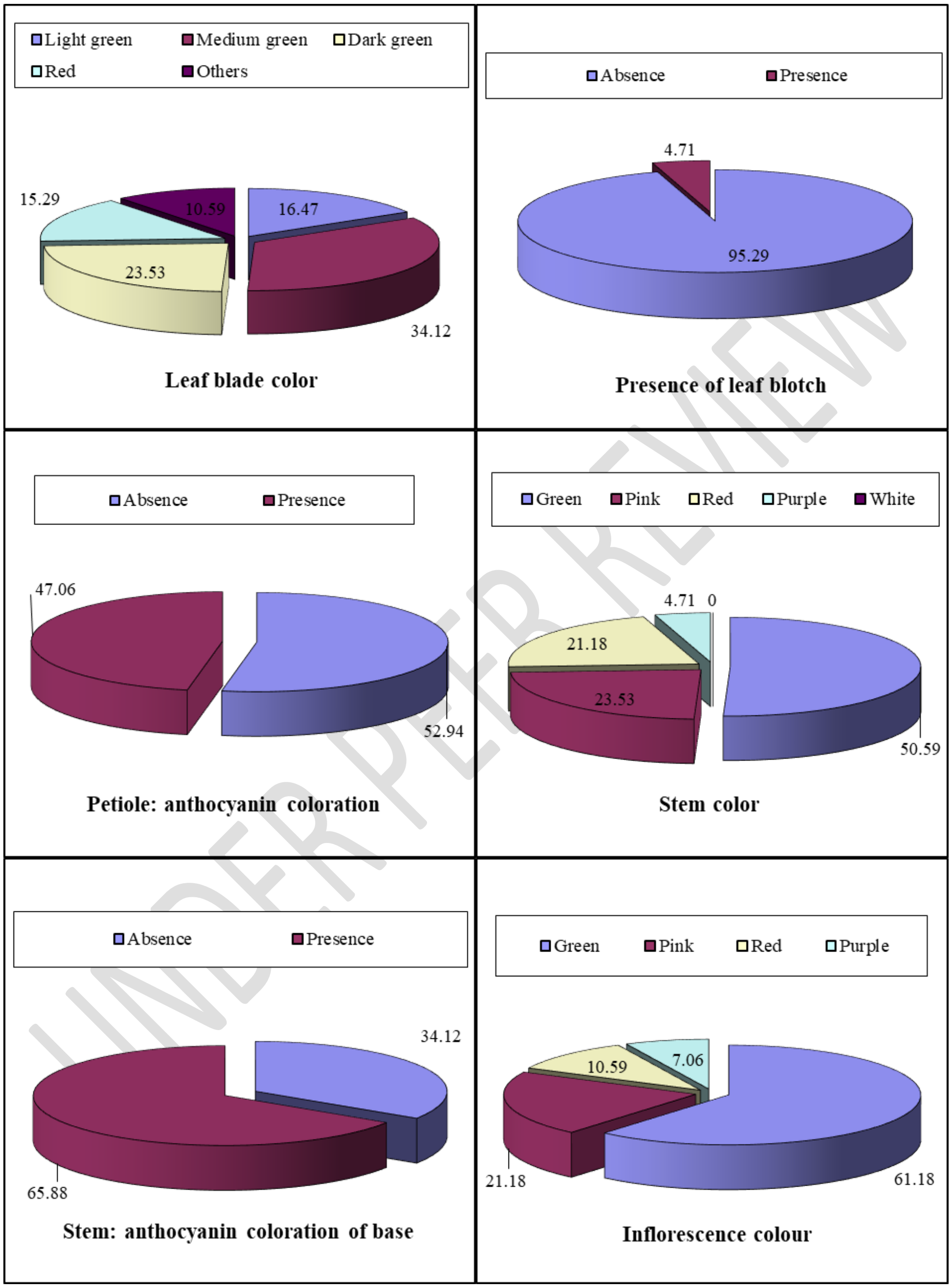

Fig. 2: Frequency distribution of morphological traits in vegetable amaranth germplasm

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