

COTYLEDONARY LEAF ARCHITECTURE IN RELATION TO TAXONOMY : APIACEAE

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A detailed investigation has been made for the first time on major as well as minor venation patterns, areoles, veinlets and vein terminations in the cotyledonary leaves of eleven genera of Apiaceae with a view to examine their utility in the systematics of this taxon. The cotyledonary leaf architecture is exclusively acrodromous excepting *Hydrocotyle rotundifolia* where it is pinnate brochidodromous. The cotyledonary leaf architectural features of Apiaceae are of taxonomic significance and helpful for distinguishing the taxa studied from one another. Accordingly a key has been presented for this purpose.

Keywords: Apiaceae, cotyledon leaf, architecture, taxonomy.

A perusal of the literature on foliar architectural studies clearly demonstrates that very little is known so far about the cotyledonary leaf architecture in comparison to that of adult leaves (Shenoy and Inamdar 1985). Sampath Kumar (1982) highlighted the usefulness of the cotyledonary leaf architecture in taxonomy.

One of the earlier works in this line appears to be that of Urban (1873) who distinguished *Trigonella* and *Melilotus* from *Medicago* based on characters of cotyledons. Some of the works of cotyledonary architecture include those of *Argyreia nervosa* (Govil 1972), Trifolieae (Gupta 1978), Convolvulaceae (Sampath Kumar 1982, Shenoy and Inamdar 1985), *Crotalaria* (Shailja and Trivedi 1984), Caesalpinioideae (Smith and Scott 1985), *Eucalyptus* (Carr *et al.*1986), Mimosoideae (Smith and Scott 1995) and Acacieae (Scott and Smith 1998, Dengler *et al.* 2001, Roth-Nebelsic *et al.* 2001, Runions *et al.* 2005).

Survey of the literature reveals that so far there is no work on the cotylerdonary leaves of the Apiaceae excepting for a cursory report on the gross morphology (Cerceau- Larrival 1962).

A detailed investigation has been made presently on major as well as minor venation patterns, areoles, veinlets and vein terminations in the cotyledonary leaves of eleven genera of Apiaceae with a view to examine the cotyledonary architectural data in its systematics.

MATERIALS AND METHODS

Details of the observations of the taxa studied with reference to the cotyledonary leaf architectural characters have been presented in Table 1. The cotyledons were collected from the seedlings grown in pots and fixed in Carnoy's fixative consisting of alcohol and glacial acetic acid (3:1) (Johansen 1940). After one week, the fixative was replaced by 70% alcohol for preservation of the material. Slides have been prepared after washing the leaves with water and kept in 5% KOH solution at 25° C for 4-6 hours (Thakur 1988). Then the material was again washed with water and gently cleared off the mesophyll, and then stained with safranine and mounted in glycerine.

The terminology used in the description of venation pattern, in general, is after Hickey (1973, 1979) and Ellis et al. (2009). However, for vein terminations, the terminology is after Tucker (1964). For the purpose of determining the status of a lateral vein as primary or secondary, the method followed is after Smith and Scott (1985) in view of its adaptability to the architectural features of Apiaceae.

RESULTS AND DISCUSSION Gross morphological features

The cotyledonary leaves studied are simple, symmetrical with an entire margin. However, the shape of cotyledonary leaf is variable i.e. ovate in *Apium*, *Centella* and *Hydrocotyle* (Plate1E, 1H, 2A) but variously oblong to elliptical in rest of the taxa. The apex varies from acute or obtuse but slightly retuse in *Centella* and *Hydrocotyle* (Plate 1H, 2A). The base is also acute to obtuse. However, the texture of the cotyledonary leaves in all taxa studied is chartaceous.

Type of venation

The venation is generally differentiated into a number of size classes. Primary (1°) , secondary (2°) and tertiary (3°) veins are considered as the major veins while the subsequent categories i.e. quaternary (4°) onwards constitute the minor veins (Pray, 1955a, 1955b).

The cotyledonary leaf architecture is mainly acrodromous (two or more primary or strongly developed secondary veins running in convergent arches towards the leaf apex) and basal with three primary veins (1[°]) arising from the petiole/base excepting in *Hydrocotyle rotundifolia* wherein pinnate brochidodromous condition (secondaries joined together in a series of prominent arches) (Plate 2A) is present and it is diagnostic in distinguishing this taxon from the rest of the Apiaceae studied.

The study also shows that the acrodromous condition is of perfect nature (= acrodromous veins running at least two thirds of the distance to the leaf apex) in all the taxa except in Centella asiatica. In this taxon, the lateral acrodromous veins abruptly merge with the secondary veins or its branches or with mid primary vein in the distal lamina instead of forming the convergent arches at the leaf apex. When the whole cotyledonary leaf is viewed in totality, the proximal part is clearly acrodromous but it cannot be said with conviction in the distal part. Similar mixed situations, though not of this combination have also been encountered in the mature foliar architectural patterns (involving other combinations) in Amaranthaceae (Shanmukha Rao and Narmada 1994).

The highest vein order in the cotyledons studied is either 3° or 4° (Table 1) except in *Cuminum cyminum* which is with 2° as the

highest vein order (Plate 2I) and taxonomically significant to delimit the taxon from rest of the species studied. Observations made earlier in *Crotalaria* (Shailja and Trivedi 1984) and Convolvulaceae (Shenoy and Inamdar 1985) revealed that 4[°] as the highest vein order in general, in the cotyledonary leaves.

Major venation

Primary veins (1°) : The primaries are usually three (Plate 1A-H) but Hydrocotyle rotundifolia shows a single primary vein (Plate 2A). The mid primary vein is with variable thickness i.e. moderate (Centella and Apium), thick (Daucus and Pimpinella), massive (Coriandrum and Cuminum) (Plate 2I) and stout in rest of the taxa investigated. The lateral primaries are relatively thinner than the mid primary vein in all the taxa investigated except in *Centella asiatica* where all the three primaries show the same thickness (Plate 1H). In Apiaceae, the lateral primary veins always emerge from the petiole/base of the leaf independently on either side of the mid vein. Another interesting aspect of the lateral primaries is that, most of the time they converge distantly with the mid vein without producing any secondaries at all. This is a strong indication for recognizing these laterals as primary veins in spite of their being less thick than the mid vein. This phenomenon appears to be very rare and peculiar in comparison to other dicotyledonous taxa studied. It would be interesting to survey further taxa from this angle. Presently, the lateral primaries are always basal (= veins originating at the base of the leaf / petiole) excepting for the exclusive presence of suprabasal lateral primaries (= veins originating some distance above the leaf base) in Coriandrum sativum. This character renders this taxon highly diagnostic among the Apiaceae studied.

Secondary veins (2°) : The number of the secondary veins among the different taxa of the Apiaceae is variable and diagnostic. They range from 2-9 (Table 1). Normally the

SI. No.	Name of the taxon	No.Of	No. of 2° Veins on	Range of angle	Predominent	Highest vein	No.of areoles	No.of Vein-	No .of Veinlets	Marginal Ultimate	Venation Pattern
		J	either side of mid primary	between 1° & 2°	vein origin	order	per Sq. mm.	lets per Sq. mm.	entering the areoles	venation	
Sub-fa	Sub-family : Hydrocotyloideae										
	Tribe : Hydrecetyleae										
1	Centella asiatica(L.) Urben (=Hydrocotyle astaticaL.)	3	3-4	Wide acute	0	3°	1.6	1	0.4	Looped	Acrodromous
2	Hydrocotyle rotundifoliaRoxb.	1	2-3	Acute- right angle	0	3°	44	0.1	*	Looped	Brochłdodromous
Sub-fa	Sub-family : Apioideae										
	Tribe : Danceae										
3	Daucus carotaL.	3	6-7	Acute	AO,OR	3°	0.8	1.4	*	Fimbriate	Acrodromous
	Tribe : coriandreae										
4	Corlandrum sativumL.	3	3-4	Acute- obtuse	OA,RA,RO	40	1.4	2.8	1.2	Fimbriate	Acrodromous
	Tribe : Apicae										
5	Annel majusL.	3	6-8	Acute- obtuse	OA,AO	40	6	4	1.2	Fimbriate	Acrodromous
9	Anethum graveolens [Peucedanum graveolens(L.) Hicm]	6	7=8	Acute Barrow- Wide acute	OA,AO	40	2	1	0.4	Finderiate	Acrodromous
7	Apium graveolensL.	9	4-5	Acute-wide acute	A,0	3°	1	0.4	0.2	Fimbriate	Acrodromous
80	Cuntinum cyntaund.	9	6-7	Acute		20	1.6	0.1	*	Fimbriate	Acrodromous
9	Foeniculum vulgaveMiller	9	4-5	Acute	OA, A	30	:	0.6	*	Fimbriate	Acrodromous
10	Pimpinella monoicaDalz.	ю	8-9	Acute-wide acute	OR,00,0A	3°	1.2	5.4	2	Fimbriate	Acrodromous
11	Trachyspernum annu(L.) Sprague [=Carum copticum(L.) Hierm]	e	6-8	Acute-wide acute	OA,RA,OR	6	1.8	3.8	1	Finderkate	Acrodromous

Table 1 : Cotyledonary Leaf Characteristics in the Apiaceae

Note: * The veinlet count is low and often missing in the microscopic field of 1 sq.mm. **Though the arcoles are present they are large and could not be viewed in 1 sq.mm.

secondary veins are produced on all the primaries. However, in Apiaceae this phenomenon is interesting and taxonomically important. Though, several of them show secondaries both on the mid and lateral primaries (Plate 1H) several others viz. Daucus carota, Anethum graveolens, Cuminum cyminum, Foeniculum vulgare show secondaries only on the mid primary vein, but not on the two lateral primary veins (Plate 1F, 1G, 2A). In general, secondary veins are sparse and produced distantly from one another in Apiaceae. This character is much pronounced in Foeniculum vulgare (Plate 1A, Table1). The angle of divergence of secondary veins ranges from narrow acute, wide acute to obtuse (Table 1). The relative thickness of secondary veins is thick, moderate or thin. Their course is straight or curved.

Intersecondary veins : Intersecondary veins are very rare and found only in *Anethum graveolens* (simple with a single vein segment) as well as in *Ammi majus* and *Coriandrum sativum* (simple or composite).

Tertiary veins (3°) : They are present in all the taxa studied excepting in Cuminum cyminum. The predominant angle of origin from the secondary vein is mostly OA, AO, OR and rarely AR or RA. Interestingly, tertiaries are very meagre in some taxa and arise only from the admedial (upper) side of the secondary veins (Table 1, Plate 1 A, 1 E). Hickey (1979) recognized three patterns of tertiary veins viz., ramified, reticulate and percurrent. In the present work ramified (tertiary veins branching into higher orders without rejoining the secondary veins), random reticulate (Tertiary veins anastomosing with other tertiary veins or with the secondary veins) and percurrent (tertiaries from the opposite secondaries joining) patterns are witnessed besides the formation of loops and areoles. However, the number of tertiary veins is extremely meagre in Foeniculum vulgare, Apium graveoleus and Hvdrocotyle rotundifolia in comparison to other taxa.

Minor Venation

Quaternary Veins (4°) : The quarternary veins are always thin and randomly oriented. They form the highest vein order in 4 of the taxa studied in the Apiaceae (Table 1).

Marginal ultimate venation : Hickey (1979) recognized three types of marginal ultimate venation viz., looped (major portion of the marginal ultimate venation recurved to form loops), fimbriate (higher vein orders fused into a vein running just inside of the margin and incomplete (freely ending veinlets directly adjacent to the margin). Of these, the first two types are presently observed, the incomplete being totally absent. They are mostly fimbriate but, looped only in Centella asiatica and Hydrocotyle rotundifolia (Table 1, Plate 2A). The loops are formed only by two secondary arches in Hydrocotyle rotundifolia whereas in Centella asiatica, number of loops are many and prominent. **Areoles :** Areoles are formed by $1^{\circ} - 4^{\circ}$ veins. They are present in all the taxa investigated but their number is meagre. Further, the size of the areole is in general large (Table 1). The development of areoles is always imperfect (= meshes of irregular shape, more or less variable in size - Hickey 1979) with polygonal, pentagonal, rectangular (Plate 2 D, 2 F) to triangular in shape or incompletely closed. It is further observed that the size of the areoles is not constant even within the same species. Earlier, the same situation was observed in the cotyledons of the Convolvulaceae (Shenoy and Inamdar 1985). The areoles are with or without veinlets (Plate 2 D, 2 F) and the number of veinlets entering the areoles is variable in the Apiaceae studied (Table 1).

Veinlets : These are freely ending ultimate veins of the leaf and veins of same order which occasionally cross areoles (Hickey 1979). In the present study, they are either simple [= without branches] or branched once or twice (Plate 2E). They are uniseriate or biseriate in the present study. Further, loop formation by vein endings (Plate 2H) is also observed in some Apiaceae as was observed



Plate 1 (A- H): Cotyledonary leaf venation patterns in the Apiaceae

A. Foeniculum vulgare - Acrodromous venation **B.** Anethum graveolens - Acrodromous venation. **C.** Coriandrum sativum - Acrodromous venation; mid primary vein branched suprabasally. **D.** Pimpinella monoica - Acrodromous venation. **E.** Apium graveolens - Acrodromous venation. **F.** Cuminum cyminum - Acrodromous venation; marginal ultimate venation fimbriate. **G.** Daucus carota - Acrodromous venation. **H.** Centella asiatica - Acrodromous venation; marginal ultimate venation looped.



Plate 2 (A- I) : Cotyledonary leaf venation in the Apiaceae

A. *Hydrocotyle rotundifolia* - Brochidodromous venation X25. **B.** *Coriandrum sativum* - Convergence of mid and lateral primary Veins at the distal end of cotyledon X109. **C.** *Trachyspermum ammi* - Arrows showing vein terminations X94. **D.** *Anethum graveolens* - Large areole showing a simple veinlet X94. **E.** *Ammi majus* - Arrows showing branched veinlets X20. **F.** *Pimpinella monoica* - Areoles without veinlets X77. **G.** *Ammi majus* - Thick mid primary vein and thin lateral primary veins X83. **H.** *Ammi majus* - Loop formation by 3° veins X104. **I.** *Cuminum cyminum* – Massive mid primary vein; fimbriate marginal ultimate venation X88. (M = Mid primary vein; LP = Lateral primary vein; A = Areole; L = Loop).

earlier in cotyledons of Convolvulaceae (Shenoy and Inamdar 1985).

Vein terminations : Cells terminating the veins show considerable variations. According to Tucker (1964), veinlet terminations are of six types, viz. tracheoidal elements, conventional tracheids, dilated tracheids, reticulate walled tracheids, terminal sclereids and secretory cells. Of these, only conventional tracheids are found in the present study. Further, the tracheids are uniseriate (Plate 2D) or biseriate but multiseriate vein terminations observed in *Trachyspermum ammi* (Plate 2C). They are linear, elongated or juxtaposed (Plate 2C) with variable shapes and sizes.

Isolated vein endings and Isolated tracheids : Kasapligil (1951) for the first time reported the occurrence of the isolated veins in dicotyledonous mature leaves. They were also observed in the cotyledonary leaves of some dicotyledons (Shailja and Trivedi 1984; Shenoy and Inamdar 1985). Presently, they are meagre in the cotyledons of Apiaceae. Simple isolated vein endings are observed in *Coriandrum sativum* while branched variety in *Trachyspermum ammi*. Isolated tracheids are completely absent in the present study.

Taxonomic importance :

Analysis of the cotyledonary venation in Apiaceae shows that the following characters are helpful in the identification of the taxa. They are: Major venation pattern, marginal ultimate venation, number of 2[°] veins, highest vein order, shape of the cotyledon and vein endings. Therefore, based on the cotyledonary architecture a key is presented in the following for the identification of the Apiaceae investigated.

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Key for the identification of taxa based on cotyledonary architecture :

1. Venation pattern brochidodromous
1. Venation pattern acrodromous.
2. Suprabasal branching of mid primary vein present
2. Suprabasal branching of mid primary vein absent.
3. Highest vein order 2 [°]
3. Highest vein order 3° or 4° .
4. Highest vein order 3° .
5. Cotyledonary leaves ovate.
6. Marginal ultimate venation looped Centella asiatica
6. Marginal ultimate venation fimbriate Apium graveolens
5. Cotyledonary leaves other than ovate.
7. Number of secondary veins 4 or 5 only Foeniculum vulgare
7. Number of secondary veins 6 or above.
8. Loop formation present. Biseriate vein endings present
8. Loop formation absent. Uniseriate vein ending present Pimpinella monoica
4. Highest vein order 4 [°] .
9. Number of veinlets per sq. mm. less than two Anethum graveolens
9. Number of veinlets per sq. mm. more than three
10. Isolated free vein ending present; multiseriate vein endings present
Trachyspermum ammi
10. Isolated free vein ending absent; uni or biseriate vein endings present Ammi majus

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^{*} original not seen.