

Early identification and phenetic analysis of eight species of sub-tribe Cassiinae [Leguminosae : Caesalpinioideae] found in Tripura in relation to their seedling morphology

Mandakranta Roy¹ and B. K. Datta

Taxonomy and Biodiversity Laboratory, Department of Botany, Tripura University,
Suryamaninagar-799130, Tripura, India

¹Corresponding author; E-mail: mandakranta.roy@gmail.com

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Abstract

A study was conducted to analyse the phenetic relationship of eight species of sub-tribe Cassiinae (Fabaceae), found in various parts of Tripura based on their seedling morphological characters and to prepare a synoptic key for their early identification. All the taxa showed Phanerocotylar Epigeal Foliaceous (PEF) type of germination, with marked difference in their cotyledonary morphological characters. For preparation of the artificial key various parameters like - shape of cotyledons, venation pattern, hypocotyls, epicotyls, eophylls, leaves etc. were considered. The phylogenetic status of the studied taxa had been determined on the basis of seedling morphological characters and presented through a dendrogram.

Key words: Seedling morphology, *Cassia*, Phanerocotylar Epigeal Foliaceous (PEF), dendrogram.

INTRODUCTION

Seedling stage is a critical phase in the life cycle of an individual (Harper 1977; Angevine and Chabot 1979; Silvertown *et al.* 1993). It manifests such diversity of morphological characters which are as useful and reliable as that of floral features that can be used for early identification of any taxa (Duke 1965; Duke 1969; Duke & Polhill 1981; Burger 1972; Ye 1983). Morphology of seedling stage can help to find derived or primitive transitory structures those disappear during the phylogenetic connections (Ricardi *et al.* 1977; Torres, 1985). Emphasis on seedling morphology was given since the beginning of the 20th century (Compton 1912); however, data are still insufficient (Garwood 1995). Little investigation and progress in this field had been there particularly in the context of Indian flora (Paria 1998). At present this field is of growing interest for scientists as it offers valuable information not only in taxonomy but also in ecology, forestry, silviculture and crop weed interaction. In context to this, a survey was carried out to investigate species relationship of eight members of sub-tribe *Cassiinae* (Leguminosae : Caesalpinioideae) found in Tripura [viz., *Cassia fistula* Linnaeus, *C. javanica* subsp. *nodosa* (Roxburgh) K. Larsen & S.S. Larsen, *C. renigera* Bentham, *Senna alata* (Linnaeus) Roxburgh, *S. occidentalis* (Linnaeus) Link, *Senna siamea* (Lamark) H.S. Irwin & Barneby, *Senna sophera* (Linnaeus) Roxburgh and *Senna tora* (Linnaeus) Roxburgh] based on their seedling morphological characters and to prepare a synoptic key for the early identification and characterization of the studied taxa at their juvenile stages.

The sub-tribe *Cassiinae* comprises of 3 genera namely *Cassia*, *Chamaecrista* and *Senna* (Irwin & Barneby 1981). According to the monographic study on Cassiinae by Singh (2001) there are 43 species of *Senna*, 11 species of *Chamaecrista* and 8 species of *Cassia* found in India. However, other studies based on morphological and molecular data have reported

that the monophyletic relationship among *Cassia*, *Chamaecrista* and *Senna* remains unclear (Doyle *et al.* 2000; Bruneau *et al.* 2001; Kajita *et al.* 2001). According to Deb (1981), there are 9 species of sub-tribe *Cassiinae* found in Tripura belonging to two genera *Cassia* and *Senna*, namely *Cassia fistula*, *C. javanica* ssp. *nodosa*, *C. renigera*, *Senna alata*, *S. occidentalis*, *S. siamea*, *S. sophera*, *S. tora* and *S. timorensis*. They are frequently occurring both in wild as well as in cultivated condition at almost all parts of the state. Out of them 2 are undershrubs [*S. occidentalis* and *S. tora*], 2 shrubs [*S. alata* and *S. sophera*] and the rest 5 are trees [*C. fistula*, *C. javanica* ssp. *nodosa*, *C. renigera*, *S. siamea* and *S. timorensis*]. *S. siamea* and *S. fistula* are most commonly available trees and *S. timorensis* being one of the rarest among *Cassiinae* in Tripura, no record of it was obtained during the present survey.

The members of *Cassiinae* found in Tripura have a wide range of importance. They possess a significant amount of medicinal properties and a few among them are of great economic importance. *Senna siamea* and *Cassia fistula* are commonly found as avenue trees and are also planted in tea gardens for shade and to maintain soil nitrogen. The leaves of *Senna alata* are exclusively used by the Chakma and Darlong tribal communities of Tripura in the treatment of ringworm infections (Deb *et al.* 2012; Oladele *et al.* 2010). It has also been reported in the treatment of leprosy and syphilis (Kritikar & Basu 1935). The use of *Senna sophera* leaves as an antidote for snake bites is well documented in the Indian literature (Kritikar & Basu 1935). *Cassia fistula*, also known as the Golden Shower tree for its beautiful yellow showy flowers, is well known in Ayurvedic medicine as *aragvadhā* (“disease killer”). It has hepatoprotective, anti-inflammatory, antitussive, antibacterial, antifungal activities and is also used in wound healing (Gupta 2010). The seed extracts of *Cassia javanica* ssp. *nodosa* has effective purgative nature and haemagglutinating activity and the seed gum has rheological activity (Goncalves *et al.* 2004). The leaves and flowers of *Senna occidentalis* are edible and the infusion of leaves has long been used as an effective medicine for the treatment of hepatitis in rainforests (Nuhu & Alio 2008). *Cassia renigera* also known as Burmese pink cassia is a rich source of flavonoids and anthraquinones (CSIR 1992). A study on free radical scavenging activity screening of medicinal plants from Tripura showed a positive result of antioxidant activity of stems of *Cassia javanica* ssp. *nodosa* and *Cassia renigera* (Kshirsagar & Upadhyay 2009). The alcoholic extract of flowers of *Senna siamea* has a potent antioxidant activity against free radicals and affords significant protection against oxidative damage in liver (Kaur *et al.* 2006). *Senna tora* is traditionally used as laxative, for the treatment of leprosy and various skin disorders and is also effective against free radical mediated diseases (Rejiya *et al.* 2009).

So far no synoptic key has been constructed for the flora of Tripura, based on seedling morphological characters. Therefore, the key prepared in the present study is completely a new initiative in Tripura for the early identification and conservation of the studied taxa. The phenogram presented in this study is the first report of implication of seedling morphological characters in phenetic analysis of the members of the sub-tribe *Cassiinae* found in Tripura.

MATERIALS AND METHOD

In the present investigation, seedlings of various stages of eight species of sub-tribe *Cassiinae* found in Tripura viz., *Cassia fistula*, *Cassia javanica* ssp. *nodosa*, *Cassia renigera*, *Senna alata*, *Senna occidentalis*, *Senna siamea*, *Senna sophera* and *Senna tora* were collected from different areas of the state and identified by comparing with the seedlings raised from identified seeds in the experimental garden. For seedling morphological study, the terminologies proposed by Burger (1972), Hickey (1973), Bokdam (1977) and Vogel (1980) were followed.

For diagnosis of seedlings characters like cotyledon, eophyll, hypocotyl, etc. the method of Vogel (1980), and Das & Paria (1999) were taken into consideration. The seedling specimens representing the different stages of development were dried and preserved as herbarium vouchers in Tripura University Herbarium.

Taking the seedling characters into count an artificial key was constructed for the early identification of the investigated taxa. Numerical evaluation of seedling morphological characters for determining phenetic relationships among the investigated taxa had been done through cluster analysis which is represented by Phenograms constructed by different linkage methods such as Unweighted Pair Group Method of Arithmetic Average (UPGMA) and Single Linkage with the assistance of PAST software program to draw affinities between the investigated taxa as far as practicable. The characters used for the cluster analysis were represented in the form of a comparative table (Table 1).

RESULT

Details of the investigation of seedling morphology on the plants under Cassiinae from Tripura are presented in Table 1.

Table 1. Comparative analyses of the morphological characteristics of the studied seedlings [Abbreviations used: **ac**: acute; **act**: actinodromous; **apc**: apiculate; **asym rnd**: asymmetrically round; **aur**: auriculate; **brd lan**: broad lanceolate; **cun**: cuneate; **del**: deltoid; **ellip**: elliptic; **lan**: lanceolate; **lin**: linear; **muc**: mucronate; **obl**: oblong; **obl obv**: oblong obovate; **obl orb**: oblong orbicular; **obl ov**: oblong ovate; **oblq**: oblique; **oblq trunc**: obliquely truncate; **obv**: obovate; **orb**: orbicular; **ov**: ovate; **pcot**: paracotyledon; **PEF**: Phenerocotylar Epigeous Foliaceous; **ren**: reniform; **rnd**: round; **rtnd**: rotund; **st-g**: straight-glabrous; **st-h**: Straight-hairy; **st-h***: straight- characteristic white hairs, up to 0.3cm long and perpendicular to stem; **slght ntchd**: slightly notched]

| Name of the taxa | Seedling characters | | | | | | | |
|---|---------------------|-----------|---------|-----------------------------|---------|--|-----------|-------------------------|
| | Seedling type | Hypocotyl | Stipule | Cotyledons / Paracotyledons | | | Eophyll | |
| | | | | Type | Shape | No. of primary veins, venation pattern | Shape | Apex, base |
| <i>Cassia fistula</i> Linnaeus [Roy & Datta 105] Fig. 1(b-c). | PEF | St-h | del | pcot | obv | 5, act | ov | ac, rnd |
| <i>Cassia javanica</i> ssp. <i>nodosa</i> (Roxburgh) K. Larsen & S.S. Larsen [Roy & Datta 112] Fig. 1(d-f). | PEF | St-g | lin | pcot | obl | 3, act | obl | muc, oblq trunc |
| <i>Cassia renigera</i> Bentham [Roy & Datta 122] Fig. 1(j-k). | PEF | St-h | ren | pcot | obl ov | 3, act | obl ov | muc, oblq trunc |
| <i>Senna alata</i> (Linnaeus) Roxburgh (Roy & Datta 081] Fig. 1(a). | PEF | St-h | aur | pcot | rnd | 7, act | obl obv | rnd – slght ntchd, oblq |
| <i>Senna occidentalis</i> (Linnaeus) Link [Roy & Datta 092] Fig. 1(g-i). | PEF | St-h* | lin | pcot | obl orb | 5, act | brd lan | apc, rtnd |
| <i>Senna siamea</i> (Lamarck) H.S. Irwin & Barneby [Roy & Datta 105] Fig. 1(l-m). | PEF | St-h | lin | pcot | obl orb | 5, act | ellip | muc, asym rnd |
| <i>Senna sophera</i> (Linnaeus) Roxburgh [Roy & Datta 097] Fig. 1(n). | PEF | St-h | lin | pcot | orb | 5, act | obl - lan | ac, oblq |
| <i>Senna tora</i> (Linnaeus) Roxburgh [Roy & Datta 099] (Fig. 1(o-p). | PEF | St-h | lin | pcot | orb | 5, act | obv | apc, cun |

Based on the seedling morphological characters a dichotomous key has been constructed to facilitate the identification of these plants in their natural habitat. The Key is presented below:

Key to the plants of Cassiinae of Tripura

- 1a. Primary veins of cotyledons 3, stipules reniform, leaflets in 4 - 6 pairs in eophylls ... 2
 1b. Primary veins of cotyledon more than 3, stipules never reniform, leaflets in 2-3 pairs in eophylls 3
 2a. Paracotyledons sessile, leaflets oblanceolate with blunt acuminate apex *Cassia javanica* ssp. *nodosa*
 2b. Paracotyledons with petioles 0.1cm long; leaflets oblong ovate with acute apex ... *Cassia renigera*
 3a. Petiolules never orange, primary veins 5 in cotyledons, stipules not auriculate 4
 3b. Petiolules characteristically orange, primary veins 7 in cotyledons, stipule auriculate .. *Senna alata*
 4a. Cotyledons 0.1 cm thick initially, leathery later, stipules deltoid, leaflets up to 8 cm long at higher stages *Cassia fistula*
 4b. Cotyledons always membranous, stipules linear, leaflets not more than 6 cm long at higher stages 5
 5a. Stem with reddish yellow lenticels; lamina elliptic, reddish brown tinged, midrib bifurcated at 2/3 length of the cotyledon and forming continuation with the adjacent primary nerves forming a characteristic heart shape *Senna siamea*
 5b. Lenticels never reddish brown, lamina not elliptic, always green, never reddish brown tinged, midrib not forming any characteristic heart shape on cotyledon 6
 6a. Stem with characteristic white simple hairs up to 0.3 cm arranged perpendicular to the stem, leaflets ovate-lanceolate, base rotund *Senna occidentalis*
 6b. Hairs if present very short, simple, colourless, up to 0.1 cm long, leaflets obovate or oblong- lanceolate, base not rotund 7
 7a. Leaflets oblong, lanceolate later, acute, base oblique *Senna sophera*
 7b. Leaflets obovate, apiculate, base cuneate *Senna tora*

DISCUSSION

The eight investigated species of sub-tribe *Cassiinae* [Leguminosae : Caesalpinioideae] in Tripura, namely *Cassia fistula* (Fig. 1b), *Cassia javanica* ssp. *nodosa* (Fig. 1d), *Cassia renigera* (Fig. 1j), *Senna alata* (Fig. 1a), *Senna occidentalis* (Fig. 1g), *Senna siamea* (Fig. 1l), *Senna sophera* (Fig. 1n) and *Senna tora* (Fig. 1o) were characterised by some important seedling morphological features which can serve as marker characters for identification of the taxa at species level. It was observed that all the eight studied species showed Phanerocotylar Epigeal Foliaceous (PEF) type of germination. PEF type of germination is advantageous over other types in many ways. Seedlings with PEF type of germination generally grow faster than seedlings with other types when they were exposed to increased light (Pooma & Bongers 1988). Such germination produce seedlings with higher maximum photosynthetic rates than Cryptocotylar Epigeal Reserve (CHR) type (Strauss-Debene-detti & Bazzaz 1991). According to Hladik & Miquel (1990), Garwood (1996), Kitajima (1996) and Green & Juniper (2004b), functional morphology of cotyledon determines how the resources are used during initial seedling growth and development which in turn is

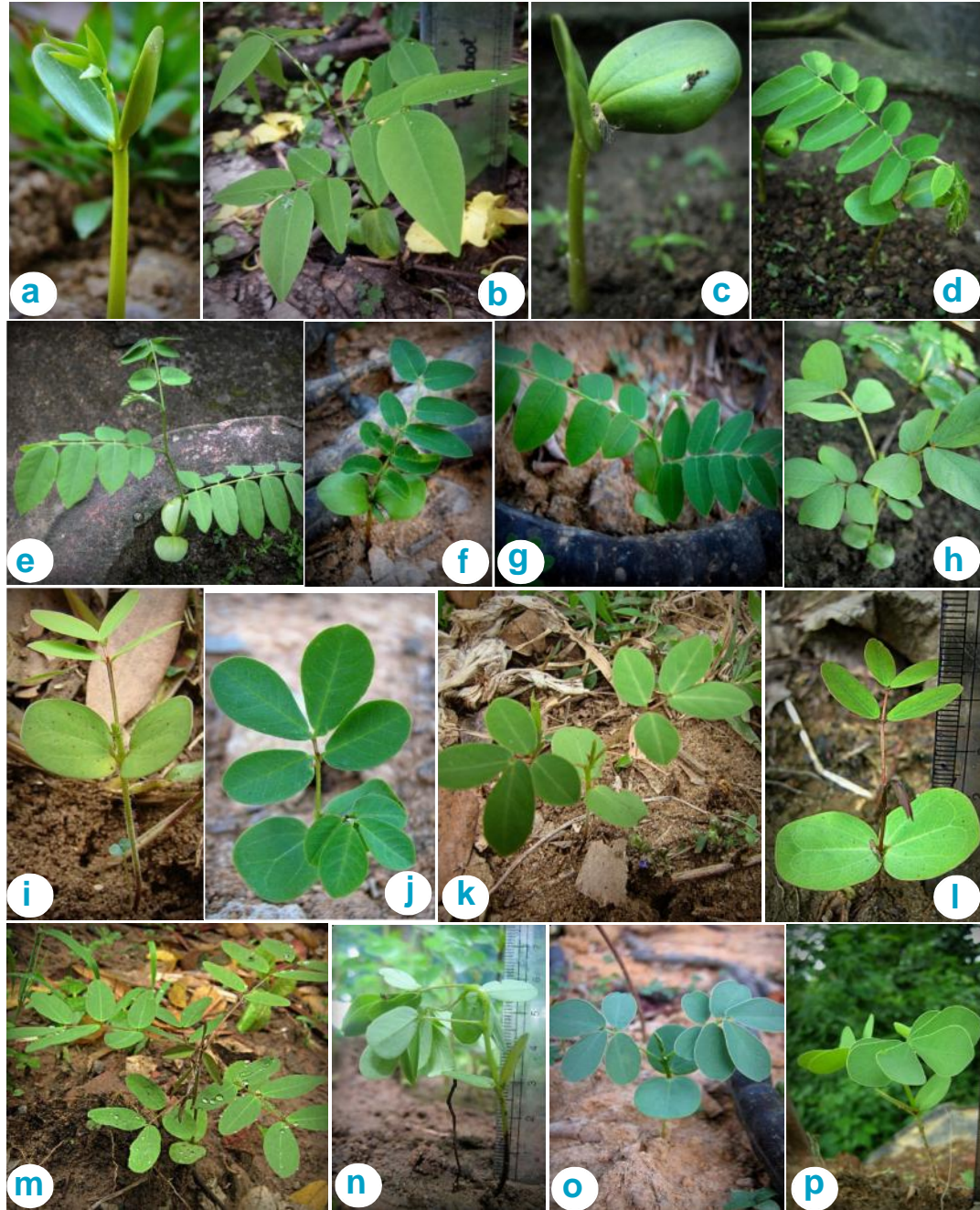


Fig. 1. Various seedling stages of the eight investigated members of two genera of sub tribe *Cassiinae* found in Tripura: **(a-b)** *Cassia fistula*; **(c-e)** *Cassia javanica* ssp. *Nodosa*; **(f-g)** *Cassia renigera*; **(h)** *Senna alata*; **(i-k)** *Senna occidentalis*; **(l-m)** *Senna siamea*; **(n)** *Senna sophera*; **(o-p)** *Senna tora*.

associated with the survival and regeneration of the species in particular habitats. In this investigation all the studied taxa had foliaceous photosynthetic cotyledons. One main advantage of having such is that foliaceous cotyledons help in faster autotrophic growth of seedlings in

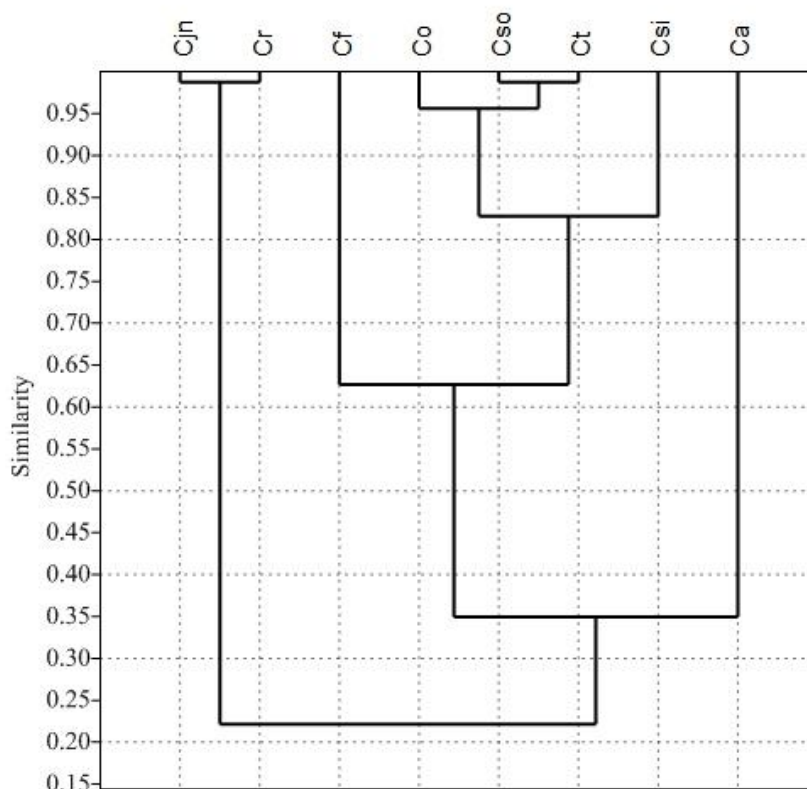


Fig. 2: Phenogram based on seedling morphological data (UPGMA)

[Abbreviations used in Fig 2, Fig. 3 & Fig. 4: Cf- *Cassia fistula*, Cjn- *Cassia javanica* subsp. *nodosa*, Cr- *Cassia renigera*, Ca- *Senna alata*. Csi- *Senna siamea*, Cso- *Senna sophora*. Ct- *Senna tora*]

highly lighted environment compared to the reserved ones (Garwood 1996). Species with photosynthetic cotyledons start using light as an energy source much earlier than those with reserve cotyledons (Kitajima 2002) and also enable the plants to use rich light resources found in forest gaps (Kitajima 1992).

Interspecific distinction between the investigated taxa (Table 1) revealed that there are 3 primary nerves in the cotyledon of *Cassia renigera*, and *C. javanica* subsp. *nodosa*, 7 in *Senna alata* and 5 in the rest of the taxa under study, viz. *Cassia fistula*, *Senna occidentalis*, *Senna siamea*, *Senna sophora* and *Senna tora*. In *Cassia renigera* the paracotyledons are sessile and the leaflets were oblanceolate (Fig. 1k) whereas in *C. javanica* ssp. *nodosa* the paracotyledons were shortly petiolate and the leaflets were oblong-ovate (Fig. 1f). *Senna alata* was distinct from rest of the studied species in having characteristic orange coloured petiolules (fig. 1a). *S. occidentalis* unlike all the other studied taxa had remarkably white hairs up to 0.3 cm long just beneath its cotyledons placed at right angle to the stem (fig. 1d). The cotyledonary mid-vein in *S. siamea* is bifurcated at 2/3 length of the blade and got joined with the adjacent primary nerves to form a characteristic clear heart shape on the blade (Fig. 1l) which was indistinct in rest of the species observed. *Cassia fistula*, *Senna occidentalis*, *S. siamea*, *S. sophora* and *S. tora* differed from each other distinctly in terms of shape and colour of the leaflets. The leaflets of *S. siamea* unlike others were elliptic with a characteristic brownish tinge (fig.

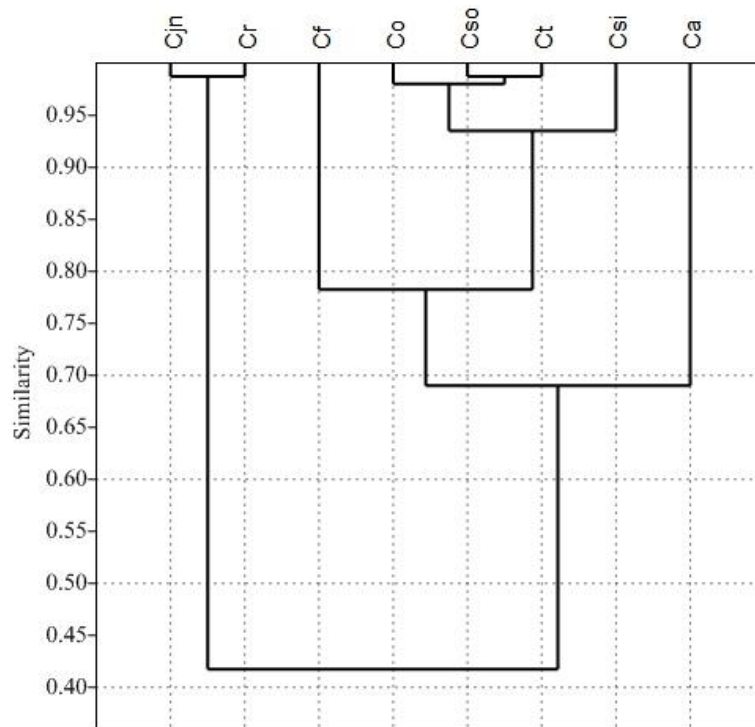


Fig. 3: Phenogram based on seedling morphological data (Single Linkage method)

1m). The leaflets of *S. sophera* were green, oblong initially, lanceolate later with acute apex and oblique base whereas leaflets (Fig. 1n) of *S. tora* were obovate with apiculate apex and cuneate base (Fig. 1p). *Cassia fistula* had ovate leaflet with acute apex and round base (Fig. 1c) whereas in *Senna occidentalis* the leaflets were broadly lanceolate with apiculate apex and rotund base (Fig. 1h & 1i). The length of epicotyls was distinctly variable in all the taxa studied (Fig. 4). A comparative analysis of epicotyl length of the studied taxa in Fig 4 showed that *C. fistula* had longest epicotyl, measuring 3.2 cm, than rest of the taxa studied, followed by *S. sophera* (3 cm) and *S. alata* (2.2 cm). *S. tora* had smallest epicotyl of only 0.3 cm long (Fig. 4). Cotyledon measurement of all the eight studied species confirmed a significant positive and linear correlation ($r = 0.867$, $p = 0.001$) between the length and breadth of cotyledons (Fig. 5). Calculation of the linear regression showed $Y = 0.94632 - 0.27485 X$ (Y being the dependent variable: Cotyledon breadth and X being the dependent variable: cotyledon length).

Taking into account of such variable seedling exomorphic characters, the phenetic analysis had been carried out to draw species affinity at their juvenile stages. Multivariate phenetic analyses have been used in classifying many plants and interpreting results of taxonomic studies (Gomez-Compo *et al.* 2001; Chiapella 2000; Sneath & Sokal 1973). Seedling features are often distinctive at the species level and correlate well with data from other source with regard to sectional and sub sectional placement of species with genus (Canne 1983). Cluster analysis based on UPGMA method revealed the presence of distinct clusters among the investigated taxa as indicated in the phenogram (Fig. 2). The ultimate outcome of the eight studied taxa through the phenogram revealed that the whole phenogram was divided into two main clades. One clade separated the two closely related taxa viz., *C. renigera* (Cr) and *C. javanica ssp nodosa* (Cjn) showing more than 95 %

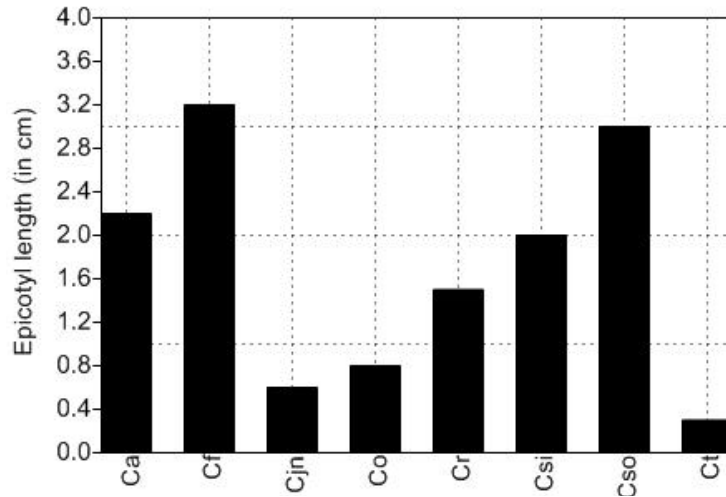


Fig. 4: Comparative analysis of epicotyl measurement of the eight investigated species of sub-tribe *Cassiinae* in Tripura

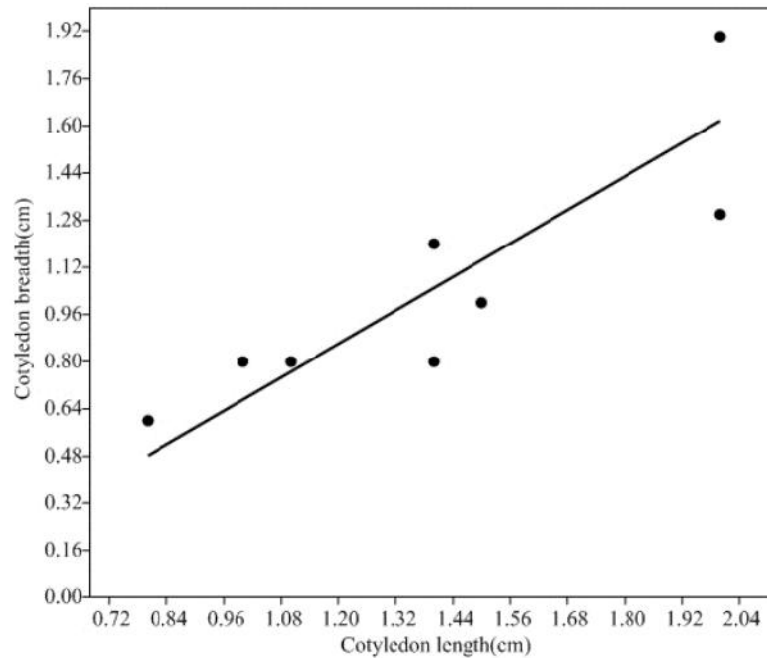


Fig. 5: A graphical representation of data showing a significant positive correlation between cotyledon length and breadth of the studied species of sub-tribe *Cassiinae* found in Tripura

similarity, from rest of the studied taxa on the basis of characteristic reniform stipules. The second clade containing the rest of the six studied taxa was again divided into two major clades separating *S. alata* (Ca) from the rest on the other clades with less than 40 % similarity. This is due to the presence of remarkable orange petiolules in (Ca) unlike the others. *S. sophera* (Cso) and *S. tora* (Ct) were closely related with more than 96 % similarity and was represented by the same clade which was joined by *S. occidentalis* (Co), followed by *S. siamea* (Csi) and *C. fistula* (Cf). It is noteworthy that *S. sophera*

(Cso) and *S. tora* (Ct) were distinguishable in few morphological characters viz., leaflet shape, apex and base. *S. occidentalis* (Co) unlike the above two species were separated from them in having remarkable white long hairs on its hypocotyl. Cs and Cf were further separated on the basis of leaflet shape. The leaflets were elliptic in the former with mucronate tips and asymmetrical base while the leaflets were ovate with acute apex and rounded base in the later. The emergence of taxa on the Phenogram remained same as well as distinct irrespective of the method used such as UPGMA or Single Linkage method, although the linkage similarity between the taxa varied (Figs. 2 & 3).

CONCLUSION

The study presented here is the first report of implication of seedling morphological characters in phenetic analysis of the members of *Cassinae* found in Tripura. Such characters are considered to be genetic markers as because these are manifested in the seedlings just after the seed germination and before attaining the secondary growth. Moreover, due to rapid urbanization, industrializations, over exploitation and jhum cultivation the forest resources of one of the richest biodiversity states like Tripura is getting lost day by day. As no synoptic keys based on seedling morphology for floristic studies in Tripura had yet been prepared till date, this initiative can be helpful in the early identification and better management of such highly medicinal plants of the state and can also help further in conservation and prevention of loss of plant resource from the forests.

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