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Comparative analysis of major phenotypical traits of some Dicotyledonous weed seedlings of crop fields of Balurghat Block, Dakshindinajpur, West Bengal

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Abstract

Juvenile traits of twenty weeds belonging to four families under Magnoliopsida have been studied among fifteen major Rabi crops of Balurghat Block of Dakshindinajpur. Seedlings are described considering both qualitative and quantitative traits. Qualitative traits of seedlings have been considered for the construction of artificial key and phenograms. However, major quantitative traits are used to compare the individual species among families through one way analysis of variance (ANOVA) using Duncan Multiple Range Test. Principal Component Analysis (PCA) also uniformly grouped all the studied qualitative and quantitative traits; hence, depicts towards their inter-relation, and importance for distinction of taxa at different levels.

Key words: Weed seedlings, phenotypic traits, artificial key, phenogram, principal component analysis

INTRODUCTION

Most of the annual weeds complete their life cycle within a very short period than crop plants dispersing large number of seeds. The viable seeds germinate immediately and establish seedlings that interact with crops in different ways. Therefore, rapid and accurate identification of weeds at the seedling stage is the first step in the design of successful weed management program that saves producers and land managers time and money (Parkinson *et al.* 2013). Identification of weed seedlings provides several benefits. First, weed management is typically much easier, less costly, and more effective at the seedling or juvenile (*e.g.* rosette) stage than on mature plants. Second, controlling a weed during early growth stages allows desirable neighbouring crops to grow better. Finally, improper identification may lead to misapplication of a management tactic, such as herbicides or failure to adequate control the weedy plant species at the time that it is most vulnerable (Parkinson *et al.*2013).

Once a species has been correctly identified, an integrated weed management (IWM) program can be designed that combines the use of biological, cultural, mechanical and chemical practices to manage the weeds. Chomas *et al.* (2001) stressed on the identification of 54 common problem weeds at their seedling stage in the North Central States in view of successful weed management program.

Besides, seedlings have long been studied mainly under morphological, ecological or taxonomic perspectives (De Vogel 1980; Garwood 2009; Leck *et al.* 2008). Seedlings are a critical stage in the plant life cycle and much of their morphological variation among different plants may reflect adaptations to varied abiotic and biotic factors that influence their growth and development (Garwood 2009; Leck *et al.* 2008). On the other hand, the study of seedlings may clarify the nature of some morphological characters or document their changes during their development from early stages to adults (Compton 1912; Fogliani *et al.* 2009; Tomlinson 1960). In addition, variation found in several seedling characters (De Vogel 1980; Garwood 2009; Lubbock 1892) provides an important source of systematic characters in delimiting groups at different taxonomic levels (Kujit 1982; Rodrigues & Tozzi 2008; Tillich 2003).

The Balurghat block is located between 25º13'0" N and 88º47'0" E within Dakshindinajpur District of West Bengal. The block is agriculturally sound possibly due to its silty-loamy to sandy-loamy soil and appropriate climates. Besides, the river Atrai flows through Balurghat forming huge river bed with fertile soil and finally joins with the river Brahmaputra in Bangladesh. More than 18 kinds of Rabi Crops grow in fertile soil of this block. But weeds are some of the most troublesome pests of major crops. They compete with the crops mainly for water, nutrients, space and light. Therefore, eradication or control of weeds in this block is significant. Considering works of Chomas *et al.* (2001) and Parkinson *et al.* (2013), an effort has been made for the identification of weeds at the juvenile stage in some major crop fields of the block.

MATERIALS AND METHODS

Thorough survey for collection of seeds and/or seedlings of the weeds in major rabi crops has been done partly from October, 2013 to June, 2014 and partly from October, 2014 to March, 2015 in different gram panchayets of Balurghat Block (e.g. – Bhatpara, Boaldar, Dakra, Danga, Jalghar, Khaspur and Malancha). The seedlings are collected in pre and post harvesting periods as well as growth stages of crops. The seeds are air-dried and sown in seedbed of 1m×1m size separately with proper tagging from time to time to raise seedlings in the experimental garden of Balurghat College. So-raised seedlings are compared to natural ones for proper identification. Besides few seedlings are identified following literatures of Chancellor (1966), Chomas *et al.* (2001) and Parkinson *et al.* (2013) where identification have been made as broad-leaved category in colour photograph. The seedlings are described with qualitative and quantitative traits following Duke (1965), Burger (1972), de Vogel (1980), Paria *et al.* (1990, 2006), Das & Kamilya (2014) and Kamilya & Das (2014).

An artificial key has been prepared considering some diagnostic qualitative traits for separating four families first and then separate keys are constructed to delimit different species within each family. A table has been prepared to show the distribution of the weed seedlings where taxa are arranged alphabetically along with author's name(s), family, exsciccatae and figure number (Table – I). Adult plants are identified following Prain (1903), Kanjilal *et al.* (1934 – 1940) and Mitra & Mukherjee (2013).

For statistical analysis, the qualitative characters of each taxon are considered as numerical form. The data has been assessed by DendroUPGMA. This is statistical utility software which creates a phenogram based on the clustering of pair-wise similarity or dissimilarity among taxa (Garcia-Vallve 1999). Separate phenograms for the four concerned families are created and displayed as Figure 1. The quantitative traits are tested through one way analysis of variance (ANOVA) using Duncan Multiple Range. All the statistical tests are performed using SPSS software (SPSS Inc., version 16.0). The entire data set of all qualitative and quantitative traits is subjected to a principal component analysis (PCA) using the Varimax method. This analysis allows the identification of interrelated variables.

RESULT

Artificial Key (valid for the taxa studied only):

***Key to the families*:**

1. Seedlings stipulate (ochreate) ……..…………………… **Polygonaceae**

1a. Seedlings exstipulate ……………..…………………………… 2

2. Seedlings always with purplish tinge at hypocotyls, petioles and undersurface of paracotyledons and first two leaves ...……….………. **Amaranthaceae**

2a. Seedlings without purplish tinge in different organs (except *Xanthium indicum* with reddish-purple tinge on petioles of paracotyledons and first two leaves) ..… 3

3. First two leaves with oblique base; paracotyledons ovate or lanceolate .… **Solanaceae**

3a. First two leaves with base otherwise; paracotyledons suborbicular or narrowly oblong or elliptic ……………………………………………. **Asteraceae**

Key to the species of Polygonaceae:

1. First two leaves subopposite with stipules scaly, not fimbrate, deciduous ……….. *Rumex dentatus* subsp. *klotzscianus*

1a. First two leaves alternate with stipules not scaly, fimbriate, persistant …. 2

2. First two leaves linear with hyphodromous venation ……………. *Polygonum plebeium*

2a. First two leaves elliptic-lanceolate with camptodromous venation ………3

3. Paracotyledons obovate to ovate; first two leaves with apex subrounded-mucronate ……………………………….…………… *Persicaria hydropiper*

3a. Paracotyledons linear; first two leaves obtuse to acute .... *Persicaria orientalis*

***Key to the species of Amaranthaceae*:**

1. First two leaves glabrous with retuse apex …………………………. 2

1a. First two leaves minutely pubescent with acute apex ...……............ 4

2. Apex of paracotyledons acute; groove on the apex of the first two leaves deeper …………………………………………..……………... *Amaranthus tricolor*

2a. Apex of paracotyledons obtuse; groove on the apex of the first two leaves less deeper …........... 3

3. Seedlings with axillary spines appearing at 3rd-4th leaf stage .... *Amaranthus spinosus*

3a.Seedlings without spines at any stage …………................... *Amaranthus viridis*

4. Paracotyledons narrow lanceolate; first two leaves oblanceolate with decurrent base …………………………………………..………. *Alternanthera polygonoides*

4a. Paracotyledons oblong-elliptic; first two leaves obovate with attenuate base ……..… *Alternanthera sessilis*

***Key to the species of Solanaceae*:**

1. Base of paracotyledons sub-rounded ……………..……………. 2

1a. Base of paracotyledons cuneate …………………..…………… 3

2. Seedlings hairy; hypocotyls and first internodes with purple tinge ....*Physalis peruviana*

2a. Seedlings glabrous hypocotyl and first internodes pale green .... *Physalis angulata*

3. Seedlings pubescent; paracotyledons lanceolate; hypocotyls green; first two leaves elliptic-ovate ……………………………………………….............. *Datura metel*

3a. Seedlings glabrous; paracotyledons ovate; hypocotyls with blackish tinge; first two leaves broadly ovate ……………………………………………….…... *Solanum nigrum*

Key to the species of Asteraceae:

1. Base of paracotyledons cuneate; first two leaves ovate, base truncate, venation actinodromous 2

1a. Base of paracotyledons attenuate or rounded; first two leaves elliptic or obovate with cuneate or sub rounded base and campto or hyphodromous venation 3

2. Paracotyledons sub-orbicular .... *Ageratum conyzoides*

2a. Paracotyledons ovate .... *Ageratum haustonianum*

3. Paracotyledons narrowly oblong or elliptic obovate; first two leaves broadly elliptic or obovate 4

3a. Paracotyledons broadly ovate, lanceolate or orbicular; first two leaves narrow elliptic 5

4. Paracotyledons narrowly oblong, obtuse and actinodromous venation; first two leaves opposite, broadly elliptic with sub rounded base and acute apex .... *Xanthium indicum*

4a. Paracotyledons elliptic-ovate, apex rounded, hyphodromous venation; first two leaves alternate, obovate, obtuse with attenuate base .... *Grangea maderaspatana*

5. Paracotyledons broadly ovate; base of first two leaves cuneate, apex obtuse and camptodromous venation .... *Eclipta prostrata*

5a. Paracotyledons elliptic or suborbicular; base of first two leaves attenuate, apex acute and hyphodromous venation 6

6. Paracotyledons elliptic; margin of first two leaves dentate …............ *Centipeda minima*

6a. Paracotyledons suborbicular; first two leaves entire .... *Gnaphalium polycaulon*

OBSERVATION AND DISCUSSION

Seedlings of twenty weeds of four families and each family with more than two species have been considered for present study. The distribution of weed seedlings in different crop fields indicated that *Amaranthus viridis, Eclipta prostrata, Polygonum plebeium, Rumex dentatus* subsp. *klotzscianus* and *Solanum nigrum* are most abundant. On the other hand, *Datura metel, Grangea maderaspatana* and *Xanthium indicum* have the least distribution. All twenty epigeal phanerocotylar seedlings are of broad-leaved type (Dangwal *et al.* 2012; Parkinson *et al.*2013) and belonging to Magnoliopsida (dicots). The term '*phanerocotylar epigeal seedling type'* has already been recognized and used in several previously published literatures (de Vogel, 1980; Duke, 1965, 1969; Ng, 1978; Clifford, 1991; Garwood, 1995). Initially various diagnostic qualitative traits of seedlings separate four families. For family level separation, characters of stipules, paracotyledons and first two leaves are considered as depicted in the key. For species level separation in four families, primary emphasis has been given to the characters of paracotyledons and first two leaves, and secondary emphasis is addressed to hypocotyl characters. The traits are stable and conservative, and so used for the construction of artificial key for the identification of weed seedlings in the field before flowering and fruiting.

Comparative Analysis of Qualitative Traits through phenogram: In Polygonaceae, *Rumex dentatus* subsp. *klotzscianus* is first segregated from the other three taxa on the

Table 1. Crop-wise distribution of the weed seedlings

***Name of plant*; Family; *Exsciccatae*; Figure**	Occurrence in some major crop fields														
	Wheat	Potato	Tomato	Brinjal	Cabbage	Cauliflower	Mustard	Lentil	Garlic	Bottle gourd	Onion	Raddish	Til	Tishi	Oal cobi
Ageratum conyzoides Linnaeus; Asteraceae; *Das & Kamilya 032;* [Plate I: 1]	+	+	-	-	+	+	+	+	-	-	-	+	-	-	+
Ageratum haustonianum Miller; Asteraceae; *Das & Kamilya 035;* [Plate I: 2]	+	+	+	-	-	+	-	-	-	-	-	+	-	-	-
Alternanthera polygonoides (Linnaeus) Robert Brown *ex* Roemer & Schultes; Amaranthaceae; *Das & Kamilya 046;* [Plate I: 7]	-	+	+	-	+	+	-	-	-	-	-	+	-	-	-
Alternanthera sessilis (Linnaeus) R. Brown *ex* de Candolle; Amaranthaceae*; Das 089*; [Plate I: 8];	+	+	+	+	+	+	-	-	-	-	-	+	+	-	+
Amaranthus spinosus Linnaeus; Amaranthaceae; *Das & Das 023*; [Plate I: 3]	+	+	+	+	-	-	+	-	+	+	-	+	-	-	-
Amaranthus tricolor Linnaeus; Amaranthaceae; *Das & Kamilya 027*; [Plate I: 4]	-	+	+	+	+	+	+	-	-	-	-	-	-	-	+
Amaranthus viridis Linnaeus; Amaranthaceae; *Das 031*; [Plate I: 5]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Centipeda minima (Linnaeus) Addison Brown & Ascherson; Asteraceae; *Das & Kamilya 037*; [Plate I: 6]	-	+	-	-	-	-	-	+	-	+	-	+	-	-	+
Datura metel Linnaeus; Solanaceae; *Das & Das 027*; [Plate I: 9]	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eclipta prostrata (Linnaeus) Linnaeus; Asteraceae; *Das 063*; [Plate I: 10]	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Gnaphalium polycaulon Persoon; Asteraceae; *Das & Kamilys 014;* [Plate I: 12]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grangea maderaspatana (Linnaeus) Poiret; Asteraceae; *Das & Kamilya 019*; [Plate I: 11]	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Persicaria hydropiper (Linnaeus) Delabre; Polygonaceae; *Das & Kamilya 021;* [Plate I: 15]	+	+	+	+	-	-	-	+	-	+	+	+	-	+	+
Persicaria orientalis (Linnaeus) Assenov; Polygonaceae; *Das & Kamilya 023;* [Plate I: 16]	+	+	+	-	-	-	+	-	-	-	+	+	+	-	-
Physalis angulata Linnaeus; Solanaceae; *Das & Das 016*; [Plate I: 13]	+	+	+	+	-	-	-	+	+	+	-	+	-	-	+
Physalis peruviana Linnaeus; Solanaceae; *Das & Das 019*; [Plate I: 14]	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+
Polygonum plebeium Robert Brown; Polygonaceae; *Das 028*; [Plate I: 17]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rumex dentatus Linnaeus subsp. *klotzscianus* (Meissner) Rechinger *f.;* Polygonaceae; *Das 035*; [Plate I: 18]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Solanum nigrum Linnaeus; Solanaceae; *Das & Das 008*; [Plate I: 19]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Xanthium indicum Koenig; Asteraceae; Das & Das 017; [Plate I: 20]	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-

character of scaly and deciduous stipules. The linear shape and hyphodromous venation pattern separate *Polygonum plebeium* from two species of *Persicaria* in the secondary branch. Two species of *Persicaria i.e., P. hydropiper* and *P. orientalis* form tertiary branch and separated from each other by characters of paracotyledons and first two leaves.

Plate I: 1. *Ageratum conyzoides*; 2. *A. haustonianum*; 3. *Amaranthus spinosus*; 4. *A. tricolor*; 5. *A. viridis*; 6. *Centipeda minima*; 7. *Alternanthera polygonoides*; 8. *A. sessilis*; 9. *Datura metel*; 10. *Eclipta prostrata*; 11. *Grangea maderaspatana*; 12. *Gnaphalium polycaulon*; 13. *Physalis angulata*; 14. *Physalis peruviana*; 15. *Persicaria hydropiper*; 16. *P. orientalis*; 17. *Polygonum plebeium*; 18. *Rumex dentates* subsp. *klotzscianus*; 19. *Solanum nigrum*; 20. *Xanthium indicum* [Photographs with suffix 'a' of each corresponding figure number lower case of the plate indicates highlighted paracotyledons and first two leaves where paracotyledons are displayed on the left hand side and first two leaves on the right hand side]

In the Amaranthaceae, one primary branch of phenogram accommodates *Alternanthera sessilis* and *A. polygonoides* one side and three species of *Amaranthus i.e., A. tricolor, A. viridis, A. spinosus* on the other branch. Two species of *Alternanthera* makes secondary branch where *A. sessilis* differs from *A. polygonoides* by having oblong-elliptic paracotyledons and first two leaves obovate with attenuate base. Similarly, *Amaranthus* *tricolor* in the second primary branch makes its separation secondarily from *A. spinosus* and *A. viridis* by characters of paracotyledons and first two leaves as depicted in the key. Similarly, spine as major conservative character separates remaining two species of *Amaranthaceae i.e., A. viridis* and *A. spinosus.*

The Solanaceae having only four weed taxa forms two parallel primary branches each having two species. Two species of *Physalis i.e., P. angulata* and *P. peruviana* form secondary clades and separated by having glabrous seedlings without purple tinge in the first internode in the former and with hairy seedlings and purple tinge in the first internode in the later one. Similarly, *Solanaum nigrum* and *Datura metel* form secondary clade where the former species separates the later one by its glabrous seedlings, ovate paraccotyledons, blackish tinge of hypocotyls and broadly ovate shape of first two leaves.

Lastly, two species of *Ageratum* separate remaining five taxa of Asteraceae by their similarity in characters of base of paracotyledons (cuneate) and ovate shape, truncate base and actinodromous venation of first two leaves. Among other five taxa, *Xanthium indicum* and *Grangea maderaspatana* make their secondary grouping from *Eclipta prostrata*, *Centipeda minima* and *Gnaphalium polycaulon*. Similarly, *Eclipta prostrata*

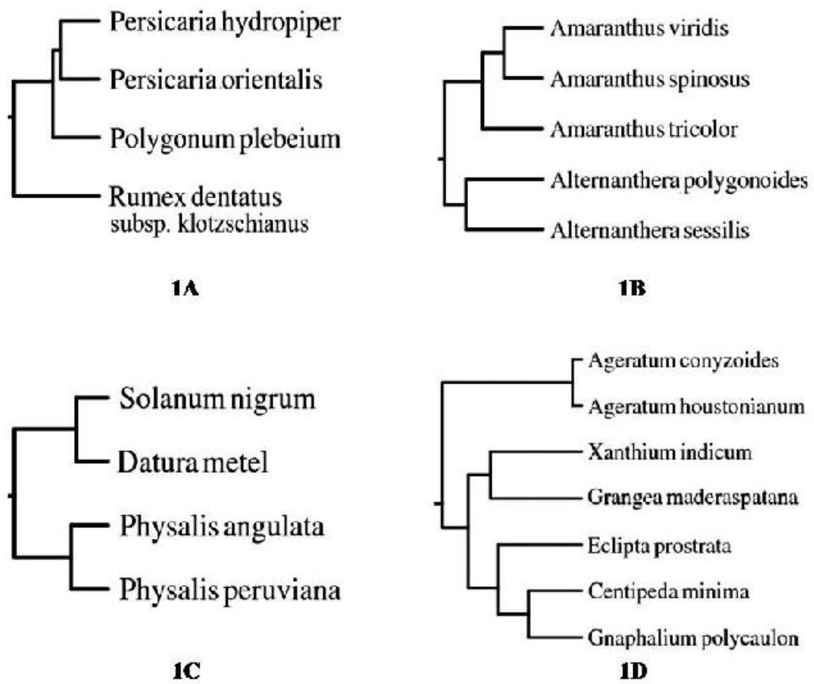

Figure 1: Phenograms based on qualitative traits of seedlings: **1A.** Polygonaceae; **1B.** Amaranthaceae; **1C.** Solanaceae; and **1D.** Asteraceae)

forms tertiary branches separating *Centipeda minima* and *Gnaphalium polycaulon* by characters as displayed in the artificial key.

Thus, phenogram construction shows parallel behaviour with artificial keys among four families. Therefore, seedling data not only helps to identify different species in the crop field but also help in numerical analysis indicating the close relationships. However, such correlation may be ambiguous when adult vegetative and reproductive characters considered for the above studied taxa in each family.

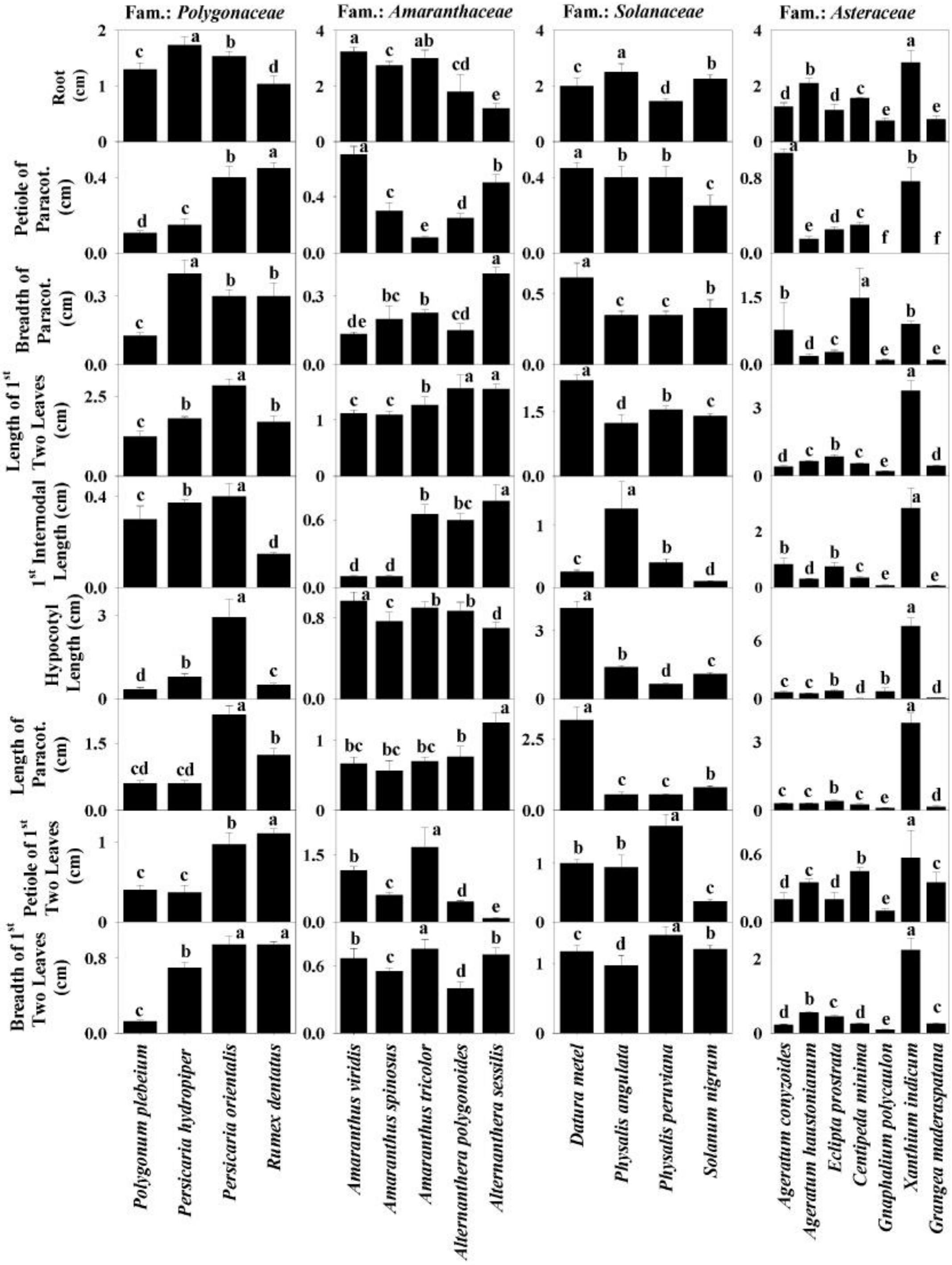

Figure 2: Analysis of different quantitative traits observed in studied seedling species of – Polygonaceae, Amaranthaceae, Solanaceae, and Asteraceae. Values represent mean ± SE. Bars showing *different letters* indicate significant differences according to Duncan's Multiple Range Test at $p < 0.05$. (*Rumex dentatus* = *Rumex dentatus* subsp. *klotzscianus*)

Analysis of Quantitative Traits: Observed results show comparative changes in the magnitude of all the studied quantitative traits within each family (Figures 2). The four different species of Polygonaceae show significant variation in most of the quantitative traits at $p < 0.05$. However, *Polygonum plebeium* and *Persicaria hydropiper* show non-significant variation in - length of paracotyledons and petiole length of first two leaves. Even breadth of paracotyledons also varies non-significantly within *Persicaria orientalis* and *Rumex dentatus* subsp. *klotzschianus* (Figure 2). Similarly, in other three families, *i.e.* Amaranthaceae, Solanaceace and Asteraceae, except few, most of the traits show significant variation among major species at $p < 0.05$ (Figure 2). Ibarra-Manríquez *et al.* (2001) also reported similar variation with the functional types of seedling from rain forests of Mexico. From the present results, it can be depicted that the variation in the magnitude of quantitative traits might have significant importance in determination of taxa in seedling stage; but may not be the independent parameters to conclude. So, along with the qualitative traits, quantitative traits should also be taken care of during the study of seedling especially from virgin areas (*i.e.* where no reports are available). Even, the present trends show that if a proper dimension can be set, both qualitative and quantitative traits should complement each other.

Principal component analysis (PCA): In the PCA of both the qualitative and quantitative traits major numbers of characters show a uniform and consistent distribution among axis 1, *i.e.* PC1, and axis 2, *i.e.* PC2 (Figure 3). All the traits commonly form two major groups. The larger group is mainly inclines towards the PC2 axis; whereas, the smaller group towards PC1 axis. This PCA clearly depicts significant inter-relationships (*i,e.* one trait is depending and / or complementing other) at different levels among major numbers of qualitative and quantitative traits in present study of the twenty seedlings species from four different dicotyledonous families. Only two qualitative characters, *i.e.* apex of paracotyledons and apex of first two leaves, remain outside the group, which might indicate their less important status for taxonomic correlation or separation of taxa. Zanne et al. (2005) also found similar relation between diverse seedling traits and their ecological and evolutionary consequences.

CONCLUSION

Identification of weeds at the juvenile stage is one of the vital directions for weed management program in crop field (Chomas *et al.* 2001; Parkinson *et al.* 2013). Since, seed production rate, seed vigour, viability and resistance to environment are higher in weeds, so rain water or irrigation in crop fields make vigorous germination of seeds establishing abundant seedlings among crops. Even flowering appears in some seedlings of Amaranthaceae (*e.g., Amaranthus viridis, A. spinosus, Alternanthera sessilis*) where the paracotyledons remain still attached indicating their partly ephemeral behaviour. This creates problems for their total eradication from the field because they immediately disperse their seeds. However, eradication at the seedling stage prevents the seed setting of weeds as well as minimizing competition of weeds with crops for light, nutrients, water, *etc.* Thus, within this limited scope of study, the artificial key for above seedlings will be helpful for immediate identification in the field and then helps the farmers to take decision for their eradication by minimizing the use of herbicide or any other means.

Phenogram analysis of the taxa within each family again indicates their phenetic relationships that highlights their separation at different levels even in this juvenile stage where few genes have expressed to adapt within the environment. Quantitative trait analysis showing different bar diagrams having different letters like a, b, c, d, *etc.* against any taxon of any family indicate their significant differences according to Duncan's MRT at $p < 0.05$.

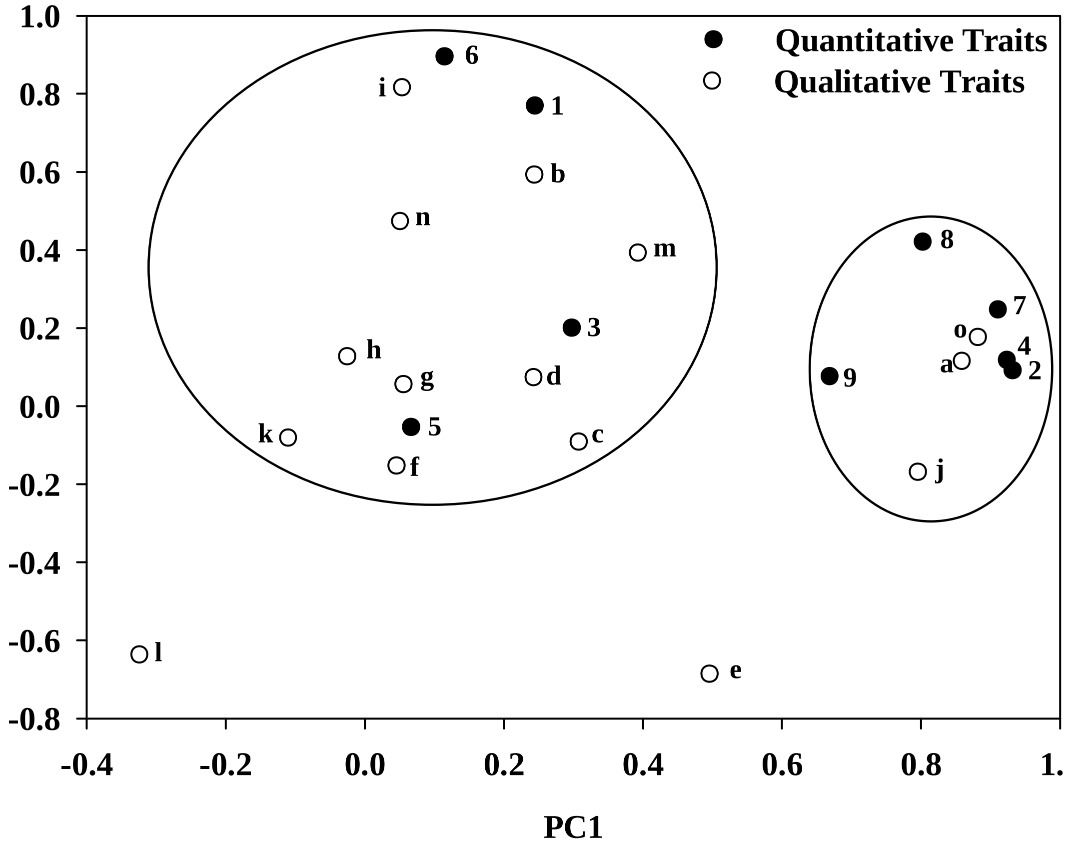

Figure 3: The principal component analysis (PCA) of qualitative and quantitative traits in twenty studied plant species of four different families. The scatter plot of both the traits on the PC1 and PC2 side. Closed (black circle) and open (white circle) symbols denote quantitative and qualitative traits, respectively. Each symbol represents the mean values for the PC scores of the three replicates of each parameter. For the convenience, the parameters are denoted by numbers and alphabets like – (1) root length, (2) hypocotyl length, (3) petiole length of paracotyledons, (4) length of paracotyledons, (5) breadth of paracotyledons, (6) petiole length of first two leaves, (7) length of first two leaves, (8) breadth of first two leaves, (9) first internode length; and (a) petiole types of paracotyledons, (b) surface of paracotyledons, (c) shape of paracotyledons, (d) base of paracotyledons, (e) apex of paracotyledons, (f) venation types of paracotyledons, (g) phyllotaxy of first two leaves, (h) stipule types of first two leaves, (i) surface of first two leaves, (j) shape of first two leaves, (k) base of first two leaves, (l) apex of first two leaves, (m) margin of first two leaves, (n) types of primary of first two leaves, (o) venation pattern of first two leaves.

These differences indicate to consider various quantitative traits for artificial key construction and separation/ or inclusion of taxa in a particular rank along with qualitative traits. Similarly, PCA of both quantitative and qualitative traits indicate their considerable dependence and significance for delimitation of the taxa.

One example of parallel significance of seedling characters of three species of *Amaranthaceae e.g. A. viridis, A. spinosus* and *A. tricolor* has been encountered from their palynomorph behaviour as studied by Zhingila *et al.*(2014). They observed psilate ornamentation in all the three species. But *A. tricolor* differs from *A. viridis* and *A. spinosus* by possessing oval shaped and triporate aperture while spherical shape and monolete apertures present in the later two. Similarly, our dendrogram analysis of the Amaranthaceae separates *A. tricolor* from *A. viridis* and *A. spinosus* with some seedling traits as seen in the phenogram.

Thus, the comparative analysis of various qualitative and quantitative traits of seedlings of such limited number of weed taxa have potential value for identification as well as partial taxonomic treatment.

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