

## STUDY OF SEEDLING DIVERSITY OF SOME MEMBERS OF LEGUMINOSAE AND CORRELATION OF SEED AND SEEDLING TRAITS

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Seedling morphology and diversity of sixteen taxa of Leguminosae (*sensu lato*) have been studied in the Danga forest under Balurghat Block of Dakshindinajpur District. Distribution of seedlings and their mother plants have been recorded using Garmin-GPS. Seeds have also been collected and characterised. Both seed and seedling characters are used for the preparation of artificial key and correlation study at species level. In the artificial key, seedlings of these sixteen taxa are primarily separated as cryptocotylar (four) and phanerocotylar (twelve). Further distinction of taxa of both the categories have been made using the character and characterstates of seeds and seedlings. Considerable parameters for the description of the seeds are texture, shape, color, surface and weight. Important seedling parameters are shape and surface of hypocotyls; presence of petiole; shape, base, apex, margin, number of primary veins and venation of paracotyledons; phyllotaxy, nature, shape, base, apex, margin and venation of first two leaves; heteroblasty, etc. A dendrogram has been made using UPGMA method for correlation study. Relation between seed weight and seedling types has also been drawn.

**Key words:** artificial key, correlation, dendrogram, GIS- map, seeds, seedlings

Development of seedlings is an early juvenile stage in plant life cycle. Seedling characters which are non-volatile are crucial for understanding initial life processes (Paria *et al.* 1990). Different taxa within the same or different groups exhibit various types of diversity at the seedling stage. To determine the correlation of such taxa, we need to understand the variability in the natural characters among them. According to Sneath and Sokal (1973), the determination of correlation between characters of two different species yields undesirable ambiguous results. So, there must be some determinative relationships among the characters.

In this work, morphological characters of seeds and seedlings of sixteen forest tree and climber taxa of Leguminosae (*sensu* Bentham and Hooker) have been studied. Numerical evaluation of the taxa depending on different parameters has been carried out and statistical analysis has been done. The objective is to find out mathematical similarities or dissimilarities among them. In addition, the goal is to find out whether this correlation supports traditional system of classification or not. Besides,

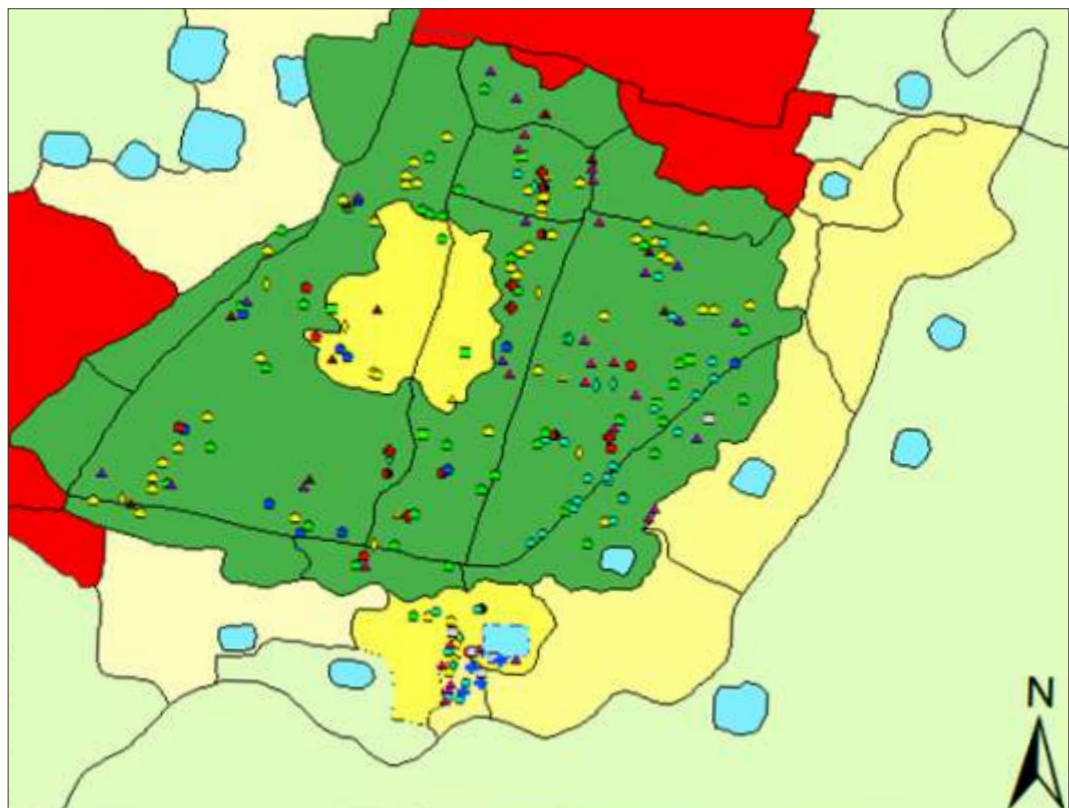
ecological behavior of seedlings in relation to seed weight and size have been traced partially.

### MATERIALS AND METHODS

The study was conducted at Danga forest located along the eastern bank of river Atreyi in Balurghat block of Dakshindinajpur district of West Bengal. It is a seminatural type of forest with predominantly tropical deciduous vegetation. The forest area is about 207.08 hectare. Mean annual rainfall is 1847.8 mm and mean annual temperature varies from 23-29°C. There are about 167 species of dicots under 125 genera within 45 families as recorded by Kamilya (2011).

We identified the target 16 species (only trees and climbers of Leguminosae) in the forest and recorded their distribution using Germin GPS. The GIS map is created using TNTmips 2013 software and displayed in fig. 1.

We collected the seeds and seedlings from September, 2012 to October, 2013 in connection with UGC Major Research Project [F. No. 41-484/2012(SR)dt. 16.07.2012]. Seeds were cleaned, air dried, characterized and weighed and sown separately in 1m×1m size



**Legend: Landuse**

- Brick kiln
- Cultivated land
- Forest area
- Open forest
- Pond
- Settlement
- Swamp forest

**Legend: Waypoint**

- Abrus precatorius*
- Acacia auriculiformis*
- Adenanthera pavonina*
- Albizia lebbeck*
- Bauhinia variegata*
- Caesalpinia bonduc*
- Cajanus scarabaeoides*
- Clitoria ternatea*
- Dalbergia sissoo*
- Delonix regia*
- Erythrina variegata*
- Mucuna pruriens*
- Leucaena latisiliqua*
- Peltophorum pterocarpum*
- Pongamia pinnata*
- Senna fistula*

Figure 1: Distribution map of leguminous plants in Danga forest

seedbeds in Departmental Garden to raise seedlings. The seedlings raised in seed beds are compared with the natural ones for proper identification. Herbarium sheets are made by

dried seedlings and diagnosed following Duke 1965, Burger 1972, DeVogel 1980 and Paria 2006.





Figure 2. Photograph of seedlings in natural habitat I- *Abrus precatorius*; II- *Acacia auriculiformis*; III- *Adenanthera pavonina*; IV- *Albizia lebeck*; V- *Bauhinia variegata*; VI- *Caesalpinia bonduc*; VII- *Cajanus scarabaeoides*; VIII- *Clitoria ternatea*; IX- *Dalbergia sissoo*; X- *Delonix regia*; XI- *Erythrina variegata*; XII- *Leucaena latisiliqua*; XIII- *Mucuna pruriens*; XIV- *Peltophorum pterocarpum*; XV- *Pongamia pinnata*; XVI- *Senna fistula*.

Various parameters are considered for characterization of seeds and seedlings in numerical form displayed in Table – 1 and Table –2 respectively.

Table1. Characters of seeds of taxa in numerical form

Name of the Species	texture	shape	color	surface	weight	Correlation with seedling type (Fig. 4) with respective weight at the end of the bars
<i>Abrus precatorius</i> L.	1	3	6	1	2	P1
<i>Acacia auriculiformis</i> A. Cunn. ex Benth.	1	2	1	1	2	P2
<i>Adenanthera pavonina</i> L.	1	2	2	1	2	P3
<i>Albizia lebeck</i> (L.) Benth.	1	4	3	1	2	P4
<i>Bauhinia variegata</i> L.	2	6	4	1	2	P5
<i>Caesalpinia bonduc</i> (L.) Roxb.	1	2	3	2	1	C1
<i>Cajanus scarabaeoides</i> (L.) du petit Thouars	1	1	4	1	1	C2

<i>Clitoria ternatea</i> L.	2	6	4	1	2	P6
<i>Dalbergia sissoo</i> Roxb.	1	3	1	2	2	P7
<i>Delonix regia</i> (Boj.) Rafin	1	5	4	2	2	P8
<i>Erythrina variegata</i> L.	1	6	5	1	1	P9
<i>Mucuna pruriens</i> (L.) DC.	1	1	1	1	1	C3
<i>Leucaena latisiliqua</i> (L.) Gillis	1	4	1	1	2	P10
<i>Peltophorum pterocarpum</i> (DC.) Backer	1	1	4	2	2	P11
<i>Pongamia pinnata</i> (L.) Pierre	2	1	1	3	1	C4
<i>Senna fistula</i> L.	1	3	4	1	2	P12

texture: hard 1, soft 2; shape: oblong 1, round 2, elliptic 3, ovate 4, narrowly elliptic 5, reniform 6; color: dark brown 1, red 2, grayish green 3, light brown 4, purple 5, red with black 6; surface: smooth 1, rough 2, wrinkled 3; weight: heavier 1, lighter 2.

Table 2. Characters of seedlings of the taxa in numerical form

Name of the Species	Nature	Hypocotyl		Paracotyledons							First two leaves							Subsequent leaves	First internode shape	
		shape	surface	petiole	shape	base	apex	margin	No. of primary veins	venation	phyllotaxy	nature	stipule	Shape	base	apex	margin			venation
<i>Abrus precatorius</i>	1	1	1	0	2	1	1	1	2	2	1	1	1	1	1	2	1	1	2	1
<i>Acacia auriculiformis</i>	1	2	3	0	2	1	1	1	2	2	1	3	1	1	3	2	1	1	4	1
<i>Adenanthera pavonina</i>	1	2	3	0	2	1	1	1	2	2	2	1	1	1	4	1	1	1	1	1
<i>Albizia lebbek</i>	1	2	3	0	2	1	1	1	4	1	1	3	1	1	3	3	1	1	1	1
<i>Bauhinia variegata</i>	1	1	1	1	2	3	1	1	3	1	1	2	1	4	2	5	1	2	3	1
<i>Caesalpinia bonduc</i>	2	0	0	0	0	0	0	0	0	0	1	1	2	3	4	3	1	1	1	1
<i>Cajanus scarabaeoides</i>	2	0	0	0	0	0	0	0	0	0	2	2	1	2	1	1	2	2	2	2
<i>Clitoria ternatea</i>	1	1	1	1	3	2	1	1	1	1	2	2	1	2	1	1	1	2	2	1
<i>Dalbergia sissoo</i>	1	1	2	1	3	2	1	1	1	1	1	1	1	3	4	3	1	1	2	2
<i>Delonix regia</i>	1	2	2	0	1	1	1	1	1	1	1	1	1	4	2	1	1	1	1	1
<i>Erythrina variegata</i>	1	1	1	0	3	1	1	1	2	2	2	2	2	2	1	1	2	2	2	1
<i>Mucuna pruriens</i>	2	0	0	0	0	0	0	0	0	0	2	2	1	2	2	1	1	2	2	2
<i>Leucaena latisiliqua</i>	1	2	3	1	2	1	1	1	1	1	1	3	1	1	3	3	1	1	1	1
<i>Peltophorum pterocarpum</i>	1	2	2	0	1	1	1	1	1	1	1	1	1	4	2	1	1	1	1	1
<i>Pongamia pinnata</i>	3	0	0	0	0	0	0	0	0	0	1	2	1	2	1	1	1	1	2	2
<i>Senna fistula</i>	1	2	2	1	2	3	1	1	1	1	1	1	1	3	4	1	1	1	2	1

Nature: phanerocotylar/ epigeal 1, cryptocotylar/ hypogeal 2, cryptocotylar/ geal 3. Hypocotyl: shape- round 1, 4-angular 2; surface- glabrous 1, pubescent 2, minutely pubescent 3. Paracotyledons: petiole absent 0, present 1; shape- oblong 1, obovate 2, reniform 3, absent 0; base- auriculate 1, cuneate 2, oblique 3, absent 0; apex- round 1, absent 0; margin- entire 1, absent 0; no. of primary veins- three 1, one 2, five 3, seven 4, absent 0; venation- actinodromous 1, hyphodromous 2, absent 0. First two leaves: Phyllotaxy- alternate 1, opposite 2; nature- compound unipinnate 1, simple 2, first unipinnate second bipinnate 3; stipule- only stipule present 1, stipel and stipule present 2; shape- oblong 1, ovate 2, elliptic 3, bilobed 4; base- rounded 1, cordate 2, oblique 3, cuneate 4; apex- acute 1, rounded 2, obtuse 3, mucronate 4, emarginated 5, acuminate 6; margin: entire 1; venation- camptodromous 1, actinodromous 2. Subsequent leaves: bipinnate 1, unipinnate 2, simple 3, phyllode 4; First internode: shape- 4-angular 1, round 2.



Color photographs of each taxon showing above characters are shown in fig. 2 with highlighted seeds, paracotyledons and first two leaves. **Artificial key: (Key valid for the taxa studied only)**

1. Seedlings phanerocotylar; seed weigh mostly lighter, rarely heavier.....2

1a. Seedlings cryptocotylar; seed weigh strictly heavier.....13

2. Paracotyledons strictly obovate, base strictly auriculate.....3

2a. Paracotyledons may be obovate or oblong or reniform, base may be auriculate or cuneate or oblique.....6

3. Hypocotyl minutely pubescent; apex of first two leaves either obtuse or mucronate, subsequent leaves bipinnate, never transforms into phyllodes.....4

3a. Hypocotyl glabrous; apex of first two leaves rounded, subsequent leaves transforms into phyllodes.....*Acacia auriculiformis*

4. Venation of paracotyledons actinodromous; first two leaves alternate, first leaf unipinnate, second leaf bipinnate, leaf base oblique, apex obtuse; seeds ovate in shape.....5

4a. Venation of paracotyledons hyphodromous; first two leaves opposite, both leaves unipinnate, leaf base rounded, apex mucronate; seeds round.....*Adenantha pavonina*

5. Paracotyledons sessile, number of primary veins seven; seeds grayish green in color.....*Albizia lebbeck*

5a. Paracotyledons petiolate, number of primary veins three; seeds dark brown in color.....*Leucaena latisiliqua*

6. Hypocotyl pubescent; venation of paracotyledons strictly actinodromous with three primary veins; first two leaves strictly unipinnate, base cuneate.....7

6a. Hypocotyl glabrous; venation of paracotyledons may be actinodromous or hyphodromous with one or three or five primary veins; first two leaves may be simple or

unipinnate, base rounded or cordate.....10

7. Hypocotyl 4-angular; paracotyledons obovate or oblong, base cuneate or auriculate; apex of first two leaves acute or rounded; internodes 4-angular; seeds light brown in color.....8

7a. Hypocotyl round; paracotyledons reniform, base oblique; apex of first two leaves obtuse; internodes round; seeds dark brown in color.....*Dalbergia sissoo*

8. Paracotyledons oblong, sessile, base auriculate; apex of first two leaves rounded; subsequent leaves bipinnate; seeds oblong or narrowly elliptic.....9

8a. Paracotyledons obovate, petiolate, base cuneate; apex of first two leaves acute; subsequent leaves unipinnate; seeds elliptic.....*Senna fistula*

9. Few glandular hair present on hypocotyl; paracotyledons shorter in length (17mm); seeds oblong.....*Peltophorum pterocarpum*

9a. Glandular hair absent on hypocotyls; paracotyledons longer in length (26mm); seeds narrowly elliptic.....*Delonix regia*

10. Base of paracotyledons auriculate or oblique; first two leaves oblong or ovate, apex rounded or acute; subsequent leaves unipinnate.....11

10a. Base of paracotyledons cuneate; first two leaves bilobed, apex emarginated; subsequent leaves simple.....*Bauhinia variegata*

11. Paracotyledons reniform; first two leaves opposite, simple, ovate, apex acute, venation actinodromous; seeds reniform in shape.....12

11a. Paracotyledons obovate; first two leaves unipinnate, simple, oblong, apex rounded, venation camptodromous; seeds elliptic in shape.....*Abrus precatorius*

12. Paracotyledons petiolate, base oblique,



seeds oblong, dark brown in color.....14

13a. First two leaves compound, elliptic, base cuneate, apex obtuse, spiny stipel present; subsequent leaves bipinnate; seeds round, grayish green in color..... *Caesalpinia bonduc*

14. Seedlings crypto-hypogeal; first two leaves opposite, venation actinodromous; seeds smooth.....15

14a. Seedling crypto-geal; first two leaves alternate, venation camptodromous; seeds

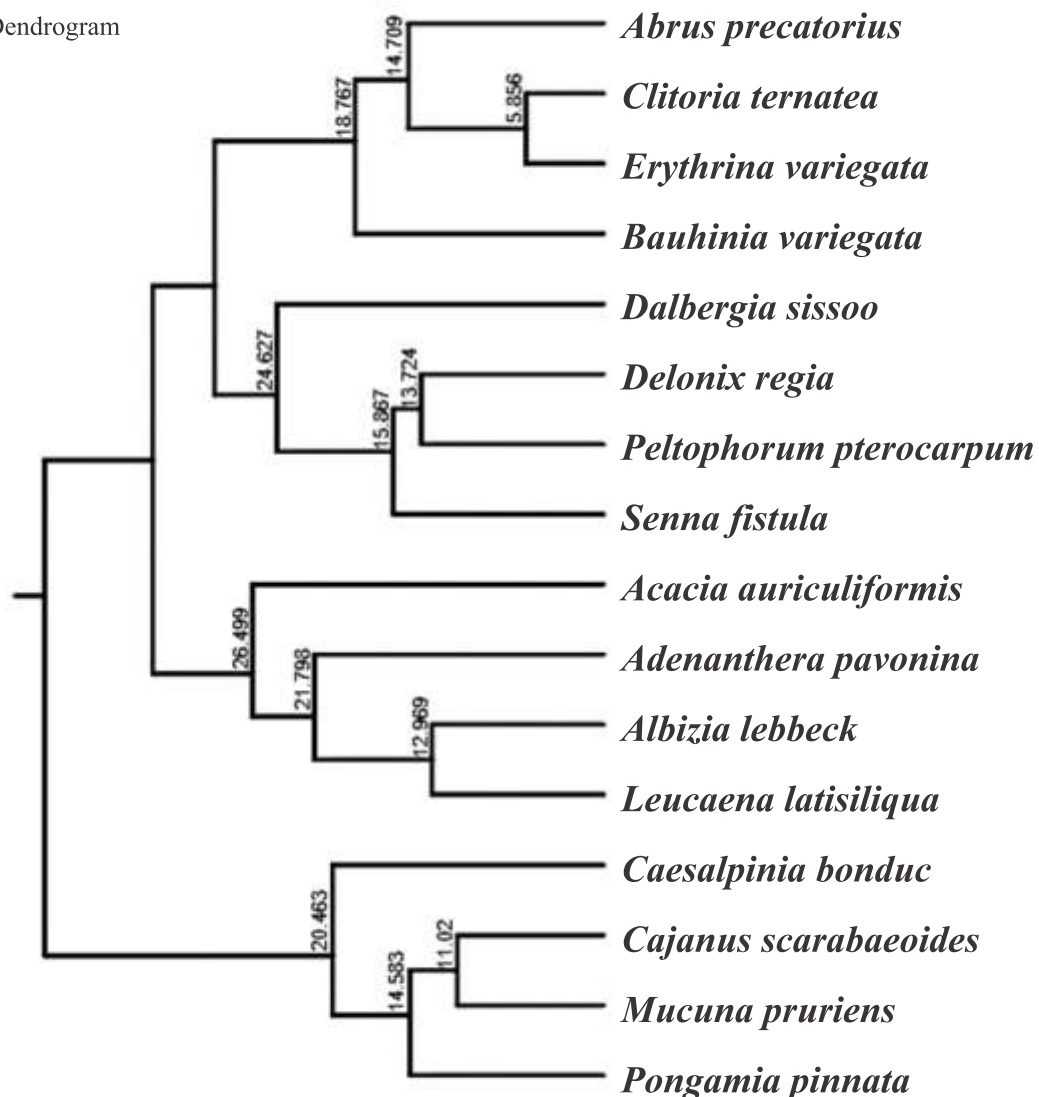
wrinkled..... *Pongamia pinnata*

15. Base of first two leaves rounded, seeds light brown in color..... *Cajanus scarabaeoides*

15a. Base of first two leaves cordate, seeds dark brown in color..... *Mucuna pruriens*

Statistical analysis and dendrogram are made by DendroUPGMA software which calculates a similarity or dissimilarity matrix from a set of variables and makes clustering (Garcia-Vallve, 1999). The software is available in internet and it uses Unweighted Pair Group method with

Figure 3. Dendrogram



Numerical data indicates Mean Branch Divergence value



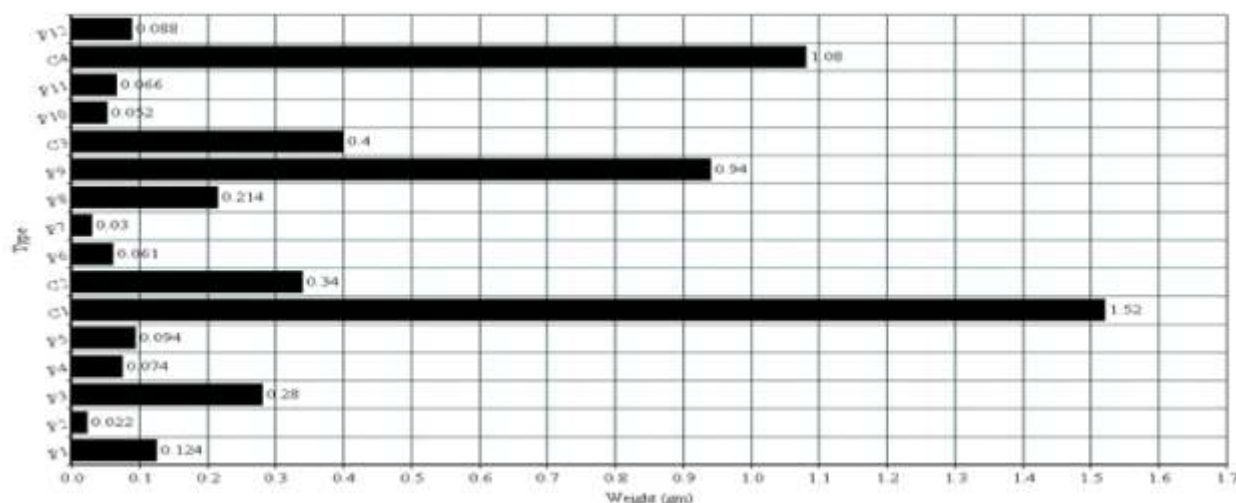


Figure 4. Correlation of seed weight with seedling type

Arithmetic mean (UPGMA) algorithm and constructs a dendrogram. We put the data (numerical form of characters) in fasta- like format and run the system in Pearson coefficient which measures linear correlation between those variables and shows the result through a distance matrix (Table-3) in phylip format where mean branch divergence value between the taxa is displayed. Finally we got the dendrogram.

**DISCUSSION:**

Interpretation on the correlation of taxa in the dendrogram has been made with the help of the artificial key. The dendrogram primarily displays two principal clades. It separates four taxa *Cajanus scarabaeoides*, *Mucuna pruriens*, *Pongamia pinnata* and *Caesalpinia bonduc* for their cryptocotylar mode of germination from the rest twelve taxa being their phanerocotylar mode of germination.

In the cryptocotylar clustering, *Cajanus scarabaeoides* and *Mucuna pruriens* show maximum proximity (mean branch divergence value 11.02) and they are only distinguished by subrounded base of first two leaves associated with light brown color of seeds in the former and cordate base of first two leaves associated with dark brown color of seeds in the later. *Pongamia pinnata* is distinctive (14.583) from *C. scarabaeoides* and *M. pruriens* on the basis

of its cryptocotylar-geal nature, alternate first two leaves with camptodromous venation and wrinkled seeds. The last taxon in this group, *Caesalpinia bonduc* shows contrasting attributes from the rest by characters of first two leaves and subsequent leaves; color and shape of seeds.

The other primary clade consisting of phanerocotylar members shows secondarily two clusterings; one subclade with eight taxa traditionally recognized as members of Faboideae and Caesalpinioideae, and the other subclade with four taxa traditionally recognized as Mimosoideae. The first secondary clade or subclade forms two lineages; in the first of which *Bauhinia variegata* separates (18.767) from the rest by possessing cuneate base of paracotyledons, bilobed first two leaves with emarginate apex and simple subsequent leaves. Further separation has been made in this clade where *Clitoria ternatea* and *Erythrina variegata* show closer proximity (5.586) differing only in the characters of paracotyledons, stipel, first two leaves and seeds. These two taxa again separated out from *Abrus precatorius* (14.709) on the shape of paracotyledons; nature, phyllotaxy, shape apex, venation of first two leaves and shape of seeds. In the other lineage of this secondary clade, *Delonix regia* and *Peltophorum pterocarpum* show maximum

level of proximity (13.724) but distinguishable only on the absence of glandular hair on hypocotyl, longer paracotyledons and narrowly elliptic seeds in *D.regia* while presence of glandular hair on hypocotyl, shorter paracotyledons and oblong seeds in *P. pterocarpum*. These two are again distinguished from *Senna fistula* (15.867) having oblong, sessile paracotyledons with auriculate base; rounded apex of first two leaves; bipinnate subsequent leaves and oblong seeds. *Dalbergia sissoo* has been isolated from the rest three taxa of secondary lineage on the shape of hypocotyls; shape and base of paracotyledons; apex of first two leaves; shape of internodes and color of seeds with mean branch divergence value 24.627.

In the other secondary subclade among the phanerocotylar members, there are four taxa i.e., *Acacia auriculiformis*, *Adenanthera pavonina*, *Albizia lebeck* and *Leucaena latisiliqua*. *A. auriculiformis* shows separate lineage from others (26.499) by glabrous hypocotyl, subsequent leaves becoming phyllode while in the rest taxa, hypocotyl is minutely pubescent and subsequent leaves never becoming phyllode. Furthermore, *A. lebeck* and *L. latisiliqua* are in the same lineage (12.969) but distinguishable only by sessile paracotyledons with seven primary veins and grayish green seeds in the former, and petiolate paracotyledons with three primary veins and dark brown seeds in the later. *Adenanthera pavonina* shows a different lineage (21.798) from these two taxa on venation of paracotyledons; nature and phyllotaxy of first two leaves with their base and apex; and seed shape.

From taxonomic point of view, there are some noteworthy points to fulfill our goal. Among the phanerocotylar clusterings, it is displayed that *Abrus precatorius*, *Clitoria ternatea* and *Erythrina variegata* represent the same subclade as they belong to subfamily Faboideae while the other one, where *Bauhinia variegata* belongs to subfamily Caesalpinioideae. *C. ternatea* and *E. variegata* being in the same

tribe Phaseoleae show the correlation of seed and seedling characters. In the later group, *Delonix regia*, *Peltophorum pterocarpum* and *Senna fistula*, all from subfamily Caesalpinioideae, have more correlation among their seed and seedling characters than the other member like *Dalbergia sissoo* of subfamily Faboideae. Again *D. regia* and *P. pterocarpum* are in the same lineage and possess characterstate correlation possibly by their presence in the same tribe Caesalpinieae. Moreover, the four species traditionally belonging to subfamily Mimosoidae represent the secondary clade or subclade among the phanerocotylar members in the dendrogram. In the cryptocotylar clustering, *Cajanus scarabaeoides*, *Mucuna pruriens* and *Pongamia pinnata* being from subfamily Faboideae form closer affinity than *Caesalpinia bonduc* of subfamily Caesalpinioideae. Moreover, *C. scarabaeoides* and *M. pruriens* of tribe Phaseoleae display same lineage.

But there are some negative correlations. *Dalbergia sissoo* of subfamily Faboideae does not correspond to its inclusion within the clade having other members of that subfamily. Other botanical disciplines like chromosome number, palynology, etc. support the inclusion of this genus in Faboideae, but it is contradictory to the seedling correlation. Similarly, *Bauhinia variegata* from subfamily Caesalpinioideae coexists with members of subfamily Faboideae in the dendrogram. Among the Faboideae clustering, *Adenanthera pavonina* and *Leucaena latisiliqua* of the tribe Mimoseae do not show close relation in seedling characters. Whereas it is evident from the dendrogram that *L. latisiliqua* exists with *Albizia lebeck* on their seedling characters disfavoring tribe-wise separation. It is also evident that *D. sissoo* and *P. pinnata* of the tribe Dalbergieae placed at two extremes in the cladogram as the former one shows phanerocotylar type and the later shows cryptocotylar type. Four taxa from tribe Phaseoleae viz., *Clitoria ternatea*, *Erythrina variegata*, *Cajanus scarabaeoides* and *Mucuna pruriens* do not coexist due to their differences in the basic developmental nature of

seedlings as the first two show phanerocotylar type against the later two of cryptocotylar type. Also *Delonix regia* and *Peltophorum pterocarpum* segregate out from *Caesalpinia bonduc* on their phanerocotylar nature although they are belonging to the same tribe *Caesalpinieae*.

Within this limited scope of study, it can be concluded that in some cases, traditional tribe-wise separation corresponds to seedling features. To arrive at concrete decision, we need to study of seedling characters of more taxa within this family. Thus, present study partially favours the separation of subfamilies of *Leguminosae* as *sensu lato*.

### Seed weight and nature of seedlings

We have also found that the seed weight of the cryptocotylar taxa is mostly heavier than the phanerocotylar ones except the seed weight of *Erythrina variegata* although from phanerocotylar group, matches with the cryptocotylar ones. Duke (1969) remarked that cryptocotylar species often have seedlings whose first leaves are cataphylls and Ng (1976) showed that for Malayan forest trees the seeds of the cryptocotylar species were in general larger and took longer time to germinate than did those of the phanerocotylar species. The same generalizations were applicable to Australian species (Clifford, 1984). Two other characters strongly associated with cryptocotyly are the lack of endosperm in the seed and the tree habit. In the present investigation, in addition to the above, some notable exceptions are found. For example, cryptocotyly is also associated with climber taxa like *Caesalpinia bonduc*, *Cajanus scarabaeoides*, *Mucuna pruriens*. Furthermore first leaves in seedlings of the above cryptocotylar taxa are not cataphylls except *Pongamia pinnata*.

### Ecological behavior of seedlings

The high proportion of cryptocotylar species in the *Magnoliflorae* usually regarded as rich in primitive taxa suggests that the character may be primitive, a view already proposed by

Grushvitskyi (1963). Popma and Bongers (1988) showed that phanerocotylar seedlings generally grew faster than seedlings with other types when they were exposed to increased light. Strauss-Debenedetti and Bazzaz (1991), working their congeneric species of *Moraceae* and found that phanerocotylar seedlings have higher maximum photosynthetic rate than cryptocotylar seedlings. In our observation, most phanerocotylar taxa having paracotyledon size and thickness are somewhat larger than the size of cotyledons in seeds. Cryptocotylar seedling is presumably related to plant life histories adjusted to light limited environments (Foster, 1986). It has been found that seedling survival in the shade increases with seed weight (Leishman and Westoby, 1994; Saverimuttu and Westoby, 1996; Paz, Mazer and Martinez-Ramos, 1999). Furthermore, bigger seeds produce more vigorous seedlings that better withstand physical and biotic damages (Clark and Clark, 1991). In Danga forest, we found that the species with cryptocotylar seedling type possess greater seed weight (Table-1 and Fig. 4) and most of them are growing in shady places. Moreover, large seeds are constrained to disperse the seeds near the neighborhood of maternal plants where the probability of landing in a shaded site is high (Howe and Smallwood, 1982; Fenner, 1985; Gonzalez-Mendez, 1995). We also observed most of the seedlings of large seeds in the vicinity of mother plants in the forest.

Besides such study is helpful for identification of taxa before flowering and fruiting condition at natural habitat. After that they may be considered for phytodiversity management to increase the diversity of flora in the traditional forest.

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