AMBIENT APPRAISAL

Phenetic Analysis of Seedling Morphology of Some Members of Cucurbitaceae from Tripura, North-east India

Rashmi Rani*, Badal Kumar Datta

Plant Taxonomy and Biodiversity Laboratory, Department of Botany, Tripura University, Suryamaninagar, 799022, Tripura.

Study Area: Tripura, India

Coordinates: 23.84°N; 91.28°E

Keywords: Taxonomy,
Clustered analysis

Study area

Introduction:

Seedling is an important stage in the plant life cycle and is arguably the busiest phase in a plant's lifetime (Farnsworth, 2008). The seedling of a species is usually uniform in morphology. The seedlings of many taxa, particularly herbs, are so small as to escape attention in their native habitats. But juvenile stages of plants, particularly of trees, are often so strikingly different from the adult stages, that even with good field knowledge of plants it is difficult to correlate the seedling plant with an adult shoot of the same species. Seedling is a critical phase in the life cycle of an individual (Harper, 1977). Seedling characters are as reliable as the floral ones. A critical examination of the correlation between the group of characters of both the adult as well as the juvenile plant has repeatedly shown results in a better understanding of taxonomically difficult taxa (Bokdam, 1977). The study of seedling morphology, which had been a less explored field in flowering plants, particularly in angiosperms, has now emerged as an essential discipline for taxonomic research at present. Moreover, the worldwide loss of biodiversity is a symptom of indiscriminate human exploitation of natural resources and developing land for human use (Savard et al. 2000). So, proper studies on seedling and their morphology will be helpful for their early identification and conservation of natural resources which in turn shall help in the conservation of biodiversity. In this study *Coccinia grandis* (L.) Voigt, *Diplocyclos palmatus* (L.) Jeffrey, *Gymnopetalum chinense* (Lour.) Merr., *Mukia maderaspatana* (L.) Roem. and *Trichosanthes tricuspidata* Lour. have been investigated based on seedling characteristics. Juice of the whole plant of *C. grandis* is

Abstract

Seedling characters are important and promising from the taxonomic as well as evolutionary point of view. The seedling morphology of five medicinal plants of Cucurbitaceae was studied from different parts of Tripura, North-east India. Seedling morphology of five species of Cucurbitaceae has been investigated on the basis of their germination pattern, the position of cotyledon, hypocotyl, epicotyl, and para-cotyledon and eophylls development. An artificial key has been constructed for easier identification of plants in their juvenile stage. Phenogram has been prepared to investigate the correlation between the investigated taxa. Cluster analysis summarized the relationship among the investigated taxa into a phenogram.

taken by Chakma for diabetes. (Morshed, 2013.). *D. palmatus* is used as reproductive medicine (Vadnere et al., 2013). The decoction of the root *G. chinense* is taken as an antidote to poisoning by ripe fruits and against tetanus after a miscarriage. (Rahman, 2013.). *M. maderaspatana* used in epilepsy and rheumatism (Rahman & Keya, 2015). *T. tricuspidata* used for curing asthma, migraine, fever, diabetic carbuncles and other maladies (Bhandari et al., 2008). Medicinal plants can be identified in the seedling stage in the field and can be helpful in safeguarding medicinal plants by transplanting to a compatible environmental condition. The key prepared in the present study is completely a new initiative for the early identification and conservation of the studied taxa. The phenogram presented in this study is the first report of the implication of seedling morphological character in the phenetic analysis of the members of the family Cucurbitaceae found in Tripura.

Materials and methods:

Study area: Study area: Tripura is a state in North-East India and considered as a biodiversity hotspot. It borders Bangladesh, Mizoram, and Assam. Tripura is surrounded by Bangladesh on its north, south and west: the length of its international border is 856 km (84% of its total border). The state lies between 22°56' to 24°32' North latitudes and 91°09' to 92°20' East longitudes with an aerial extent of 10,491.69 sq. km.

Our study: the mature seedling specimens *Coccinia grandis*, *Diplocyclos palmatus*, *Gymnopetalum chinense*, *Mukia maderaspatana* and *Trichosanthes tricuspidata* were

*Corresponding Author: rashmi.botany@tripurauniv.in

collected from different localities of Tripura. The specimens were photographed and documented in the form of herbarium sheets. They were compared and identified with the help of seedling raised from identified seeds. At least eight to ten specimens of each growth form were studied from various habitats. Morphological observation and description of seedlings were done according to Duke (1965), Burger (1972), Bokdam (1977), Vogel (1980). Artificial keys were prepared for the identification of investigated taxa in the juvenile stage. Phenograms were prepared based on UPGMA method in PAST software. Soil pH, electrical conductivity has been calculated based on the Soil water suspension method. (Soil survey staff, 2004). Soil organic carbon had been calculated based on the Walkley-Black method (Reference add). Soil available phosphorus had been calculated based on Olsen and Sommers, and Bray and Kurtz method (Reference add). Available soil potash had been calculated by Hanway and Heidelberg method. Soil characters had been calculated to correlate soil properties with the seedling.

Table-1: GPS data of the site of sample collection

#	Voucher #	Species	Locality	Lat.	Long.
1	TUH2115	<i>C.grandis</i>	Bikramnagar	23.7472	91.2694
2	TUH2189	<i>D.palmatus</i>	Netaji Nagar Rd	23.7984	91.2631
3	TUH2217	<i>G.chinense</i>	Pandabpur	23.7654	91.2393
4	TUH 2218	<i>M. maderas-patana</i>	Madhupur	23.7612	91.2614
5	TUH 2211	<i>T.tricuspidata</i>	Madhupur	23.7617	91.2619

Results & Discussion:

Diagnoses and key to identification of the studied taxa.:

- i). *Coccinia grandis* (Linnaeus) Voigt, Hort. Suburb. Calcutt. 59. 1845. (Plate -1).

Vernacular name: Telakucha, Bimbika, Kunduru.

Seedling Morphology: (Up to 10th leaf stage)

Seedling PEF. Roots tap, elongating. Hypocotyl epigeous, elongating. Paracotyledon sessile, exstipulate, leaf oblong, $\pm 2-3 \times \pm 1-2$ cm, apex retuse, margin entire, base attenuate, venation pattern palmate. Epicotyl hairy, $\pm 0.1-0.3 \times \pm 0.1-0.2$ cm. Eophylls simple, alternate, soft/membranous, exstipulate, petiolate, leaf ovate, $\pm 2-4 \times \pm 3-4$ cm, base cordate, apex acute, margin sinuate, venation pattern palmate, surface of blade glabrous both surface. Next leaves simple, alternate, ovate, other characters more or less same as that of first two leaves except measurements.

- ii). *Diplocyclos palmatus* (Linnaeus) C. Jeffrey, Kew Bull. 15: 352. 1962. (Plate-1).

Vernacular name: Mala, Shivalingi, Put loguli, Bankakra, Lollipop Climber, Marble Vine.

Seedling Morphology: (Up to 6th leaf stage)

Seedling PEF. Roots tap, reduced. Hypocotyl epigeous, elongating. Paracotyledon epigeous, petiolate, exstipulate, leaf obovate, $\pm 2-2.8 \times \pm 1.5-1.9$ cm, apex

retuse, base attenuate, margin entire, venation pinnate, surface glabrous. Epicotyl reduced. Eophylls simple, alternate, exstipulate, petiolate, leaf trilobed, $\pm 2.5-4.3 \times 3-4.2$ cm, apex acute, base obtuse/ auriculate, margin lobed, venation palmate, surface glabrous. Third and fourth leaves simple, alternate, exstipulate, petiolate, leaf lobed, base auriculate, apex acute, symmetric, palmate. Next leaves simple, alternate, other characters more or less same as that of first two leaves except measurement

- iii). *Gymnopetalum chinense* (Loureiro) Merrill, Philipp. J. Sci. 15: 256. 1919. (Plate 1).

Vernacular name: Ribbed Orange Gourd

Seedling Morphology: (Up to 9th leaf stage)

Seedling PEF. Roots tap, elongating. Hypocotyl epigeous, elongating. Paracotyledon epigeous, sessile, leaf oblong, $\pm 2.5-2.9 \times 1.2-1.4$ cm, apex retuse, base attenuate, margin entire, venation pattern palmate, surface glabrous. Epicotyl reduced. Eophylls simple, alternate, exstipulate, petiolate, leaf ovate, $\pm 2.8-3.5 \times \pm 3-4$ cm, apex acuminate, base auriculate, margin erose, venation pattern palmate, surface of blade hairy. Third and fourth leaves simple, ovate, rough, exstipulate, petiolate, alternate, Next leaves simple, alternate, petiolate, ovate, other characters more or less same as that of first two leaves except measurements.

- iv). *Mukia maderaspatana* (Linnaeus) M. Roemer, Fam. Nat. Syn. Monogr. 2: 47. 1846. (Plate 1).

Vernacular name: Aganaki, Agumaki, Bilari, Madras pea pumpkin, Rough bryony.

Seedling Morphology: (Up to 6th leaf stage)

Seedling PEF. Roots tap, short elongating. Hypocotyl epigeous reduced. Paracotyledon epigeous, petiolate, leaf elliptic, $\pm 1.2-1.6 \times 0.7-1.2$ cm, apex retuse, base obtuse, margin entire, venation pattern pinnate, glabrous both surfaces. Epicotyl hairy, $\pm 0.3-1.3 \times \pm 0.2-0.4$ cm, Eophylls simple, alternate, exstipulate, petiolate, leaf shape ovate, $\pm 2-3.2 \times \pm 2-3.5$ cm, apex acute, base cordate/truncate, margin sinuate, venation pattern palmate, hairy both surface. Next leaves simple, ovate, alternate, other characters are more or less same as that of first two leaves except measurements.

- v). *Trichosanthes tricuspidata* Loureiro, Fl. Cochinch. 2: 589. 1790. (Plate 1).

Vernacular name: Makal, Kowaluli, Mahakal, Indrayan.

Seedling Morphology: (Up to 6th leaf stage)

Seedling CHR. Roots tap, elongating. Hypocotyl epigeous reduced. Cotyledon cryptocotylar, shape ovate $\pm 1.3-1.6 \times \pm 0.8-1.0$ cm, apices obtuse, base of cotyledon cuneate, margin entire. Epicotyl $\pm 0.4-0.8 \times \pm 0.15-0.2$ cm, glabrous. Eophylls simple, alternate, stipulate, petiolate, leaf shape ovate, $\pm 2.5-6.5 \times \pm 2.3-4.8$ cm, apex acuminate, base auriculate, margin entire/sinuate, venation pattern palmate, hairy both surfaces. Next

Table 2: Some major seedling characteristics of the investigated taxa

Taxa	Seedling type	Stipules (P*)cot	Eoph.	Nature Eoph.	Metaph.	Phyllotaxy Eoph.	Metaph.	Shape (P*)cot	Venation pattern Eoph.	Metaph.	(P*)cot	Eoph.	Metaph.
<i>C.grandis</i>	PEF	Ex	Ex	Sim	Sim	Alt	Alt	Obl	Ov	Ov	Pin	Pal	Pal
<i>D.palmatus</i>	PEF	Ex	Ex	Sim	Sim	Alt	Alt	Obov	Lob	Lob	Pin	Pal	Pal
<i>G.chinense</i>	PEF	Ex	Ex	Sim	Sim	Alt	Alt	Ov	Ov	Ov	Pal	Pal	Pal
<i>M.maderaspatana</i>	PEF	Ex	Ex	Sim	Sim	Alt	Alt	Eli	Ov	Ov	Pin	Pal	Pal
<i>T.tricuspidata</i>	CHR	Ex	St	Sim	Sim	Alt	Alt	Ov	Ov	Ov	Inc	Pal	Pal

List of abbreviations used in table: Alt: Alternate; CHR: Cryptocotylar hypogeal reserve; El: Elliptic; Ex: Exstipulate; Inc: inconspicuous; Lob: Lobed; Obl: Oblong; Obov: Obovate; Ov: Ovate; P*-para; Pal: Palmate; PEF: Phanerocotylar epigeal foliaceous; Ph.: phyll; Pin: Pinnate; Sim: Simple; St: Stipulate.

leaves simple, ovate, alternate, other characters are more or less same as that of first two leaves except measurements.

3.1. Key to the investigated taxa

- 1a. (Para)cotyledons always phanerocotylar.....2
- b. (Para) cotyledons always cryptocotylar2
Trichosanthes tricuspidata
- 2a. Eophylls are ovate.....3
- b. Eophylls are lobed.....*Diplocyclos palmatus*
- 3a. Paracotyledon shape is ovate or elliptic.....4
- b. Paracotyledon shape is oblong.....*Coccinia grandis*
- 4a. Paracotyledon shape is ovate.....*Gymnopetalum chinense*
- b. Paracotyledon shape is elliptic.....*Mukia maderaspatana*

Figure-2: Phenogram based on Seedling morphological data (UPGMA method) to draw affinities between the studied genera in family Cucurbitaceae.

Table-3: Showing soil characteristics where seedling grown.

# Species	pH	EC (ds/m)	Org. C (%)	Phos. kg/ha	Pot. kg/ha
1. <i>C.grandis</i>	5.19-5.3	0.30-0.6	0.52-0.6	4.25-4.8	±349-355
2. <i>D.palmatus</i>	±4.79	±0.16	±0.74	±1.70-	±331-
	-4.9	-0.19	-0.78	1.80	340
3. <i>G.chinense</i>	±4.85	±0.13	±0.54	±1.69	±328
	-4.90	-0.16	-0.56	1.76	340
4. <i>M.maderaspata</i>	±4.96-4.6	±0.07-0.09	±1.99-2.5	±104-140	±521-552
5. <i>T.tricuspidata</i>	±4.56-4.7	±0.43-0.5	±1.19-1.2	±19.9-22	±539-545

pH: <6.5 Acidic soil. >8.5 alkaline soil

Electrical conductivity(ds/m):<1.0 Normal. >1.0 Saline

Organic carbon (%):0.5 Low. 0.5-0.75 Medium. >0.75 High

Available P(kg/ha):<10 Low. 10-25 Medium. >25 High

Available K(kg/ha):<120 Low. 120-280 Medium. >280 High

Discussion:

We investigate five species of a family Cucurbitaceae based on seedling morphological characteristics. These seedling characters can serve as a marker characters for the

Plate-1: (1) *C.grandis* (A) Paracotyledon (B) Mature seedling (C-D) Sketches of seedling (2). *D.palmatus* (A) Paracotyledon (B) Mature seedling (C-D) Sketches of seedling (3) *G.chinense* (A) Paracotyledon (B) Mature seedling (C-D) Sketches of seedling (4) *M.maderaspatana* (A) Paracotyledon (B) Mature seedling (C-D) Sketches of seedling (5) *T.tricuspidata* (A) Cotyledon (B) Mature seedling (C-D) Sketches of seedling

identification of taxa. Out of five *Trichosanthes tricuspidata* showed Cryptocotylar Hypogeal Reserve (CHR) type of germination and rest 4 taxa showed Phanerocotylar Epigeal Foliaceous (PEF) type of germination. The PEF type of germination is advantageous over other types of germination as they generally grow faster than when exposed to increased light (Pooma & Bongers, 1988). Multivariant phenetic analyses have been used in classifying many plants and interpreting the results of taxonomic studies. Seedling features are often distinctive at the species level and correlate well with the data from other sources regarding sectional and subsectional placement of species with the genus (Cane, 1983). Seedling characters; like germination pattern, nature of root, hypocotyl, shape, size, color, apex, base, margin of paracotyledons and eophylls is considered in this investigation. The root of *Diplocyclos palmatus* is reduced, and rest species have elongating roots. Hypocotyl of

Trichosanthes tricuspidata is reduced while rest taxa have elongating hypocotyl. *Coccinia grandis* and *Gymnopetalum chinense* have oblong paracotyledon, *Diplocyclos palmatus* have obovate paracotyledon, *Mukia maderaspatana* has elliptic paracotyledon and *Trichosanthes tricuspidata* ovate paracotyledon. Eophylls of all taxa has alternate phyllotaxy. Based on these seedling characters artificial key has been constructed for the easier identification of plants in their juvenile stage. A phenogram has been constructed using UPGMA method to draw a correlation between the taxa. Phenogram shows two clusters 1A and 1B. Cluster 1A contains *Coccinia grandis*. Cluster 1B contains two sub-cluster. Subcluster 2A contains *Diplocyclos palmatus* which may be because it has reduced hypocotyl. Cluster 2B contains two clusters 3A and 3B. Subcluster 3A contains *Gymnopetalum chinense* and *Mukia maderaspatana*. Subcluster 3B contains *Trichosanthes tricuspidata* which may be because of cryptocotylar seedling. All species have been growing in acidic soil. All taxa have been growing in low soil salinity. Soil organic carbon of *Mukia maderaspatana* and *Trichosanthes tricuspidata* has been found to be growing in high soil organic carbon. An increase in SOC can increase soil aggregate stability, water retention capacity, water infiltration, plant available water, soil microbial and macro fauna biomass and activity, and cation exchange. *Mukia maderaspatana* has the highest available phosphorus and rest investigated taxa has the lowest available phosphorus. The deficiency of P in soil severely affects the plant metabolism and in turn their yields. All investigated taxa have highest available potassium. Potassium is extremely important in many ways to the productivity of the plant. It improves nitrogen use efficiency.

Conclusively, the seedling character of Cucurbitaceae plant is related to each other and the artificial key is extremely useful for early identification and restoration of plants. Seedling morphological studies of medicinal plants help to restore the wild medicinal plants in the early stage of their life cycle. These types of study are extremely useful for safeguarding biodiversity and to draw the evolutionary relationship among medicinal properties of plants.

Acknowledgements:

Author is grateful to Tripura University for RET fellowship.

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