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PHENETIC ANALYSIS OF SEEDLING MORPHOLOGY OF SOME MEMBERS OF AMARANTHACEAE

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ABSTRACT: The seedling morphology of eleven taxa of Amaranthaceae has been investigated. Among the 31 seedling morphological characters, the characters like length, surface of hypocotyl; apex, shape, surface, petiole of paracotyledons; base, apex, surface, margin, petiole of first two leaves; phyllotaxy, apex, surface, base of subsequent leaves (eophylls); length, surface of epicotyl appeared to be useful. Following the methods of numerical taxonomy, i.e. Cluster Analysis, Principal Component Analysis, the seedling morphological characters have been assessed. Cluster analysis summarized the relationship among the investigated taxa into a phenogram where the investigated taxa were sorted into five distinct groups. Seedling morphological characters are useful to distinguish between the species that are presented as artificial key.

KEYWORDS: Seedling morphology, Amaranthaceae, Numerical Taxonomy, Cluster analysis, Principal Component Analysis (PCA).

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1.INTRODUCTION

The Amaranthaceae are tropical family and most diverse in the Neo-tropics, Tropical and Southern Africa and Australia [1]. It has large number of genera as well as species that are inhabited in wide range of ecological conditions. The family exhibits diverse range of habitat, sexual orientation and

growth forms. The members of this family are much important in human life as promising food and fodder crops and medicinal plants [2,3,4,5]. Some species have economic importance being popular garden plants but some are also considered as weed as well as invasive alien species in Indian environment [6,7]. Amaranthaceae were segregated into two subfamilies, i.e. Amaranthoideae and Gomphrenoideae, which were further subdivided into several tribes and sub-tribes [8]. It was stated that subfamily Gomphrenoideae are natural in comparison to the members of subfamily Amaranthoidea [9]. Being diverse morphologically and taxonomically[1], this family have been subjected to systematic study in the context of leaf architecture[e.g. 10, 11, 12], pollen morphology [e.g. 13,14], anatomy [e.g. 15,16,17,18], cytology [e.g. 19,20], phytochemistry[e.g. 21,22,23,24], and molecular data [e.g. 8,9,25,26]. However, taxonomic investigation of the members of this family using seedling morphological data is lacking. It is generally agreed that taxonomic studies should not include morphological characters of the mature specimens only, but also the characters of the juvenile stages of the seedling plants[27]. The value of seedlings for taxonomic considerations is enormous and meaningful. Seedlings of one species are usually uniform in morphology. The organs and characters of seedlings are not many but their diversity and states are immense. Naturally, the specific combinations of morphological characteristics may assist in identification of seedling taxa. Seedling morphological characters proved useful to identify the weeds for successful weed management programme in North Central States [28] as well as Montana and the North Great Plains [29].There was record of seedling morphology of 15 common dicot weeds, of which four taxa belongs to family Amaranthaceae from the crop field of Eastern Uttar Pradesh [30]. Moreover, there is report of seedling morphology of weeds from Dakshindinajpur, West Bengal. Seedling morphology of six species of Amaranthaceae has been described to identify and eradicate as weed from crop field [31,32].The present paper represents the seedling morphological characteristics of Amaranthaceae and such character diversity has been used in systematic delimitation of investigated taxa.

2. MATERIALS AND METHODS

In the present investigation, seeds, seedlings and adult specimen of eleven species belonging to six genera of Amaranthaceae were collected from natural habitats representing different parts of West Bengal, India. The collected seeds were gently washed, air dried and sown in the earthen pots. After germination, different developmental stages of seedlings were recorded through drawing and photography, and studied. Seedlings of different stages having morphological diversity were also collected from natural habitats which were studied and compared with those of raised ones[33]. At least ten to fifteen specimens of different growth forms were studied from different habitats. The seedlings were photographed and documented in the way of herbarium sheets. The voucher specimens were deposited in Calcutta University Herbarium (CUH), India. For the method of the description of seedlings, several literatures [34,35,36,37,38,39,40] were followed. The reliable

characters of seedling are germination type, paracotyledons, hypocotyl, epicotyl, first two leaves and subsequent leaves or eophylls. The relative growth and elongation of different parts were expressed as "reduced" (root 0.9-2.4 mm; hypocotyl 0.1-1.9 mm; epicotyl 0.1-1.5 mm), and "elongating" (root 2.5-6 mm; hypocotyl 2-5 mm; epicotyl 1.6-3 mm). Dimension and colour refer to fresh materials. In the figures, each division of the scale represents one cm.

2.1. Multivariate analysis: In numerical analysis, the investigated eleven taxa were regarded as Operational Taxonomic Units (OTUs) for determining the phenetic relationships among the investigated taxa following Mundhra and Paria [41], and Kolodziejek [42]. Analyses of 31 morphological characters, which are selected as good characters for differentiating taxa, were used in numerical analysis (Table II). Some of them were binary (zero-one) and some quantitative multi-state [43]. The morphometric analyses were performed through Cluster Analysis and Principal Component Analysis (PCA) and the aim of these analyses were preliminary segregation of the material to distinguish between groups of OTUs studied. The software package STATISTICA PL. ver. 6 [44] was used for numerical analyses.

3. RESULTS AND DISCUSSION

3.1. Morphology of Seedlings

The seedling morphology of the eleven investigated taxa from the family Amaranthaceae exhibits some interesting characters and reveals diversities in phyllotaxy; shape of first two and subsequent leaves; shape and other details of paracotyledons; characters of hypocotyl and internodes, etc(Table I). Using these features, the investigated taxa under different genera as well as species can be distinguished with the help of an artificial key.

Table I: Seedling morphological diversity of the investigated species of Amaranthaceae

Taxa / **Characters**	***Achyranthes aspera* L.**	***Achyranthes bidentata* Bl.**	***A1ternanthera paronychioides* St. Hil.**
Seedling type	epigeal, phanerocotylar	epigeal, phanerocotylar	epigeal, phanerocotylar
Taproot development	elongating	elongating	reduced
length (cm)	3.5-4.5	2.5-3.5	0.9-3
nature	hardy	hardy	herbaceous
colour	creamish white	creamish white	creamish white
collet	distinct	distinct	not distinct
Hypocotyl development	elongating	elongating	reduced
length (cm)	2- 2.6	3-5	0.7- 1

Taxa / **Characters**	***Achyranthes aspera* L.**	***Achyranthes bidentata* Bl.**	***A1ternanthera paronychioides* St. Hil.**
Hypocotyl shape in t.s.	ribbed	ribbed	terete
surface	scabrous	glabrous	scabrous
colour	purplish green	deep purple	deep purple
Paracotyledons position	opposite	opposite	opposite
nature	herbaceous	thick herbaceous	thick herbaceous
persistency stage	5^{th} – 6^{th} leaf	6^{th} - 8^{th} leaf	10^{th} – 12^{th} leaf
shape	lanceolate	lanceolate	narrow lanceolate
length (cm)	2.4-2.6	2-2.5	0.5-1
width (cm)	0.5-0.6	0.5-0.8	0.1-0.2
apex	acute	acute	obtuse
base	cuneate	cuneate	attenuate
surface	glabrous	glabrous	glabrous
margins	entire	entire	entire
primary Vein	one	one	one
venation	brochidodromous	brochidodromous	reticulo-camptodromous
petiole	present	present	present
Paracotyledons length (cm)	0.3-0.4	0.7-1	0.2-0.3
petiole surface	hirsute	glabrous	glabrous
Epicotyl development	reduced	elongating	reduced
length (cm)	0.5-1.5	2.2- 3	0.5- 0.7
surface	hirsute	patently hairy	woolly
shape in t.s.	angular	narrowly 4-gonous	terete
colour	purplish green	purplish green	purplish green
First Two Leaves nature	simple	simple	simple
phyllotaxy	opposite	opposite	opposite
shape	broadly elliptic	elliptic	oblanceolate

Taxa / Characters	*Achyranthes aspera* L.	*Achyranthes bidentata* Bl.	*A1ternanthera paronychioides* St. Hil.
First Two Leaves length (cm)	2-2.2	2.5-4	1.2-2
width (cm)	1-1.5	1.5-2	0.3-0.5
apex	acute	acuminate	obtuse
base	cuneate	cuneate	decurrent
margin	undulating	undulating	entire
nature	herbaceous	herbaceous	herbaceous
venation	brochidodromous	brochidodromous	camptodromous
petiole	present	present	present
length (cm)	0.2-0.5	1-2	0.4-0.5
petiole colour	purplish green	deep purplish	light purple
petiole surface	densely hairy	hairy	softly hairy
Subsequent Leaves	simple	simple	simple
phyllotaxy	opposite-decussate	opposite-decussate	opposite-decussate
shape	elliptic-suborbicular	broadly elliptic	oblanceolate- spathulate
apex	acute-apiculate	acuminate	obtuse
base	cuneate	cuneate	narrowed into a long petiole
margin	sinuate	slightly undulating	entire
surface	velvately tomentose	scarcely hairy	hairy
petiole	present	present	present

Taxa / Characters	*Alternanthera sessilis* (L.) DC.	*Amaranthus spinosus* L.	*Amaranthus tricolor* L.	*Amaranthus viridis* L.
Seedling type	epigeal, phanerocotylar	epigeal, phanerocotylar	epigeal, phanerocotylar	epigeal, phanerocotylar
Taproot development	reduced	elongating	elongating	elongating
length (cm)	0.9-1.5	2.5-3	2.5-3.5	3-3.5
nature	weak	slightly hardy	moderately soft	herbaceous
colour	brownish	creamish white	creamish white	creamish
collet	not distinct	distinct	not distinct	distinct

Taxa / Characters	*Alternanthera sessilis* (L.) DC.	*Amaranthus spinosus* L.	*Amaranthus tricolor* L.	*Amaranthus viridis* L.
Hypocotyl development	reduced	reduced	reduced	reduced
length (cm)	0.6- 0.8	0.6- 0.9	0.8- 1	0.8- 1
shape in t.s.	terete	ridges	ribbed	glabrous
surface	glabrous	glabrous	glabrous	ridges
colour	purplish green	green	purplish green	deep purple
Paracotyledons position	opposite	opposite	opposite	opposite
nature	thick herbaceous	thick herbaceous	herbaceous	thick herbaceous
persistency stage	8^{th} – 10^{th} leaf	2^{nd}- 3^{rd} leaf	7^{th} – 8^{th} leaf	5^{th} – 6^{th} leaf
shape	elliptic	lanceolate	lanceolate	lanceolate
length (cm)	1-1.5	0.3-0.8	0.6-0.8	0.5-0.8
width (cm)	0.35-0.45	0.2-0.4	0.2-0.25	0.12-0.15
apex	obtuse	obtuse	obtuse	obtuse
base	attenuate	cuneate	cuneate	cuneate
surface	glabrous	glabrous	glabrous	glabrous
margins	entire	entire	entire	entire
primary Vein	one	one	one	one
venation	brochidodromous	brochidodromous	brochidodromous	camptodromous
petiole	present	present	present	present
length (cm)	0.4-0.6	0.2-0.4	0.1-0.15	0.6-0.8
petiole surface	thinly hairy	glabrous	glabrous	glabrous
Epicotyl development	reduced	reduced	reduced	reduced
length (cm)	0.5- 1.2	0.1-0.5	0.1- 1. 3	0.1- 1.4
surface	softly hairy	scaberulous	glabrous	glabrous
shape in t.s.	narrowly 4-gonous	narrow ridges	narrowly angular	ridges & furrow
colour	green	purple green	often tinged with magenta	purplish green

Taxa / Characters	***Alternanthera sessilis* (L.) DC.**	***Amaranthus spinosus* L.**	***Amaranthus tricolor* L.**	***Amaranthus viridis* L.**
First Two Leaves nature	simple	simple	simple	simple
phyllotaxy	opposite	alternate	alternate	alternate
shape	obovate	ovate	obovate	ovate
length (cm)	1.4-1.7	1-1.2	1-1.5	1-1.2
width (cm)	0.6-0.8	0.5-0.6	0.6-0.9	0.5-0.8
apex	obtuse	retuse	obcordate	retuse
base	decurrent	attenuate	attenuate	attenuate
margin	entire	entire	entire	entire
nature	herbaceous	herbaceous	herbaceous	herbaceous
venation	brochidodromous	camptodromous	craspedodromous	brochidodromous
petiole	present	present	present	present
length (cm)	0.05-0.1	0.5-0.7	1-2.5	1-1.3
petiole colour	green	green	pinkish	tinged with purple
petiole surface	softly hairy	glabrous	glabrous	glabrous
Subsequent Leaves	simple	simple	simple	simple
phyllotaxy	opposite-superposed	alternate	alternate	alternate
shape	obovate	ovate	obovate	ovate
apex	obtuse	retuse	obcordate	retuse
base	long attenuate	cuneate	attenuate	attenuate
margin	entire	entire	entire	entire
surface	glabrous	glabrous	glabrous	glabrous
petiole	present	present	present	present

Taxa / Characters	*Celosia argentea* L.	*Digera muricata* (L.) Mart.	*Gomphrena serrata* L.	*Pupalia lappacea* L.
Seedling type	epigeal, phanerocotylar	epigeal, phanerocotylar	epigeal, phanerocotylar	epigeal, phanerocotylar
Taproot development	reduced	shortly elongating	elongating	elongating
length (cm)	1.3-1.5	2-5	3.5-5	5-6
nature	slight hardy	slightly hardy	slightly hardy	hardy
colour	creamish white	creamish white	creamish white	whitish
collet	not distinct	not distinct	distinct	not distinct
Hypocotyl development	elongating	elongating	reduced	elongating
length (cm)	2- 2.2	2.5- 3.5	0.5- 0.8	2- 2.5
shape in t.s.	glabrous	minutely hairy	scabrid	minutely hairy
surface	terete	terete	with striations	terete
colour	purplish green	deep purple	purplish green	purplish green
Paracotyledons position	opposite	opposite	opposite	opposite
nature	herbaceous	herbaceous	thick herbaceous	herbaceous
persistency stage	6^{th} - 7^{th} leaf	up to initiation of flowering	4^{th} leaf	7^{th}- 8^{th} leaf
shape	lanceolate	linear	narrow oblanceolate	lanceolate
length (cm)	0.5-1	1.9-2.2	1.6-1.8	1.8-2.2
width (cm)	0.2-0.4	0.2-0.3	0.1-0.3	0.3-0.4
apex	acute	acute	sub-acute	acute
base	cuneate	cuneate	attenuate	cuneate
surface	glabrous	glabrous	slightly mealy	glabrous
margins	entire	entire	entire	entire
primary vein	one	one	one	one
venation	semi-camptodromous	brochidodromous	eucamptodromous	brochidodromous

Taxa / Characters	***Celosia argentea* L.**	***Digera muricata* (L.) Mart.**	***Gomphrena serrata* L.**	***Pupalia lappacea* L.**
Paracotyledons petiole	present	present	sessile	present
length (cm)	0.3-0.4	0.1-0.15	-	0.6-0.8
petiole surface	glabrous	glabrous	absent	minutely hairy
Epicotyl development	reduced	reduced	reduced	reduced
length (cm)	0.1- 0.3	0.5- 1.2	0.5- 1	0.4-1.3
surface	glabrous	minutely hairy	hirsute	finely hairy
shape in t.s.	sulcate-ribbed	ribbed	terete	terete
colour	tinged with purple	reddish green	purplish green	reddish green
First Two Leaves nature	simple	simple	simple	simple
phyllotaxy	alternate	alternate	opposite	opposite
shape	ovate	ovate	oblanceolate	elliptic
length (cm)	1.5-1.6	2-2.8	1.1-1.5	2-2.3
width (cm)	0.6-0.7	1-2	0.2-0.3	1-1.3
apex	acuminate	acute	obtuse	acuminate
base	cuneate	cuneate-subrounded	long attenuate	attenuate
margin	entire	entire	entire	entire
nature	herbaceous	herbaceous	herbaceous	herbaceous
venation	eucamptodromous	cladodromous	brochidodromous	brochidodromous
petiole	present	present	sessile	present
length (cm)	0.6-0.7	1-1.3	-	0.5-0.7
petiole colour	tinged with purple	pinkish	absent	reddish green
petiole surface	glabrous	minutely hairy	absent	hairy

Taxa / Characters	*Celosia argentea* L.	*Digera muricata* (L.) Mart.	*Gomphrena serrata* L.	*Pupalia lappacea* L.
Subsequent Leaves	simple	simple	simple	simple
phyllotaxy	alternate	alternate	opposite	opposite-decussate
shape	ovate	ovate	oblanceolate	elliptic
apex	acuminate	acute	acute	acuminate
base	cuneate	cuneate	long attenuate	attenuate
margin	entire	entire	entire	entire
surface	glabrous	hairy	hairy	hairy
petiole	present	present	sessile	present

Key to the investigated taxa

(valid for taxa mentioned)

1a. Hypocotyl elongating (1.6- 3.2 mm); apex of first two leaves acute-acuminate 2

1b. Hypocotyl shortly elongating (0.1- 1.5 mm); apex of first two leaves retuse-obtuse 6

2a. Eophylls alternate ……..…… 3

2b. Eophylls opposite ……..…… 4

3a. Paracotyledons linear; eophylls obtuse at apex .....……*Digera muricata*

3b. Paracotyledons with acute apex; eophylls acuminate at apex….....*Celosia argentea*

4a. Eophylls elliptic, base attenuate, margin entire…….....….....…. *Pupalia lappacea*

4b. Eophylls other than elliptic, base other than attenuate, margin slightly undulating to sinuate.....5

5a. Eophylls suborbicu1ar, obtusely apiculate at apex, surface of blade slightly corrugated.....*Achyranthes aspera*

5b. Eophylls ovate-elliptic to broadly elliptic, sub-acuminate to acuminate at apex, surface of blade not corrugated …….....…….....……*Achyranthes bidentata*

6a. Leaf blade narrow oblanceolate-obovate …….....…… 7

6b. Leaf blade other than narrow oblanceolate-obovate …….....….....…… 9

7a. Paracotyledons sessile; eophylls sessile, alternate …..….....….....… *Gomphrena serrata*

7b. Paracotyledons distinctly petiolate; eophylls petiolate, opposite …….....……. 8

8a. Eophylls oblanceolate to spathulate; paracotyledons narrow lanceolate .....

..... *Alternanthera paronychioides*

8b. Eophylls obovate; paracotyledons elliptic-oblong……..….....… *Alternanthera sessilis*

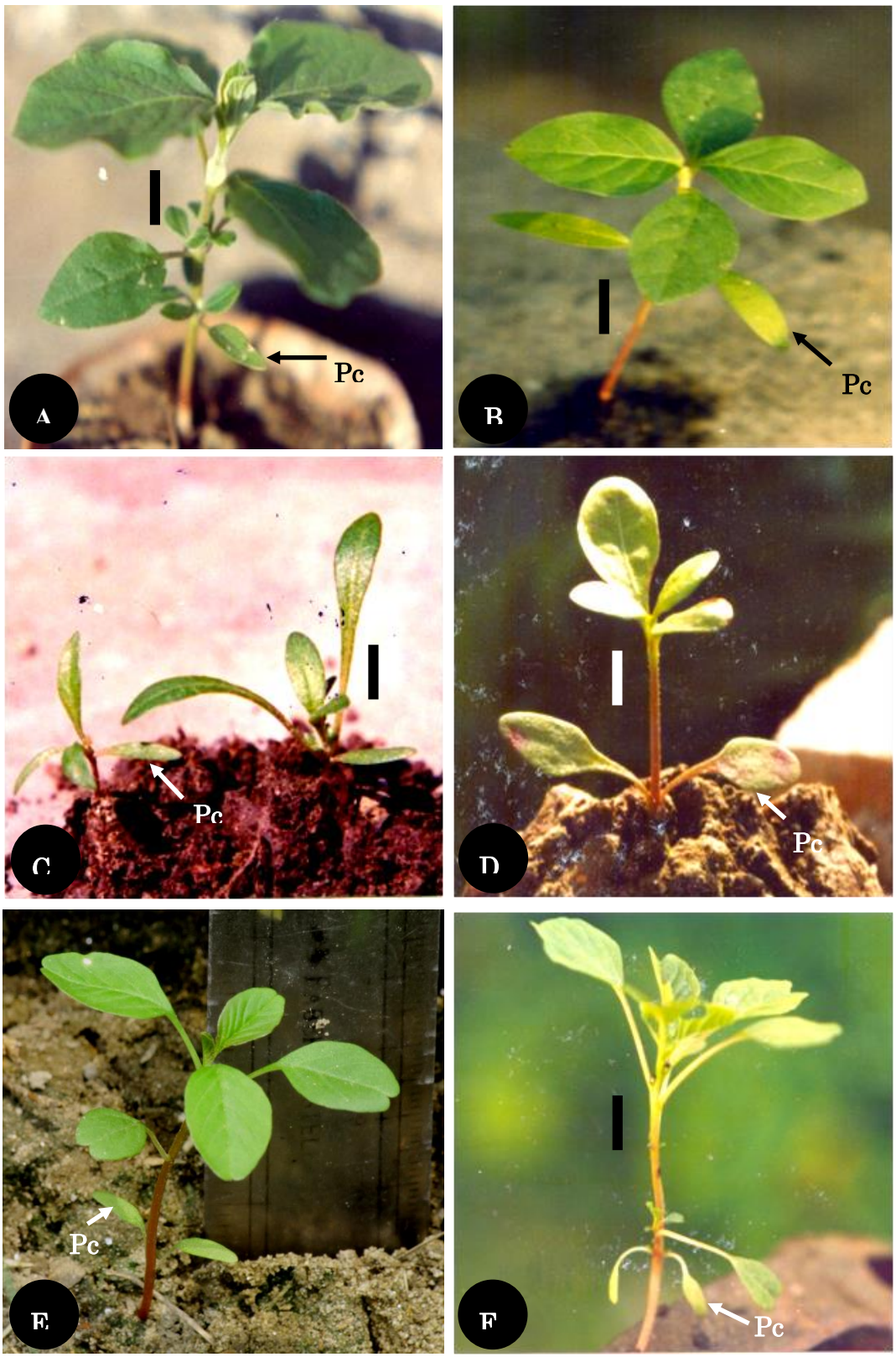

Figure I: Photographs of diversity of Seedling morphology

(A) *Achyranthes aspera* L., (B) *Achyranthes bidentata* Bl., (C) *Alternanthera paronychioides* St. Hil., (D) *Alternanthera sessilis* (L.) DC., (E) *Amaranthus spinosus* L., (F) *Amaranthus tricolor* L., (Scale bar= 1 cm, Pc= Paracotyledons).

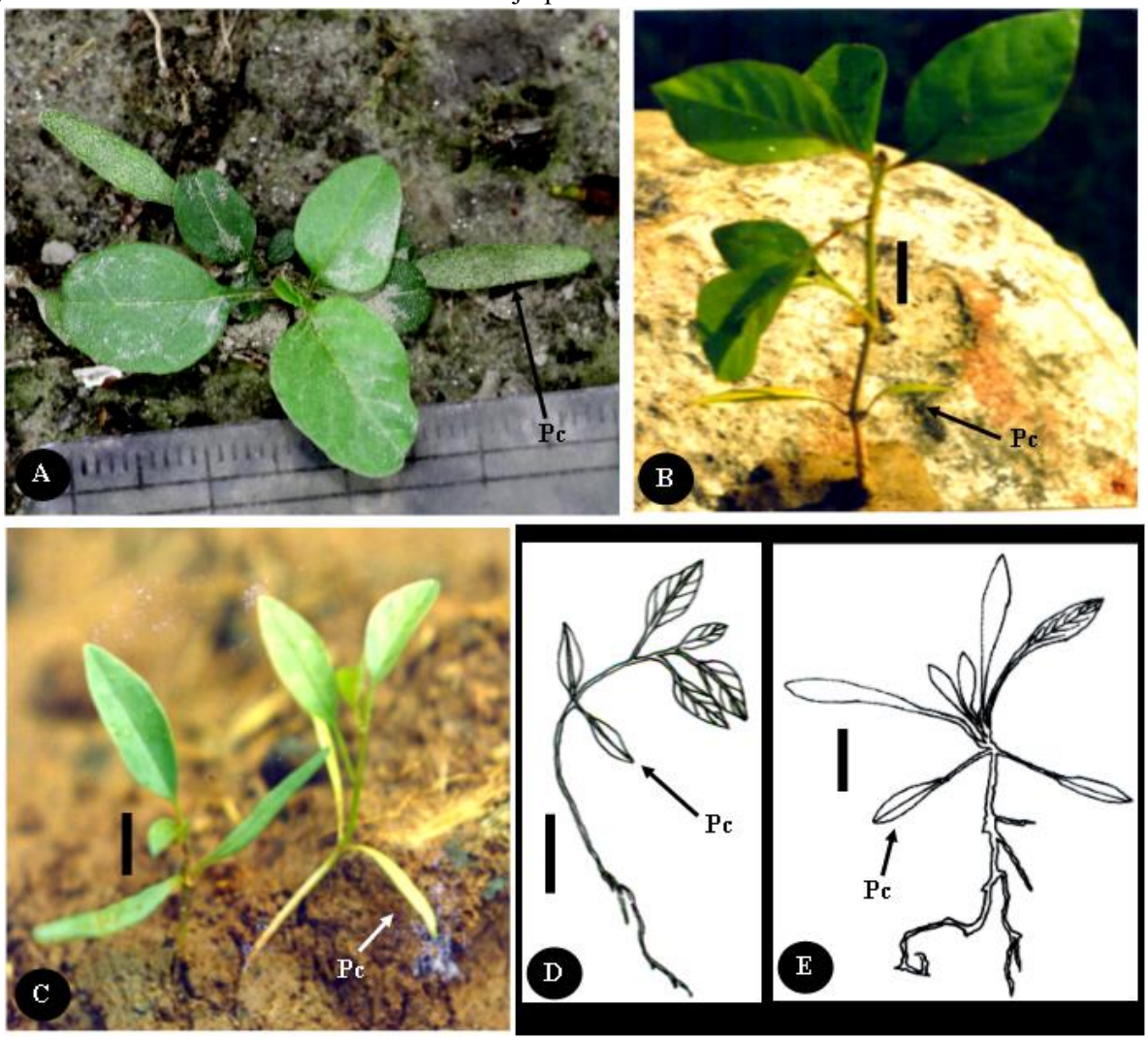

Figure II: Photographs of diversity of Seedling morphology

(A) *Amaranthus viridis* L., (B) *Pupalia lappacea* L., (C) *Digera muricata* (L.) Mart, (D) *Celosia argentea* L., (E) *Gomphrena serrata* L., (Scale bar= 1 cm, Pc= Paracotyledons)

9a. Seedlings with axillary spines appearing at 3rdto 4thinternodal stage…..…. ..... *Amaranthus spinosus*

9b. Seedlings without axillary spines ……..…… 10

10a. Eophylls obovate with obcordate apex…….....……*Amaranthus tricolor*

10b. Eophylls ovate with shallow retuse apex…….…..…… *Amaranthus viridis*

It is remarkable to note that eleven taxa represent epigeal-phanerocotylar germination, in which the cotyledons emerge from seed above the soil, borne on prominent hypocotyls (Figures I and II). Collet is distinct in *Achyranthes* sp, *Amaranthus spinosus, A. viridis*, and *Gomphrena* sp., whereas, rest of taxa do not have distinct collect.

Paracotyledons of all investigated taxa are herbaceous in nature and opposite in phyllotaxy. These are equal in size. They are persistent up to fifth to sixth leaves stages. *Gomphrena serrata* shows sessile paracotyledons. There are differences in morphology of paracotyledons as in shape, form of

bases and apices. Margin is usually entire and veins are prominent. The length of hypocotyl is reduced (0.5 cm – 1.1 cm) in most of the taxa except *Achyranthes* sp. and *Pupalia* sp. having elongated (2 cm- 5 cm) hypocotyl. Nature of first two and subsequent leaves are simple, exstipulate. *Achyranthes* sp. have slightly undulating margin but the rest of taxa have entire margin of first two and subsequent leaves. The phyllotaxy of first two and subsequent leaves is alternate in investigated taxa of *Amaranthus*, *Celosia*, and *Digera*, while other taxa reveal opposite phyllotaxy in first two and subsequent leaves (Figures I and II).

3.2. Multivariate Analyses

An assessment of similarity among individuals, species, and higher taxa is an essential component of biological classification. The methods of numerical taxonomy have been used in classifying many plants as well as interpreting results of taxonomic studies [45, 46]. Cluster analysis in multivariate analytical techniques, is commonly used in numerical classifications and produces a hierarchical classification of entities (taxa) based on the similarity of seedling characters. Different seedling morphological characters were selected for investigation. These exomorphic characters were taken covering all parts of seedling plants such as hypocotyl, internodes, paracotyledons, first two leaves and subsequent leaves (Table II). Cluster analysis, was conducted using STATISTICA Programme [44], based on UPGMA method which revealed the presence of two distinct clusters among the investigated taxa as indicated in phenogram (Figure III). The result appears interesting and promising from taxonomic point of view.

3.2.1. Cluster analysis

Two main clusters divided into several sub-clusters were distinguished within the Amaranthaceae (Figure III). The major OTU cluster on the right-hand side of the graph encompasses seedlings with elongating hypocotyl (2-5 mm) and with apex of first two leaves acute-acuminate. In this cluster, starting from right are included the following taxa: *Digera muricata*, *Celosia argentea*, *Pupalia lappacea, Achyranthes aspera* and *A. bidentata*. On the other hand, the major clusters on the left-hand side include seedlings with reduced hypocotyl (0.1-1.9 mm) and with apex of first two leaves retuse-obtuse. This group includes, starting from right, the following taxa: *Amaranthus tricolor*, *A. viridis*, *A. spinosus*, *Gomphrena serrata*, *Alternanthera sessilis*, and *A. paronychioides*. Within the cluster, conspicuous phenetic groups are distinguished which can be classified to the following *Groups-I, II, III, IV, V* (Figure III).*Group-I* composed of *Achyranthes* and *Pupalia*. They have paracotyledons as lanceolate, apex acute, base cuneate, venation brochidodromous-camptrodromous; first two and subsequent leaves opposite, shape elliptic, apex acute-acuminate, base cuneate, venation brochidodromous. These taxa belong to the tribe Aervinae (Figures IA, IB, IB).

Table II. Description of 31 morphological characters used in the cluster analysis

Sl. no.	Characters	*Character states*
1.	Tap root development	elongating 1; reduced 0
2.	collet	distinct 1; not distinct 0
3.	Hypocotyl development	elongating (2- 3.5 cm) 1; reduced (0.5- 1 cm) 0
4.	shape in t.s.	terete 0; ribbed 1
5.	surface	scabrous 1; glabrous 0; minutely hairy 2
6.	colour	purplish green 1; deep purple 0; green 2
7.	Paracotyledons nature	herbaceous 0; thick herbaceous 1
8.	shape	lanceolate 0; linear 1; elliptic 2; oblanceolate 3
9.	apex	acute 1; obtuse 0;
10.	base	cuneate 0; attenuate 1;
11.	surface	glabrous 0; slightly mealy 1
12.	petiole	present 1; sessile 0
13.	petiole surface	hairy 1; glabrous 0
14.	Epicotyl development	reduced (0.1-1.5) 0; elongating (2.2- 3 cm) 1
15.	surface	hairy 1; glabrous 0
16.	shape in t.s	terete 0; other than terete 1
17.	colour	purplish green 0; green 1; often tinged with magenta 2
18.	First Two Leaves phyllotaxy	opposite 0; alternate 1
19.	shape	ovate 0; elliptic 1; oblanceolate 2; obovate 3
20.	apex	acute 3; acuminate 4; obtuse 2; retuse 1; obcordate 0
21.	base	decurrent 0; attenuate 1; cuneate 2
22.	margin	undulating 1;entire 0
23.	surface	hairy 1; glabrous 0
24.	venation	brochidodromous 0; camptodromous 2; craspedodromous 1
25.	petiole	present 1; sessile 0
26.	petiole surface	hairy 1; glabrous 0
27.	Subsequent Leaves phyllotaxy	opposite-decussate 2; opposite-superposed 1; alternate 0
28.	apex	acute-apiculate 3; acuminate 4; obtuse 2; retuse 1; obcordate 0
29.	base	cuneate 2; narrowed into a long petiole 0; attenuate 1
30.	margin	sinuate 1; entire 0
31.	surface	hairy 1; glabrous 0

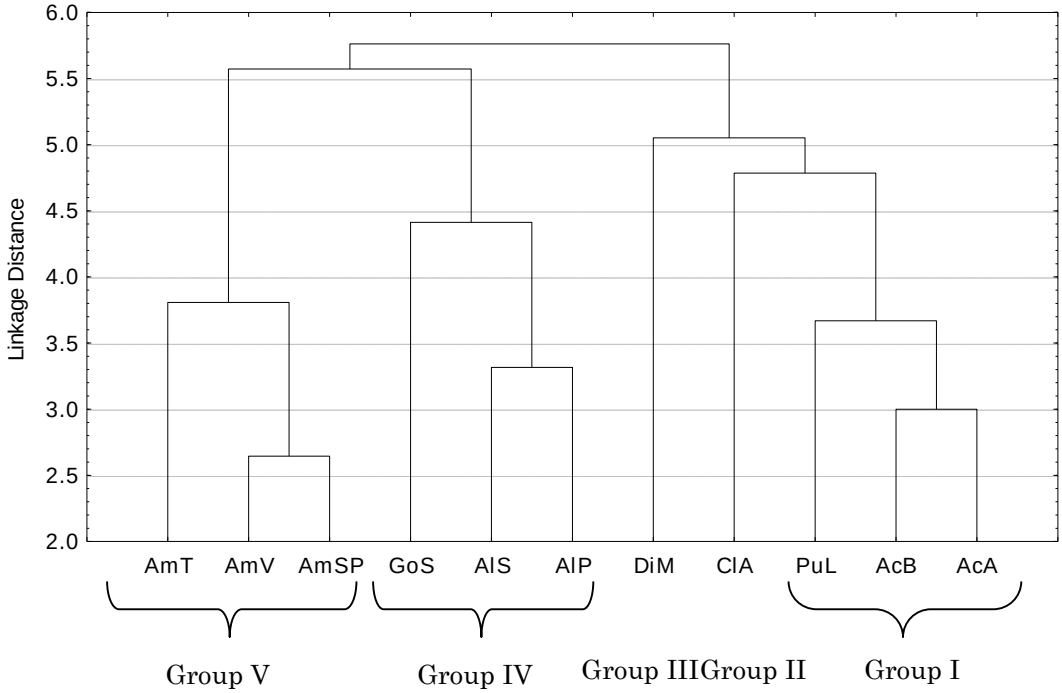

Figure III: UPGMA Phenogram based on the seedling morphological characters of investigated taxa

[Abbreviations: AcA- *Achyranthes aspera* L., AcB- *Achyranthes bidentata* Bl., AlP- *A1ternanthera paronychioides* St. Hil., AlS- *Alternanthera sessilis* (L.) DC., AmSP- *Amaranthus spinosus* L., AmT- *Amaranthus tricolor* L., AmV- *Amaranthus viridis* L., ClA- *Celosia argentea* L., DiM- *Digera muricata* (L.) Mart., GoS- *Gomphrena serrata* L., PuL- *Pupalia lappacea* L.]

Group-II includes *Celosia*. It is characterised by Paracotyledons as lanceolate, apex acute, base cuneate, surface glabrous, venation semi-camptrodromous; first two and subsequent leaves alternate, shape ovate, apex acuminate, base cuneate, surface glabrous, venation eucamptodromous. This taxon belongs to the tribe Celosieae. (Figure IID)

Group-III consists of *Digera*. They have paracotyledons linear shaped, apex acute, base cuneate, surface hairy, venation cladodromous; first two and subsequent leaves alternate, shape ovate, apex acute, base cuneate, surface hairy, venation cladodromous. This taxon belongs to the tribe Amaranthinae. (Figure IIC)

Table III. Eigenvalues, percentages and cumulative variances in PCA of seedling data matrix

Principal components (PC)	Eigenvalue	% Total variance	Cumulative Eigen value	Total Cumulative variance (%)
1	4.817790	43.79809	4.81779	43.7981
2	2.038713	18.53375	6.85650	62.3318
3	1.483029	13.48208	8.33953	75.8139

3.2.2. Principal Component Analysis (PCA)

A group of 31characters were selected which were most useful to establish differences between investigated OTUs (Table II). The best separation of groups is achieved along PC1 and PC2axes having sufficient characters to describe the relationship among the taxa (Figure IV). The three component axes, i.e. PC1, PC2and PC3, explain properly 75.81% of the total variance: the first component – 43.79% of the variation, the second– 18.53% and the third one – 13.48% (Table III). The highest loading value for the first principal component was length, surface of hypocotyl, apex of paracotyledons; apex, base, margin, surface of first two leaves; surface of petiole; apex, base, margin of subsequent leaves; for the second principal component – shape, surface, base, petiole of paracotyledons; shape in t.s., surface of epicotyl; base, surface, petiole of first two leaves; phyllotaxy, base, surface of subsequent leaves; while for the third – length of epicotyl; collect position; t.s. of hypocotyl (Table IV).

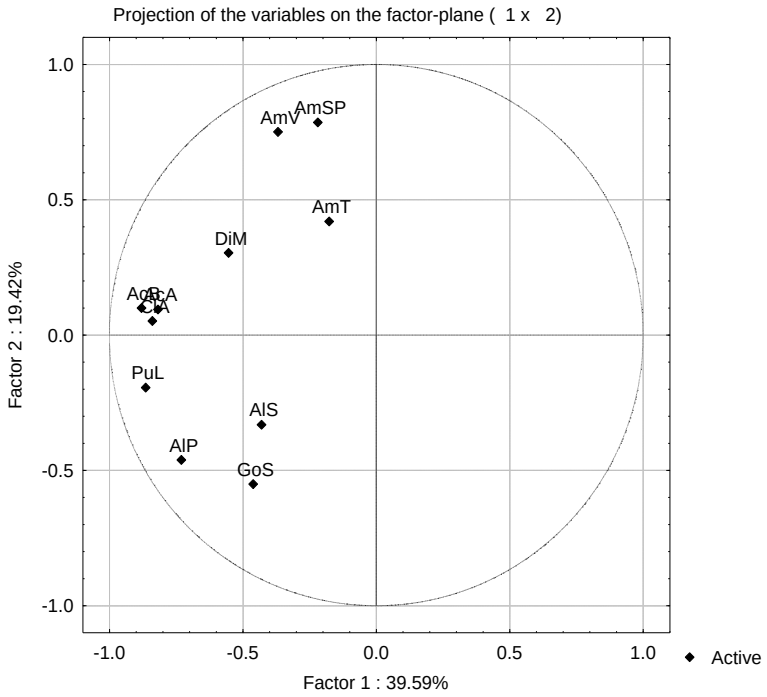

Figure IV: PCA plot based on the seedling morphological characters of investigated taxa.

[Abbreviations: AcA- *Achyranthes aspera* L., AcB- *Achyranthes bidentata* Bl., AlP- *A1ternanthera paronychioides* St. Hil., AlS- *Alternanthera sessilis* (L.) DC., AmSP- *Amaranthus spinosus* L., AmT- *Amaranthus tricolor* L., AmV- *Amaranthus viridis* L., ClA- *Celosia argentea* L., DiM- *Digera muricata* (L.) Mart., GoS- *Gomphrena serrata* L., PuL- *Pupalia lappacea* L.]

The goals of this study were to look at the range of variation of seedling morphological characters including the phyllotaxy, shape of first two and subsequent leaves; shape and other details of paracotyledons; characters of hypocotyl and internodes (epicotyl), etc. within the investigated taxa of family Amaranthaceae and to use this information to determine whether they provide additional perspectives on the taxonomy of the family. There are phanerocotylar, epigeal seedlings present in

all investigated taxa of the family Amaranthaceae. It was reported that the seedling morphology of four species of Amaranthaceae have epigeal, macaranga type of seedlings [30]. The two subfamily viz. Amaranthoideae and Gomphrenoideae of this family exhibits diverse seedling morphological characters, some of which are specific to different hierarchical circumscription and help to identify them as individual taxa with this seedling characters i.e. much before flowering and fruiting stage.

Table IV. Description of Characters with high component loading

PCA I	**Factor Scores**	**PCA II**	**Factor Scores**
length of hypocotyl	-0.923	shape of paracotyledons	0.828
surface of hypocotyl	-0.527	base of paracotyledons	0.798
apex of paracotyledons	-0.779	surface of paracotyledons	0.752
apex of first two leaves	-0.647	petiole of paracotyledons	-0.752
base of first two leaves	-0.685	surface of epicotyl	0.590
margin of first two leaves	-0.709	shape in t.s. of epicotyl	-0.809
surface of first two leaves	-0.729	base of first two leaves	-0.637
petiole surface of first two leaves	-0.746	surface of first two leaves	0.597
apex of subsequent leaves	-0.754	petiole of first two leaves	-0.752
base of subsequent leaves	-0.539	phyllotaxy of subsequent leaves	0.539
margin of subsequent leaves	-0.709	base of subsequent leaves	-0.702
surface of subsequent leaves	-0.729	surface of subsequent leaves	0.597
PCA III	**Factor Scores**	**PCA III**	**Factor Scores**
length of epicotyl	0.767	shape in t.s. of hypocotyl	0.919
collet position	0.861	venation of first two leaves	-0.602

The seedling morphology of Amaranthoideae is manifested by heterogenous assemblages of characters of different parts, including phyllotaxy, shapes of paracotyledons, first two and subsequent leaves of seedling *group I, II, III, V*. *Digera* and *Celosia* belong to Amaranthinae and Celosieae respectively and form two sub-clusters, whereas, other sub-cluster contains *Achyranthes*, *Pupalia* which belong to Aervinae. *Digera* and *Celosia* in having alternate phyllotaxy of subsequent leaves (eophylls) are separated from the Aervinae tribe, in which eophylls have opposite phyllotaxy. The seedlings of *Digera* are quite interesting. It exhibits some common characters to Amaranthinae and Aervinae. *Digera* as *type III* seedling is characterized by alternate phyllotaxy having linear paracotyledons and camptrodromous venation, elongated (2.5- 3.5 cm) hypocotyl. Cladodromous venation of first two and subsequent leaves is only present in *Digera* among all investigated taxa. *Celosia, type II* seedling is characterised by first two and subsequent leaves ovate, base cuneate,

venation eucamptodromous. Such characters help to distinguish it from *type I* seedlings having *Achyranthes aspera, A. bidentata and Pupalia lappacea*. Further, these three taxa can be separated from each other by first two and subsequent leaves characters which include shape, apex, base, margin, venation, etc. The genus *Amaranthus* as *type V* is characterised by paracotyledons with obtuse apex; eophylls retuse at apex. Further, they are identified at species level with the character-states of paracotyledons, first two and subsequent leaves. Several characters vary at species level but help to distinguish with each other within this genus. *Amaranthus* can be easily distinguished from *Alternanthera* and *Gomphrena* (Gomphrenoideae) on the basis of seedling characters as well as pollen characters [13], cytological characters [19,20] along with gross morphological characters [1].Gomphrenoideae, represented by *type IV* seedling, is characterized by reduced (0.5 cm – 1 cm) epicotyl, oblanceolate to obovate paracotyledons, first two and subsequent leaves having attenuate base. It is interesting to observe that *Alternanthera paronychioides* and *Gomphrena serrata* exhibit some common seedling characters, viz. paracotyledons, first two and subsequent leaves oblanceolate, venation camptodromous; hypocotyl surface scabrous. The shape of paracotyledons, first two and subsequent leaves are obovate having brochidodromous venation with glabrous hypocotyl. Such common relationship among *Alternanthera* and *Gomphrena* also get support from matK/*trn*K [8] and *mat*K [9] DNA sequence data.

4. CONCLUSION

From the results depicted above, we may conclude that seedling morphology of Amaranthaceae display a wide diversity at the infraspecific level for all the characters studied, that enable us to identify and combine some interesting taxa in order to obtain infrafamilial relationship among the investigated taxa. Phenetic studies based on seedling morphological data clearly indicate that most of the investigated taxa recognised at the tribal or subtribal level by different authors do not reflect natural groups which are also supported on the basis of molecular data [8] and palynological data [13]. Thus, the present study is an explanatory one involving a limited number of taxa, although this study reflects some possibilities for taxonomic consideration of seedling morphology.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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