ANNALS OF PLANT SCIENCES
ISSN: 2287-688X
OPEN ACCESS
www.annalsofplantsciences.com

Research Article

Leaf and seedling morphology: the two prospective parameters to trace phylogeny in Apiaceae subfamily Apioideae

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Received: 9/7/2017; **Accepted:** 9/28/2017

Abstract: Determination of phylogenetic affinity among the members of Apiaceae especially the subfamily Apioideae is much debatable. The morphological evidences on inflorescence, fruit and seed characters showed little concomitance with those derived from molecular parameters as far as groupings in clades are concerned. In the present study involving few selected Apiaceae members, a Dendrogram was computed and a putative evolutionary trend was outlined based on leaf margin insertion pattern and cotyledonary leaf features, general plant morphology as well as leaf surface micromorphology by SEM. The members of Apiaceae with broad-ovate pinnatifid to pinnatipartite leaves and ovate –lanceolate cotyledonary leaves were included in a distinct cluster but the members with pinnatisect – decompounds leaves and linear cotyledonary leaves were included in a separate cluster. Long Coriander (*Eryngium foetidum*) included in the subfamily Saniculoideae may represent the most primitive condition having simple entire leaf with spiny margin. The member like Long coriander might have served as progenitor stock from which Coriander, Celery, Parsley (all with ovate pinnatifid to pinnatipartite leaves) and Cumin, Fennel, Dill (all having pinnatisect to decompounds leaves with linear segments) might have originated as evidenced by gradual increase in marginal insertion. Similarly, gradual narrowing of ovate-lanceolate cotyledonary leaves might have given rise to narrow linear cotyledonary leaves. Leaf-margin insertion pattern and cotyledonary leaf features appeared to be promising in tracing phylogeny in Apiaceae especially in Apioideae. **S**imple entire leaf represents the primitive condition and evolution in Apiaceae supposed to have progressed towards gradual increase in marginal insertion leading to pinnately dissected, pinnatipartite and ultimately to decompound leaves with narrow linear segments. Dendrogram computed from morphological features showed concomitance with that phylogenetic trend as far as grouping and interrelationships of members are concerned. In the present study leaf margin insertion pattern and seedling morphology appeared to be instrumental in tracing the phylogeny in Apiaceae specially subfamily Apioideae while molecular systematics are not conclusive.

Keywords: Apiaceae; subfamily Apioideae; leaf-margin insertion pattern; seedling morphology; phylogenetic affinity

Introduction

The family Apiaceae ranks amongst the economically significant families. The family is best known as the source of important culinary herbs and spices. The family contains approximately 3730 species distributed in 434 genera usually comprising aromatic herbs and shrubs with fistular stem (Constance, 1971; Pimenov and Leonoy, 1993). The aromatic nature of these plants has led to their common use as fruits or spices.

The division of Apiaceae into three subfamilies and 12 tribes (Drude, 1897-1898) is considered as the predominant system of classification. Apioideae, the largest and economically most significant sub-family of Apiaceae is distinguishable from the other two subfamilies Hydrocotyloideae and Saniculoideae by the possession of compound umbel inflorescence, well developed vittae in seeds and free carpophores. Cladistic analysis of nuclear ribosomal DNA, Internal transcribed spacer (ITS) (Downie and Katz-Downie, 1996; Downie *et al.*, 1998) plastid *rpoC1* intron (Downie *et al.*, 1996), *rbcL* (Kondo *et al.*, 1996; Plunkett *et al.*, 1996a), *matK* (Plunkett *et al.*, 1996b) sequence provide little support for Drude's proposal (Drude, 1897-1898) on classification of Apiaceae that is based largely on morphological and anatomical characters. Phylogenetic estimations have revealed that the sub-family Apioideae is monophyletic and is a sister group to the sub-family Saniculoideae. Sub-family Apioideae comprises several well-defined sub-clades (Downie *et al.*, 1998). Morphological study has supplemented molecular and biochemical study in deriving phylogenetic linkages in very few cases (Calvino *et al.*, 2008; Jimenez-Mejias and Vargas, 2015). Seedling morphology can be very instrumental in tracing phylogeny in Apioideae. In the present investigation morphology, specially the seedling morphology, micromorphology of leaf surface, leaf margin insertion patterns were taken into consideration in tracing phylogenetic affinity among the few selected aromatic members of Apiaceae.

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Materials and Methods

Materials

The materials considered in the present investigation were – long Coriander or Culantro (*Eryngium foetidum* L.) belonging to Apiaceae subfamily Saniculoideae; Coriander or Cilantro (*Coriandrum sativum* L.), Parsley (*Petroselinum crispum* [Mill.] Nyman ex A.W. Hill), Fennel (*Foeniculum vulgare* Mill.), Ajwain (*Trachyspermum ammi* Sprague), Radhuni (*Trachyspermum roxburghianum* (DC) Craib), Cumin (*Cuminum cyminum* L.), Carrot (*Daucus carota* L.). Celery (*Apium graveolens* var. *dulce* (Mill.) pers) and Dill (*Anethum graveolens* L.) all belonging to Apiaceae subfamily Apioideae. The plants were grown in experimental station from the seeds collected from Sutton & Sons India, Pvt. Ltd in the month of December.

Methods

Leaf surface scanning: A small segment of fresh leaf of each sample was mounted on aluminum stab and without any critical point drying and gold coating, scanned under SEI 200 Quanta Scanning Electron Microscope directly.

Seedling morphology: Two weeks old seedling of each species was studied and data were taken in terms of cotyledon shape, apex, length ration of lamina and petiole etc.

General plant morphology: Thirty-four prominent external morphological characters; including the cotyledonary features were taken into consideration to differentiate different members.

Statistical analysis: On the basis of presence or absence of a particular character state in all the samples a similarity matrix was prepared giving each character state a definite number. Percentage based pairing affinity (PA) values between different combinations of sample pairs were calculated by the following formula (Das, 2012).

$$PA = \frac{\text{Character states common to sample A and B}}{\text{Total number of character states in sample A and B}} \times 100$$

From the pairing affinity values, a Dendrogram was computed using Statistica 13.2 cluster analysis software from Dell applying nearest neighbor or single linkage method.

Results

Morphological observations

Leaf surface micromorphology by SEM: The leaf surface scanning showed little variability among the genera studied. Most of the members showed epidermal cells with sinuous wall except Fennel having epidermal cells with straight walls. Radhuni and Ajwain belonging to the same genus (*Trachyspermum*) are characterized with conical warty epidermal trichomes. Dill is characterized with densely packed small leaf-like epidermal hairs and long coriander is characterized with interconnecting transverse ridges between adjacent epidermal cells (Figure 1A-D).

Seedling morphology: The seedling morphological study revealed two groups among the members studied. One group showed ovate-lanceolate to lanceolate cotyledon with distinguishable petiole, acute or rounded tips and lamina/petiole length ratio above 3.5 (Coriander, long Coriander, Celery, Parsley, Ajwain and Radhuni) while other group showed linear-lanceolate to linear cotyledon with indistinguishable petiole and lamina/petiole length ratio less than 3.5 (Carrot, Fennel, Cumin and Dill).

Study on general plant morphology: Thirty-four (34) salient morphological characters (both qualitative and quantitative) with character states were taken into consideration (Table 1). The members of Apiaceae are characterized with the features like Annual or perennial habit, Caulescent or acaulescent nature with hollow or solid stem. Leaves are exstipulate generally compound rarely simple with sheathing petiole, entire, lamina usually pinnatifid to pinnatipartite to pinnatisect-decompound. Flowers are epigynous arranged in simple or compound Umbels, Umbellules few to many flowered, rays often subtended by bract forming involucres; Pedicels long, short or obscure; Calyx 5, small to obsolete; Petals 5, monomorphic or dimorphic with inflexed lip; Ovary inferior, style with stylopodium; Fruit cremocarp with two mericarp united attached to a central axis or Carpophore.

Leaf margin insertion is of special significance in Apiaceae. Different degrees of leaf margin insertion have created 3 types of leaf lamina - pinnatifid (Celery, Coriander, Parsley, Ajwain and Radhuni), pinnatipartite (Carrot) or decompound (Fennel, Cumin and Dill). Rarely leaf may be simple with spiny margin (*Eryngium*). Pairing affinity values for different combinations of specimen pairs (Table 2) calculated on morphological character states showed highest value for pair of Ajwain and Radhuni (93.33%) and least value for the pair of Coriander and long Coriander (31.81%).

The Dendrogram (Figure 2) computed from pairing affinity values showed clear segregation of members containing pinnatifid to pinnatipartite leaves and pinnatisect to decompound leaves in two distinct separate clusters. Long Coriander (*Eryngium foetidum*) having simple leaves was totally isolated from other members. Coriander, Celery, Parsley, Ajwain and Radhuni having pinnatifid to pinnatipartite leaves showed close alliance with each other while Cumin, Dill and Fennel having pinnatisect to decompounds leaves with narrow linear segments also showed closer affinity. Carrot represented an intermediate stage. On the basis of leaf margin insertion pattern and cotyledonary leaf features a putative evolutionary trend was outlined (Figure 3) that showed emergence of members having pinnatisect to decompound leaves from the members having simple entire or pinnatifid or pinnatipartite leaves. Cotyledonary leaves also showed a probable evolutionary trend from ovate lanceolate–lanceolate form to linear-lanceolate to linear form.

Table 1: Morphological characters with character states applied

S. No.	Characters	Character states
1.	Cotyledon Shape	1. Ovate lanceolate to spathulate ovate 2. Linear lanceolate to linear
2.	Cotyledon tip	1. Acute 2. Rounded
3.	Length ration of lamina and Petiole	1. Above 3.5 2. Below 3.5
4.	Petiole of cotyledonary leaf	1. Distinguishable 2. Not distinguishable
5.	Habit	1. Annual, 2. Biennial 3. Perennial
6.	Stem	1. Creeping to erect 2. Erect
7.	Leaf lamina	1. Simple 2. Pinnatifid 3. Pinnatipartite 4. Decompound
8.	Leaf shape	1. Ovate 2. Lanceolate 3. Oblanceolate to spathulate ovate
9.	Shape of ultimate leaf segment	1. Oblong to lanceolate 2. Linear lanceolate to linear 3. Deltoid rhomboid
10.	Leaf nature	1. Cauline throughout 2. Cauline at base radical in the upper region
11.	Leaf or leaf-let tip	1. Acute to acuminate 2. Rounded
12.	Leaf margin	1. Entire 2. Serrate-spinulose 3. Glabrous to hispid 4. Serrate undulate
13.	Leaf texture	1. Herbaceous 2. Leathery coriaceous
14.	Inflorescence	1. Terminal and lateral 2. Terminal
15.	Inflorescence type	1. Compound Umbel of Umbellules 2. Compound Umbel of head
16.	Peduncle length	1. Long 2. Very short
17.	Rays	1. Equal 2. Unequal
18.	No. of rays	1. Many 2. 3-7(10)
19.	Bract and bracteoles	1. Both present 2. Bract absent bracteole present 3. Both absent
20.	Bract	1. Sublinear to lanceolate, mucronate 2. Leafy ovate-lanceolate, spinulose, serrate 3. Linear-lanceolate slightly coriaceous 4. Foliaceous pinnate with scarious margin
21.	Bracteole	1. Linear-lanceolate acute to mucronate 2. Ovate-lanceolate acuminate to spiniform 3. Linear toothed scarious
22.	Pedicels	1. Equal 2. Unequal
23.	Sepal lobe	1. Very small like ennations or absent 2. Prominent but much smaller than petal lobes 3. As long as petals
24.	Petal lobe	1. Small and equal 2. Unequal 3 longer 2 smaller
25.	Petal surface	1. Hairy abaxially 2. Not hairy
26.	Outer petals of outer flower	1. Radiant 2. Not radiant
27.	Petals with reflexed or incurved tip	
28.	Petal Colour	1. White or pinkish 2. Yellow 3. Greenish white 4. Creamy white
29.	Stamen length	1. Five equal to smaller than petals 2. Five longer than petals
30.	Style	1. Short spreading 2. Short, erect to reflexed 3. Long exceeding calyx teeth 4. Short divaricated
31.	Stylopodium	1. Conical not massive 2. Massive conical covering the apex of ovary 3. Stylopodium obscure
32.	Ovary	1. Glandular 2. Bristly hairy strigose papilose 3. Not hairy or glandular
33.	Carpophore	1. Present 2. Absent
34.	Carpophore	1. 2-Cleft to middle or base 2. 2-Cleft at the base

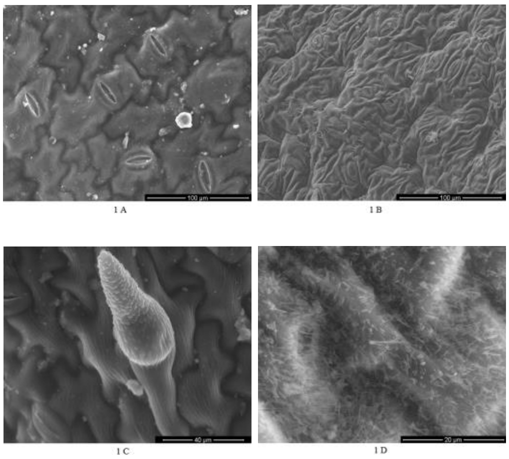
Table 2: Pairing affinity values between different combination of Apiaceae members on morphological character states

	A	B	C	D	E	F	G	H	I	J
A	100									
B	31.81	100								
C	37.20	65.90	100							
D	43.18	64.44	63.63	100						
E	29.54	62.22	54.54	46.66	100					
F	40.90	66.66	70.45	57.77	51.11	100				
G	36.36	66.66	68.18	57.77	55.55	93.33	100			
H	45.45	71.11	56.81	57.77	55.55	75.55	71.11	100		
I	39.08	58.42	63.63	55.55	55.55	62.22	57.77	68.88	100	
J	47.72	62.22	61.36	66.66	55.55	66.66	66.66	82.22	77.77	100

Sample: **A**. Long Coriander **B**. Coriander **C**. Celery **D**. Parsley **E**. Carrot **F**. Radhuni **G**. Ajowain **H**. Cumin **I**. Fennel **J**. Dill

Explanation to figures

Figure 1. A-D Leaf surface micromorphology by SEM.



A.Coriander B. Long Coriander C. Ajwain D. Dill

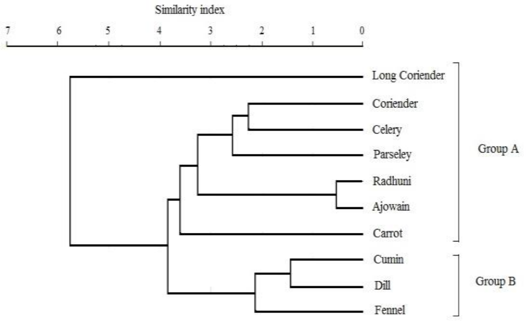


Figure 2. Dendrogram computed from pairing affinity values on morphological features.

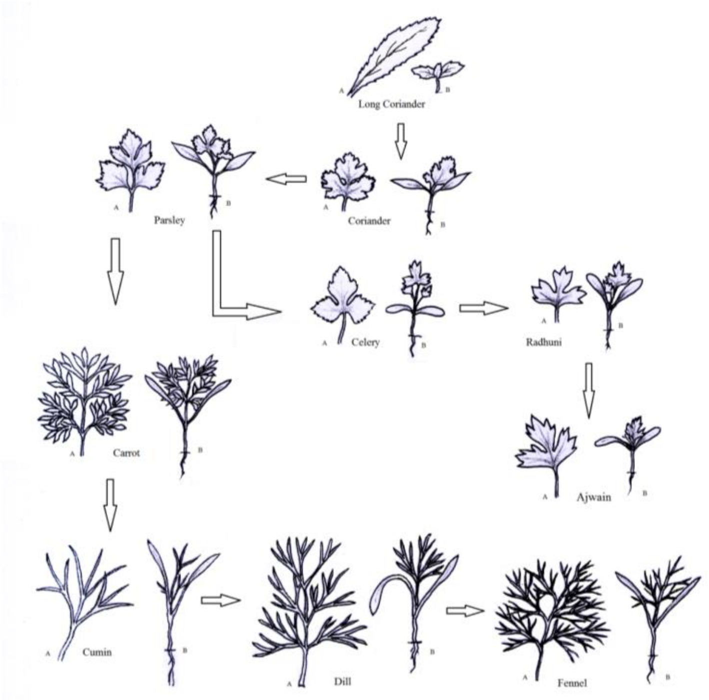


Figure 3. Putative evolutionary trend based on leaf margin insertion and cotyledonary leaf features.

Discussion

Taxonomic delimitation in the family Apiaceae has been addressed by many authors applying conventional parameters like morphology especially inflorescence pattern, seed and fruit characters, along with molecular and biochemical parameters. Subdivision of the family into three subfamilies (viz., Hydrocotyloideae, Saniculoideae and Apioideae) and 12 tribes was proposed long before (Drude, 1897-1898). Among the three subfamilies Apioideae is the largest and economically most important. The family Apiaceae has been subjected to major systematic reshuffling at various taxonomic levels. Studies on the subfamiliy apioideae especially tribe Apieae generated heterogenous assemblage of morphologically diverse data (Jimenez-Mejias and Vargas, 2015). Interrelationship between subfamilies reflected by molecular data was supported by few morphological character, but none of these morphological characters supported the monophyly of subfamily Saniculoideae or Apioideae (Calvino *et al*., 2008).

Phylogeny of Apiaceae subfamily Apioideae has been studied by many workers (Downey *et al*., 2010; Downie *et al*., 2009; Zhou *et al*., 2008; Tilney *et al*., 2009; Winter *et al*., 2008; Magee *et al*., 2008; Valiejo-Roman *et al*., 2008; Spalik *et al*., 2009). Composition of major clades based on phylogenetic analysis involving molecular data showed a great inconsistency. Taxonomic delimitation based on morphological features ~~is~~ was not also conclusive. Downie *et al.*, (2001) summarized the previous interpretations on molecular systematics of Apioideae and presented a revised classification that reflected phylogeny. He identified the congruence between different phylogenetic analyses based on Chloroplast DNA (*cp*DNA) gene, *matK* gene, intron (rpl 16, rps 16, rpo C1) sequences, *cp*DNA restriction sites, nuclear ribosomal DNA (nrDNA) and Internal Transcribed Spacer sequences. Subsequent investigation by Downie *et al.*, (2010) identified several additional clades and tribes some of these new groups have now received support from both ITS and cpDNA studies**.** The study has identified 41 major clades within Apioideae, of which 21 have already been recognized at the tribal or subtribal rank and has also helped to elucidate the natural subdivisions within the entire subfamily. Morphological and combined (morphological and molecular) data sets from 123 endemic taxa of North-American Apioideae were analysed (Sun and Downie, (2010) using maximum parsimony and Bayesian approach. Phylogenetic trees derived from morphological character showed congruence with those derived from molecular data after abolishing weak branches. Three major clades and several well-defined subclades were identified. Phylogenetic relationship between 100 species of Apiaceae subfamily Apioideae distributed mainly in China was investigated (Zhou *et al.*, 2008). Several genera were confirmed as monophyletic, though a large number of them were not monophyletic, characterized with diffuse generic boundaries and heterogeneous variation.

The most used molecular marker in Apioideae and many other angiosperms has been the Internal Transcribed Spacer (ITS) region on nuclear ribosomal DNA (Baldwin *et al.*, 1995). Maximum likelihood tree derived from ITS 1 and ITS 2 sequences from Apiaceae members showed inclusion of *Anethum graveolens* (Dill), *Foeniculum vulgare* (Fennel), *Petroselinum crispum* (Parsley) and *Apium graveolens* (Celery) in a single clade while *Trachyspermum ammi* (Ajwain), *Daucus carota* (Carrot) were included in two separate clades (Downie *et al.*, 1998). No major changes to the pre-existing phylogenetic classification were surfaced other than recognition of additional major clades to accommodate increased sampling. Inconsistency in clustering pattern in different clades is still persisting. Phylogenies estimated using parsimony, neighbor-joining and maximum likelihood methods reveal that: (1) Apiaceae subfamily Apioideae is monophyletic and is a sister group to subfamily Saniculoideae, (2) Apiaceae subfamily Hydrocotyloideae is not monophyletic, some members are strongly allied to Araliaceae and others to Apioideae and Saniculoideae and (3) Apiaceae subfamily Apioideae comprises several well-defined subclades but none of this coincides with previously recognized tribal divisions created largely on morphological and anatomical characters of the fruit (Downie *et al.*, 1998).

Molecular systematics of Apiaceae proposed by various authors is not consistent deviating from each other, though Downie *et al.*, (2010) tried to trace the congruence between them. Few genera like *Pimpinella*, *Trachyspermum*, *Angelica* etc. were assigned to two or more major clades. Phylogenetic trees derived from morphological parameters showed congruence with those derived from molecular data in very few cases. Morphology can play a vital role in solving taxonomic disputes, ambiguity in tracing phylogeny in Apiaceae, especially the seedling morphology, leaf margin insertion pattern. In the present investigation few selected members belonging to Apiaceae differing in mature leaf and cotyledonary leaf morphology were taken into consideration to trace the phylogenetic lineages. The phylogenetic lineages appeared in the present investigation displayed a significant deviation from the existing interpretation as far as grouping of taxa is concerned.

Simple leaves (entire or with different degrees of marginal incertion) are common in Saniculoideae. All the members of the tribe Steganotaenieae have compound leaves, while those of the tribe Saniculeae have simple leaves. Simple leaves are plesiomorphic in subfamily Saniculoideae and in tribe Saniculeae and compound leaves are synapomorphy for the tribe Steganotaenieae. Compound leaves also occur in subfamily Apioideae. The ancestors of the tribe Steganotaenieae and Saniculeae had nonpalmate leaves, a plesiomorphic feature also present in the ancestors of subfamily Saniculoideae, the ancestor of Apioideae (Shah and Constance, 1951).

It is presumed that simple entire leaf represents the primitive condition, evolution supposed to have progressed towards gradual increase in marginal insertion leading to pinnately dissected, pinnatipartite ultimately to decompound leaves with narrow linear segments. Similarly, ovate-lanceolate cotyledonary leaves may be considered to represent the primitive situation and gradual narrowing has given rise to linear cotyledonary leaves. Keeping in view this concept, it would be logical to interprete that the member like long Coriander (*Eryngium foetidum*) included in the subfamily Saniculoideae with simple entire spathulate-ovate to spathulate leaves represents the ancestral stock and other members having pinnatifid, pinnatipartite to pinnatisect-decompound leaves with narrow linear segments might have evolved. As the present study deals with few selected members, putative evolutionary trend derived from leaf and cotyledonary features is not comprehensive due to lack of missing links. Coriander or Cilantro is supposed to be a probable descendent (not immediate) from Long Coriander or Culantro. Two evolutionary trends are recognizable from Coriander, one giving rise to members like Celery, Ajwain, and Radhuni and other giving rise to members like Parsley, Carrot, Cumin, Dill and Fennel. The former trend represents a short branch of the main trunk characterized with pinnatifid to pinnatipartite leaves and spathulate-ovate cotyledonary leaves with rounded tips while the later is the main evolutionary trend illustrates the transition from pinnatifid, pinnatipartite to decompound leaves and transition from ovate-lanceolate acute cotyledonary leaves to narrow linear acute acute cotyledonary leaves.

Dendrogram computed from morphological features appeared supportive to the derived putative trend. Long Coriander showed wide divergence from all the other members. Members like Coriander, Celery, Parsley, Radhuni and Ajwain were clustered in a distinct group and members like Dill, Fennel and Cumin were included in a separate cluster. In molecular phylogeny (Downie *et al.*, 2010) the clades corresponding to tribes or subtribes show heterogeneous assemblage of species widely differing in morphology. The genera like *Anethum*, *Foeniculum*, *Apium*, *Petroselinum* included in the tribe Apieae differ from each other on seedling and leaf margin insertion. Cumin and Carrot are included in the clade corresponding to the subtribe Daucinae but in the present study two are placed in two separate groups. In the present investigation taxa were grouped based on leaf margin insertion pattern, seedling morphology along with other morphological features. These two morphological parameters are significant enough to minimize the differences between different phylogenetic interpretations. Delimitation of different clades or tribes or subtribes is not very sharp due to diffuse generic boundaries and heterogeneous patterns of variation. This type of study along with molecular parameters could be proved promising to derive phylogeny in Apiaceae especially in subfamily Apioideae. A comprehensive study involving a large number of taxa can reflect the overall phylogeny in Apioideae providing the missing links.

Conclusion

Application of molecular parameters alone cannot generate any comprehensive idea about phylogeny in Apiaceae subfamily Apioideae. Simple morphology could be very instrumental in deriving phylogeny especially when generic boundaries are diffuse. Interpretation from morphological parameters may or may not show congruence with that of molecular parameter.

Application of leaf lamina insertion pattern and cotyledonary leaf features along with other parameters would be very effective to trace the evolutionary trend in Apiaceae particularly in Apioideae. A comprehensive investigation involving a large number of members may provide the missing links in the trend.

Acknowledgements

The author expresses his sincere gratitude to University Grants Commission, New Delhi for the project.

References

1. Baldwin, B.G., M.J. Sanderson, J.M. Porter, M.F. Wojciechowski, C.S. Campbell and M.J. Donoghue. "The ITS region of nuclear ribosomal DNA: A valuable source of evidence on angiosperm phylogeny." *Annals of Missouri Botanical Garden* 82(1995):247-277. Print

2. Calvino, C.I., S.G. Martinez and S.R. Downie. "Morphology and biogeography of Apiaceae subfamily Saniculoideae as inferred by phylogenetic analysis of molecular data." *American Journal of Botany* 96.2 (2008): 196-214. Print

3. Constance, L. History of classification of Umbelliferae (Apiaceae). In: Heywood, V.H., (ed), *The biology and chemistry of the Unbelliferae.* Academic Press, New York, 1971, pp. 1-12.

4. Das, S. "Systematics and taxonomic delimitation of vegetable, grain and weed amaranths: a morphological and biochemical approach." *Genetic Resources and Crop Evolution* 59 (2012): 289-303. Print

5. Downie, S.R. and D.S. Katz-Downie. "A molecular phylogeny of Apiaceae subfamily Apioideae: evidence from nuclear ribosomal DNA internal transcribed spacer sequence." *American Journal of Botany* 83 (1996): 234-251. Print

6. Downie, S.R., D.S. Katz-Downie and K.J. Cho. "Phylogenetic analysis of Apiaceae subfamily Apioideae using nucleotide sequence from the Chloroplast *rpoCl* intron." *Molecular Phylogenetics and Evolution* 6(1996): 1-18.Print

7. Downie, S.R., S. Ramnath, D.S. Katz-Downie and E. Llanas. "Molecular systematic of Apiaceae subfamily Apioideae: phylogenetic analysis of nuclear ribosomal DNA internal transcribed spacer and plastid *rpoC1* intron sequence. "*American Journal of Botany* 85 (1998): 563-591. Print

8. Downie, S.R., G.M. Plunkett, M.F. Watson, K. Spalik, D.S. Katz-Downie, C.M. Valiejo-Roman, E.I. Terentieva, A.V. Troitsky, B.Y. Lee, J. Lahham and A. Ei-Oqlah. "Tribes and clades within Apiaceae subfamily Apioideae: the contribution of molecular data." *Edinburgh Journal of Botany* 58(2001): 301-330.Print

9. Downie, S.R., D.S. Katz-Downie, F.J. Sun and C.S. Lee. "Phylogeny and biogeography of Apiaceae tribe Oenantheae inferred from nuclear rDNA ITS and cpDNA psbI–5'trnK(UUU) sequences, with

emphasis on the North American Endemics clade." *Botany* 86(2008): 1039–1064. Print

10. Downie, S.R., K. Spalik, D.S. Katz-Downie and J. Reduron. "Major clades within Apiaceae subfamily Apioideae as inferred by phylogenetic analysis of nrDNA ITS sequence." *Plant Diversity and Evolution* 128.1-2 (2010): 111-136. Print

11. Drude, C.G.O. Umbelliferae. In: Engler, A. and Prantle, K. (eds), *Die Naturlichen Pflanzenfamilian*, Vol. 38. W. Engelmann, Germany, 1897-1898, pp. 63-250.

12. Jimenez-Mejias, P. and P. Vargas. "Taxonomy of the tribe Apieae (Apiaceae) revisited as revealed by molecular phylogenies and morphological characters." *Phytotaxa* 212.1 (2015): 57-79. Print

13. Kondo, K.., S. Terabayashi, M. Okada, C. Yuan and S. He. "Phylogenetic relationship of medicinally important *Cnidium officinale* and Japanese Apiaceae based on *rbcL* sequence." *Journal of Plant Research* 109 (1996): 21-27. Print

14. Magee, A.R., C.I. Calvino, M. Liu, S.R. Downie, P.M. Tilney and B.E. Van Wye. "New tribal deliminations for the early diverging lineages of Apiaceae subfamily Apioidae." *Taxon* 59(2010): 567–580. Print

15. Pimenov, M.G. and M.V. Leonoy. The genera of the Umbelliferae: A Nomenclature. Royal Botanic Garden, Kew, UK, 1993. Print

16. Plunkett, G.M., D.E. Soltis and P.S. Soltis. "Higher level relationship of Apiales (Apiaceae and Araliaceae) based on *rbcL* sequence." *American Journal of Botany* 83(1996a): 499-515. Print

17. Plunkett, G.M., D.E. Soltis and P.S. Soltis. "Evolutionary pattern in Apiaceae: Inferences based on *matK* sequence data." *Systematic Botany* 21(1996b): 477-495. Print

18. Shan, R.H. and L. Constance. "The genus *Sanicula* (Umbelliferae) in the old world and the new." University of California Publications in Botany 1951, 25: 1-78. Print

19. Spalik, K., S.R. Downie and M.F. Watson. "Generic delimitations within the Sium alliance (Apiaceae tribe Oenantheae) inferred from cpDNA rps16–5'trnK(UUU) and nrDNA ITS sequences." *Taxon* 58(2009): 735–748. Print

20. Sun, F.J. and S.R. Downie. "Phylogenetic relationships among the perennial, endemic Apiaceae subfamily Apioideae of western North America: additional data from the cpDNA trnF-trnL-trnT region continue to support a highly polyphyletic Cymopterus." *Plant Diversity and Evolution* 128(2010): 151–172.Print

21. Tilney, P.M., B.E. Van Wyk, S.R. Downie and C.I. Calvino. "Phylogenetic relationships in the genus *Lichtensteinia* (Apiaceae) based on morphological, anatomical and DNA sequence data." *South African Journal of Botany* 75(2009): 64–82. Print

22. Valiejo-Roman, C.M., E.I. Terentieva, M.G. Pimenov, E.V. Kljuykov and T.H. Samigullin. Broad polyphyly in *Pleurospermum* s.l. (Umbelliferae-Apioideae) or broad homoplasy in nrDNA ITS and chloroplast sequences?. In: Pimenov, M.G. and Tilney, P.M. (eds), *Apiales 2008*, The programme and proceedings of the 6th International Symposium on Apiales. KMK Sci Press Ltd, Moscow, 2008, pp. 140–143.

23. Winter, P.J.D., A.R. Magee, N. Phephu, P.M. Tilney, S.R. Downie and B.E. Van Wyk. "A new generic classification for African peucedanoid species (Apiaceae)." *Taxon* 57(2008): 347–364.Print

24. Zhou, J., H. Peng, S.R. Downie, Z.W. Liu and X. Gong. "A molecular phylogeny of Chinese Apiaceae subfamily Apioideae inferred from nuclear ribosomal DNA internal transcribed spacer sequences." *Taxon* 57(2008): 402–416.Print

Cite this article as:

Saubhik Das, Leaf and seedling morphology: the two prospective parameters to trace phylogeny in Apiaceae subfamily Apioideae. *Annals of Plant Sciences 6.10 (2017) pp. 1698-1704.*

doi: http://dx.doi.org/10.21746/aps.2017.10.3

Source of support: University Grants Commission, New Delhi.
Conflict of interest: Nil