



Phylogenetic Analysis of Libyan Thyme (*Thymus Capitatus*) Inferred from The Morphological Traits

Ezzudin S. Ali¹, Hesaen M. Mustafa² and Khansa A. Omaar²

¹ Department of Horticulture, Faculty of Agriculture, Omer Al-Mukhtar University, Libya; ² Department of Environmental Sciences, Faculty of Natural Resources, Omer Al-Mukhtar University, Libya.

<p>ARTICLE HISTORY</p> <p>Received: 11 August 2022</p> <p>Accepted: 9 October 2022</p> <p>Keywords: Thyme; <i>Thymus capitatus</i>; morphological trait; flower measurements; clustering analysis; genetic diversity.</p>	<p>Abstract: The genetic diversity of wild thyme (<i>Thymus capitatus</i>) which growing in southern parts of Al-Jabal Al-Akhdar region, Libya was studied by using cluster analysis of morphological traits (flower measurements). This study was aimed to establish the phylogenetic relationships based on floral parameters among accessions of thyme (<i>T. capitatus</i>). The five populations (accessions) of Libyan thyme were assigned into two clusters (clades) at the critical distance value of 22%. The 1st cluster contained three populations that were included white-flowered, dotted white-flowered and violet-flowered accession, then the 1st cluster was divided into two sub-clusters by the critical distance value of 5%, the first sub-cluster contained two populations (white-flowered, dotted white-flowered accession). While, the second sub-cluster contained one population (violet-flowered accession). The 2nd cluster contained two populations which were purple-flowered and mosaic-flowered accessions. In conclusion, The flower measurements can be a preliminary tool to classify Libyan thyme (<i>T. capitatus</i>), and floral parameters can be used in the classification of Libyan thyme accessions (populations).</p>
---	--

التحليل الوراثي للزعر اللببي (*Thymus capitatus*) المستنتج من الصفات المورفولوجية

<p>الكلمات المفتاحية: الزعر؛ الزعر البري؛ الصفات المورفولوجية؛ قياسات الزهرة؛ التحليل العنقودي؛ التنوع الجيني.</p>	<p>المستخلص: تم دراسة التباين الوراثي لنبات الزعر البري (<i>Thymus capitatus</i>)، والذي ينمو في الأجزاء الجنوبية لمنطقة الجبل الأخضر، في ليبيا باستخدام التحليل العنقودي لصفات المورفولوجية (قياسات الزهرة). تهدف هذه الدراسة إلى تحديد العلاقات التطورية القائمة على الصفات الزهرية لنباتات الزعر (<i>T. capitatus</i>). عدد خمسة عشائر طبيعية لنبات الزعر اللببي حددت في عنقودين (فرعين) عند قيمة مسافة تباعدها 22%. العنقود الأول شمل ثلاثة عشائر: عشيرة النباتات بيضاء الأزهار وعشيرة النباتات بيضاء الأزهار المنقطعة وعشيرة النباتات بنفسجية الأزهار. العنقود الثاني احتوي على عشيرتين: عشيرة النباتات أرجوانية الأزهار وعشيرة النباتات مزرکشة الأزهار. علاوة على ذلك، فقد انقسم العنقود الأول إلى تحت عنقودين: الأول شمل عشيرتين هما عشيرة النباتات بيضاء الأزهار وعشيرة النباتات بيضاء الأزهار المنقطعة، والثاني احتوي على عشيرة واحدة وهي عشيرة النباتات بنفسجية الأزهار. في الختام، يمكن أن تكون قياسات الزهور أداة أولية لتصنيف الزعر اللببي (<i>T. capitatus</i>)، ويمكن استخدام الصفات الزهرية في نباتات الزعر للتعريف والتفريق بينهم.</p>
---	--

INTRODUCTION

Since the time of Darwin, systematists have attempted to reconstruct phylogenies by looking at the distributions of character states in organisms. In general they have assumed that those organisms with more character states in common are more closely related and that

overall similarity should reflect genealogy (Baker et al., 1998; Coombs et al., 1981; Duncan et al., 1980; Hennig, 1966). A major goal in systematic biology is to examine the relationships among a group of closely related organisms to determine their evolutionary history (Manos et al., 2001). A phylogenetic tree, also called an evolutionary tree, is a

graph showing the evolutionary interrelationships among various entities that are believed to have a common ancestor. In this graph (a form of a cladogram), each node with descendants represents the most recent common ancestor of the descendants, and edge lengths correspond to time estimates (Ali and Mustafa, 2020). Traditional phylogenetic methods rely on morphological data obtained by measuring and quantifying the phenotypic properties of representative organisms (Lewis, 2001).

Many biologists agree that a phylogenetic tree of relationships should be the central underpinning of research in many areas of biology. Comparisons within or among plant species in a phylogenetic context can provide the most meaningful insights into biology. This important realization is now apparent to researchers in diverse fields, including ecology, molecular biology, physiology and evolution (Crane, 1985; Daly et al., 2001; Doyle & Donoghue, 1986; Doyle et al., 2008; Hall et al., 2002; Leht, 2009; Soltis & Soltis, 2000). One obvious importance is the value of placing organism in the appropriate phylogenetic context to obtain a better understanding of both patterns and processes of evolution. The most popular and frequently used methods of phylogenetics tree building is the unweighted pair group method with arithmetic mean (UPGMA). This method is simple, fast and has been extensively used in many studies to investigate genetic distance among populations or accessions determined by morphological traits (Doyle, 2013; Lewis, 2001 ; Ali and Mustafa; 2020).

Phenotypic characters have been and continue to be used as essential components in the final classification of living organisms. Till today, morphological characters have been the main descriptive tool characterize a given collection or germplasm, to identify and differentiate wild type populations (Ali et al., 2019; Daly et al., 2001; Doyle, 2013; Doyle & Endress, 2000; Doyle & Luckow, 2003; Ennouri et al., 2017; Schneider et al., 2009;

Soltis & Soltis, 2003). Phylogenetic analyses based on phenotypic characters have been used to identify phylogenetic relationships in various plant species, including ferns (Kenrick & Crane, 1997), morning glory (Manos et al., 2001), peonies (Zhi-Qin & Pankai-Yu, 2003), corallinoidean taxa (Kim et al., 2007). Also, the genetic distance as well as genetic variation among accessions can be determined by using cluster analysis based on phenotypic traits (Hillis & Wiens, 2000; Wiens, 2004).

Thyme(*T.capitatus*) is a perennial woody plant, 15 cm tall, with abundant stoloniferous branches. The inflorescences are composed of whorls of small, zygomorphic flowers. The pollination in *T.capitatus* is entomophilous, as in most *T.* species. The main pollinators are *Apis mellifera* and some species of *Bombus*. *T.capitatus* is gynodioecious, as for most species of *T.* (Ali et al., 2019; Gruenwald et al., 2004). Many reports have shown that *T.capitatus* possesses biological properties: antimicrobial activity (Alves et al., 2000; Bounatirou et al., 2007; Ebrahimi et al., 2008), antifungal activity (Grayer & Harborne, 1994; Kalemba & Kunicka, 2003; Ricci et al., 2005) and antioxidant activity (Bounatirou et al., 2007; Ricci et al., 2005 ; Al-mustafa and Al-thunibat, 2008). Moreover, the honey from *T.capitatus* flowers is largely appreciated for its delicacy (Figueiredo et al., 2008).

Thymus capitatus is an endemic wild plant in Al-Jabal Al-khdar area, Libya (SWECO, 1986). *T. capitatus* growing in southern parts of Al-Jabal Al-khdar showed flower color polymorphism which results in five different phenotypes: white-flowered, dotted white-flowered, purple-flowered, violet-flowered and mosaic-flowered accessions (Ali et al., 2019).

The main objective of this study was to establish the phylogenetic relationships based on floral parameters among accessions of thyme (*T.capitatus*) which growing in southern parts

of Al-Jabal Al-khdar region, Libya.

MATERIALS AND METHODS

T.capitatus growing in south parts of Al-Jabal Al-khdar region has shown a stable flower-color polymorphism (Fig. 1). There are five patterns (five populations or accessions): white-flowered, dotted white-flowered, purple-flowered, violet-flowered and mosaic-flowered plants (Ali et al., 2019). In this investigation, 5 accessions (populations) of *T.capitatus* representing the diversity of thyme, were collected during flowering period (from mid June to the end of July) from different sites in Al-Jabal Al-khdar region, Libya. For each population, 10 individual plants were selected randomly. For each plant, five floral measurements were made on 5 healthy flowers. The total length of flower, stamen length, style length, calyx length were measured using mm scale. The ratio between the calyx length and the total length of flower is determined as the percent value.

Means and standard deviations were calculated according to standard methods (Manly, 1986; Ali and Mustafa; 2020). The data matrix was converted into a matrix of dissimilarity values using standard methods (Coombs et al., 1981; Lewis, 2001; Wiens, 2004). Unweighted pair-group method using an arithmetic average (UPGMA) cluster analysis was performed. The standardized mean values of every floral trait were used to perform the cluster analysis using appropriate procedures of the program SPSS (version 14). During tree construction process, all measurements of flower for five populations were unordered and equally weighted, with multi-states interpreted as uncertainty.

RESULTS

Name accessions (populations) and means of floral traits (flower measurements) of Libyan thyme (*T.capitatus*) were presented in Table 1. Flower measurements were flower length

(ranged between 6.38 and 3.31 mm), Calyx length (ranged between 2.40 and 1.08 mm), Stamen length (ranged between 4.65 and 3.41mm), Style length (ranged between 5.37 and 4.20 mm) and Calyx/ flower length (ranged between 0.58 and 0.19 %).

Based on average values of floral traits (flower measurements), the five populations of Libyan thyme (*T.capitatus*) were classified into two clusters (clades) at the critical distance value of 22%, the 1st cluster (the early or medium-flowering cluster) and the 2nd cluster (the late flowering cluster). The 1st cluster (the early or medium-flowering cluster) contained three populations that were: white-flowered, dotted white-flowered and violet-flowered accession. Furthermore, the 1st cluster was divided into two sub-clusters by the critical distance value of 5%, the first sub-cluster contained two populations (white-flowered, dotted white-flowered accession) while the second sub-cluster contained one population (violet-flowered accession). On the other hand, the 2nd cluster (the late flowering cluster) contained two populations which were purple-flowered and mosaic-flowered accessions (Table 1 and Fig. 2).

Results of the present study showed that, there were a closer relationship between white-flowered and dotted white-flowered population than between either of them and any other populations of *T.capitatus*. These two populations first formed their own monophyletic group, and then together formed a monophyletic clade with violet-flowered population. In addition, purple-flowered and mosaic-flowered formed a monophyletic group, which was then joined by other thyme's populations.

In this study, the accessions that have large flowers size, were grouped into early or medium-blooming cluster, while the accessions that have small flowers size were clustered into late or medium- blooming cluster, based on floral traits cluster analysis.

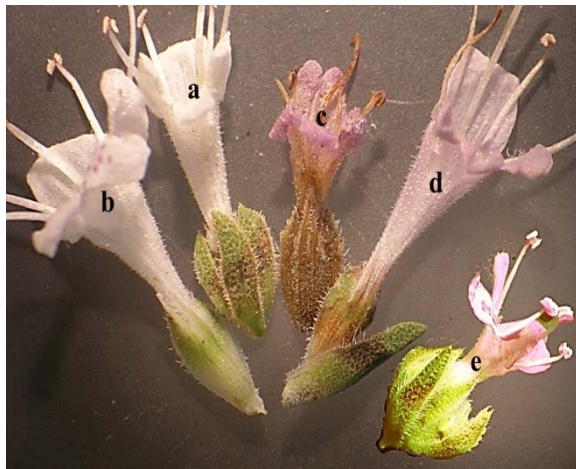


Figure: (1). Flower color polymorphism in Libyan thyme (*T. capitatus*): (a) white- flower (b) dotted-white flower (c) purple flower (d) violet-flower and (e) mosaic-flower.

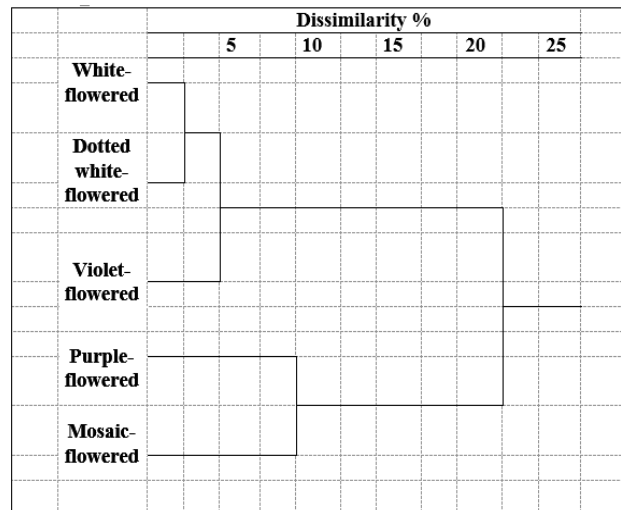


Figure: (2). Dendrogram for five accessions of Libyan thyme (*T. capitatus*) by floral traits data.

Table:(1). Means of floral traits (flower measurements) of the five accessions Libyan thyme (*T. capitatus*)

Accessions (Populations)	Blooming time	Means of floral measurements				
		Flower length (mm)	Calyx length (mm)	Stamen length (mm)	Style length (mm)	Calyx / flower length (%)
White-flowered	Early	5.83	1.08	3.41	4.46	.19
Dotted white-flowered	Early	5.65	1.26	3.55	4.20	0.22
Purple-flowered	Late	3.31	2.13	4.33	4.86	0.64
Violet-flowered	Medium	6.38	1.54	4.56	5.37	0.24
Mosaic-flowered	Late	4.15	2.40	4.65	4.53	0.58

Table:(2). Distance matrix of the Libyan thyme (*T. capitatus*) populations.

		Populations				
	White	Dotted white	Violet	Purple	Mosaic	
White	0.00	0.33	1.30	23.45	18.31	
Dotted white	0.33	0.00	1.67	18.93	14.79	
Violet	1.30	1.67	0.00	25.35	17.25	
Purple	23.45	18.93	25.35	0.00	2.51	
Mosaic	18.31	14.79	17.25	2.51	0.00	

DISCUSSION

The current study indicated that flower measurements (morphological characters) can generate a well-supported phylogenetic tree for Libyan thyme (*T. capitatus*). These results are in agreement with several studies, which demonstrated that morphological characters are informative for phylogenetic studies (Kenrick & Crane, 1997; Renzaglia et al., 2007; Schneider et al., 2002 ; Ali and

Mustafa, 2020). It is common for morphological data to be considered less important than DNA sequence data in phylogenetic studies (Bowe et al., 2000; Endress & Igersheim, 2000). However, morphological characters (such as flower measurements) differ substantially from DNA sequence characters in their complexity and their frequency of evolutionary change. Many morphological characters including floral parameters show a much lower mutational rate

than DNA sequence and thus may be less prone to problems such as saturation (Cracraft & Donoghue, 2004; Donoghue & Yang, 2016; Endress & Doyle, 2009; Hillis & Wiens, 2000). Therefore, some phenotypic parameters are likely to be ideal phylogenetic characters because they allow us to identify single evolutionary events (Bateman et al., 2006; Lee & Palci, 2015; Mathews, 2009).

Moreover the results showed that phylogenetic analysis using floral parameters is an easy, inexpensive, useful and powerful approach in taxonomic purposes. Several previous studies demonstrated that phenotypic parameters were reasonable tool to build phylogenetic tree for many plant species (Doyle et al., 2008; Giribet, 2015; Hall et al., 2002; Lewis, 2001 ; Ali and Mustafa, 2020).

In this study, all of the large flower size accessions (populations) were grouped into early or medium-blooming cluster, while the small flower size accessions were grouped into late blooming cluster, based on flower measurement cluster analysis. In addition, the violet-flowered accession was assigned into unique sub-cluster. It can be suggested that floral parameter analysis is a useful tool in studying the difference in ecological type. Therefore, morphological trait analysis (such as flower measurements) may be helpful to identify patterns of combinations between the diverse ecological type variety groups (Ali et al., 2019; Doyle, 2013; Schneider et al., 2009).

The current results indicate that flower measurements (morphological characters) can be a preliminary tool to classify Libyan thyme (*T. capitatus*). Moreover, the present research has revealed that floral parameters can be used in the classification of Libyan thyme accessions (populations). This result was confirmed by other researches which reported that the morphological characters have an important role in the classification

and identification for many species (Doyle, 2013; Manos et al., 2001; Zhi-Qin & Pankai-Yu, 2003 ; Ali and Mustafa, 2020).

The result of our cladistic analysis indicates that The success of a phylogenetic analysis depends upon the discovery of character sets that provide accurate information. This result was confirmed by other researches which reported that many character sets are molecular but the value of morphological data to reconstruct phylogenetic relationships was reasonable tool to build phylogenetic tree for many plant species (Davis et al., 1998; Doyle, 2013; Ennouri et al., 2017; Hu et al., 2018; Wiens, 2004; Wodniok et al., 2011 ; Bremer *et. al.*, 2009).

Phylogenetic tree based on morphological characters was also reported in other plant species like apple (Forte et al., 2002), Theaceae (Luna & Ochoterena, 2004), vicia (Leht, 2009) and olive (Ali and Mustafa, 2020). However, the phylogenetic tree based on morphological data could not reveal the genetic relationship among cultivars adequately (Keating et al., 2020; Lewis, 2001; Soreng et al., 2017). Furthermore, the morphological variation does not always reflect real genetic variation because of genotype-environment interaction and the largely unknown genetic control of polygenic morphological and agronomic traits (Ali et al., 2019; Ayed et al., 2015; Doyle, 2013; Wiens, 2004).

CONCLUSION

By using cluster analysis of morphological traits (flower measurements) for five natural populations *Thymus capitatus* (accessions) were assign into two clusters. The 1st cluster contained three populations that were white-flowered, dotted white-flowered and violet-flowered accession. Furthermore, the 1st cluster was divided into two sub-clusters distance value the first sub-cluster contained two populations (white-flowered, dotted white-flowered accession) while the second

sub-cluster contained one population (violet-flowered accession). On the other hand, the 2nd cluster contained two populations which were purple-flowered and mosaic-flowered accessions

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

Author contributions: Ali Contributed data, Performed the analysis; Ali and Mustafa Drafting the manuscript; Mustafa Conceived & design of the study, Collected the data; Ali, Mustafa and Omar Performed the analysis; Both Mustafa and Omar approval of the final version of the manuscript.

Funding: The authors do not have funding for the work reported in their manuscript.

REFERENCES

- Ali, E. S., Mustafa, H. M., & Blkasem, K. A. O. (2019). Morphological variation of Libyan carob (*Ceratonia siliqua* L.). *Al-Mukhtar Journal of Sciences*, 34(2), 126-133 .
- Alves, T. M. d. A., Silva, A. F., Brandão, M., Grandi, T. S. M., Smânia, E. d. F. A. S., Smânia Júnior, A., & Zani, C. L. (2000). Biological screening of Brazilian medicinal plants. *Memórias do Instituto Oswaldo Cruz*, 95, 367-373 .
- Ayed, R. B., Ennouri, K., Hassen, H. B., Triki, M., & Rebai, A. (2015). Comparison between DNA-based, pomological and chemical markers accomplished by bioinformatic tools to distinguish within Tunisian olive cultivars. *Journal of Fundamental and Applied Sciences*, 7(3), 408-421 .
- Baker, R. H., Yu, X., & DeSalle, R. (1998). Assessing the relative contribution of molecular and morphological characters in simultaneous analysis trees. *Molecular Phylogenetics and Evolution*, 9(3), 427-436 .
- Bateman, R. M., Hilton, J., & Rudall, P. J. (2006). Morphological and molecular phylogenetic context of the angiosperms: contrasting the 'top-down' and 'bottom-up' approaches used to infer the likely characteristics of the first flowers. *Journal of Experimental Botany*, 57(13), 3471-3503 .
- Bounatirou, S., Smiti, S., Miguel, M. G., Faleiro, L., Rejeb, M., Neffati, M., Costa, M., Figueiredo, A., Barroso, J., & Pedro, L. (2007). Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian *Thymus capitatus* Hoff. et Link. *Food chemistry*, 105(1), 146-155 .
- Bowe, L. M., Coat, G., & DePamphilis, C. W. (2000). Phylogeny of seed plants based on all three genomic compartments: extant gymnosperms are monophyletic and Gnetales' closest relatives are conifers. *Proceedings of the National Academy of Sciences*, 97(8), 4092-4097 .
- Coombs, E. A., Donoghue, M. J., & McGinley, R. J. (1981). Characters, computers, and cladograms: A review of the Berkeley cladistics workshop. *Systematic Botany*, 359-372 .
- Cracraft, J., & Donoghue, M. J. (2004). Charting the tree of life. *Assembling the Tree of Life*, 1-4 .
- Crane, P. R. (1985). Phylogenetic analysis of seed plants and the origin of angiosperms. *Annals of the Missouri Botanical Garden*, 716-793 .

- Daly, D. C., Cameron, K. M., & Stevenson, D. W. (2001). Plant systematics in the age of genomics. *Plant physiology* 133(4), 1333-1328.
- Davis, J. I., Simmons, M. P., Stevenson, D. W., & Wendel, J. F. (1998). Data decisiveness, data quality, and incongruence in phylogenetic analysis: an example from the monocotyledons using mitochondrial atp A sequences. *Systematic Biology*, 47(2), 282-310.
- Donoghue, P. C., & Yang, Z. (2016). The evolution of methods for establishing evolutionary timescales. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 371(1699), 20160020.
- Doyle, J. A. (2013). Phylogenetic analyses and morphological innovations in land plants. *Annual Plant Reviews online*, 1-50.
- Doyle, J. A., & Donoghue, M. J. (1986). Seed plant phylogeny and the origin of angiosperms: an experimental cladistic approach. *The Botanical Review*, 52(4), 321-431.
- Doyle, J. A., & Endress, P. K. (2000). Morphological phylogenetic analysis of basal angiosperms: comparison and combination with molecular data. *International Journal of Plant Sciences*, 161(S6), S121-S153.
- Doyle, J. A., Endress, P. K., & Upchurch, G. R. (2008). Early Cretaceous monocots: a phylogenetic evaluation. *Sborník Národního muzea v Praze. Acta Musei nationalis Pragae*, 64(2-4), 61-87.
- Doyle, J. J., & Luckow, M. A. (2003). The rest of the iceberg. Legume diversity and evolution in a phylogenetic context. *Plant physiology*, 131(3), 900-910.
- Duncan, T., Phillips, R. B., & Wagner Jr, W. H. (1980). A comparison of branching diagrams derived by various phenetic and cladistic methods. *Systematic Botany*, 264-293.
- Ebrahimi, S. N., Hadian, J., Mirjalili, M., Sonboli, A., & Yousefzadi, M. (2008). Essential oil composition and antibacterial activity of *Thymus caramanicus* at different phenological stages. *Food chemistry*, 110(4), 927-931.
- Endress, P. K., & Doyle, J. A. (2009). Reconstructing the ancestral angiosperm flower and its initial specializations. *American Journal of Botany*, 96(1), 22-66.
- Endress, P. K., & Igersheim, A. (2000). Gynoecium structure and evolution in basal angiosperms. *International Journal of Plant Sciences*, 161(S6), S211-S223.
- Ennouri, K., Rayda, B., Ercisli, S., Fathi, B., & Triki, M. A. (2017). Evaluation of variability in Tunisian *Olea europaea* L. accessions using morphological characters and computational approaches. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 45(1), 269-262.
- Figueiredo, A. C., Barroso, J. G., Pedro, L. G., Salgueiro, L., Miguel, M. G., & Faleiro, M. L. (2008). Portuguese *Thymbra* and *Thymus* species volatiles: chemical composition and biological activities. *Current Pharmaceutical Design*, 14-3120(29), 3140.

- Forte, A., Ignatov, A., Ponomarenko, V., Dorokhov, D., & Savelyev, N. (2002). Phylogeny of the *Malus* (apple tree) species, inferred from the morphological traits and molecular DNA analysis. *Russian Journal of Genetics*, 38(10), 1150-1161 .
- Giribet, G. (2015). Morphology should not be forgotten in the era of genomics—a phylogenetic perspective. *Zoologischer Anzeiger-A Journal of Comparative Zoology*, 256, 96-103 .
- Grayer, R. J., & Harborne, J. B. (1994). A survey of antifungal compounds from higher plants, 1982–1993. *Phytochemistry*, 37(1), 19-42 .
- Gruenwald, J., Brendler, T., & Jaenicke, C. (2004). Physicians Desk Reference (PDR) for Herbal Medicines. Third Edition. Montvale, New Jersey: Thomson. *Medical Economics Company* .
- Hall, J. C., Sytsma, K. J., & Iltis, H. H. (2002). Phylogeny of Capparaceae and Brassicaceae based on chloroplast sequence data. *American Journal of Botany*, 89(11), 1826-1842 .
- Hennig, W. (1966). Phylogenetic systematics. Urbana, IL: *University of Illinois Press*. [Google Scholar .]
- Hillis, D., & Wiens, J. (2000). Molecules versus morphology in systematics: conflicts, artifacts, and misconceptions. *Phylogenetic analysis of morphological data*, 1-19 .
- Hu, G.-X., Takano, A., Drew, B. T., Liu, E.-D., Soltis, D. E., Soltis, P. S., Peng, H., & Xiang, C.-L. (2018). Phylogeny and staminal evolution of *Salvia* (Lamiaceae, Nepetoideae) in East Asia. *Annals of botany*, 122(4), 649-668 .
- Kalemba, D., & Kunicka, A. (2003). Antibacterial and antifungal properties of essential oils. *Current medicinal chemistry*, 10(10), 813-829 .
- Keating, J. N., Sansom, R. S., Sutton, M. D., Knight, C. G., & Garwood, R. J. (2020). Morphological phylogenetics evaluated using novel evolutionary simulations. *Systematic Biology*, 69(5), 897-912 .
- Kenrick, P., & Crane, P. R. (1997). The origin and early evolution of plants on land. *Nature*, 389(6646), 33-39 .
- Kim, J. H., Guiry, M. D., Oak, J. H., Choi, D. S., Kang, S. H., Chung, H., & Choi, H. G. (2007). Phylogenetic relationships within the tribe Janieae (Corallinales, Rhodophyta) based on molecular and morphological data: a reappraisal of *Jania* 1. *Journal of phycology*, 43(6), 1310-1319 .
- Lee, M. S., & Palci, A. (2015). Morphological phylogenetics in the genomic age. *Current Biology*, 25(19), R922-R929 .
- Leht, M. (2009). Phylogenetics of *Vicia* (Fabaceae) based on morphological data. *Feddes Repertorium*, 120(7 - 8), 379-393 .
- Lewis, P. O. (2001). A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology*, 50(6), 913-925 .
- Luna, I., & Ochoterena, H. (2004). Phylogenetic relationships of the genera of Theaceae based on morphology. *Cladistics*, 20(3), 223-270 .

- Manly, B. F. (1986). Randomization and regression methods for testing for associations with geographical, environmental and biological distances between populations. *Researches on Population Ecology*, 28(2), 201-218 .
- Manos, P. S., Miller, R. E., & Wilkin, P. (2001). Phylogenetic analysis of Ipomoea, Argyreia, Stictocardia, and Turbina suggests a generalized model of morphological evolution in morning glories. *Systematic Botany*, 26(3), 585-602 .
- Mathews, S. (2009). Phylogenetic relationships among seed plants: persistent questions and the limits of molecular data. *American Journal of Botany*, 96 .236-228 ,(1)
- Renzaglia, K. S., Schuette, S., Duff, R. J., Ligrone, R., Shaw, A. J., Mishler, B. D., & Duckett, J. G. (2007). Bryophyte phylogeny: advancing the molecular and morphological frontiers. *The bryologist*, 179-213 .
- Ricci, D., Fraternali, D ,Giamperi, L., Bucchini, A., Epifano, F., Burini, G., & Curini, M. (2005). Chemical composition, antimicrobial and antioxidant activity of the essential oil of Teucrium marum (Lamiaceae). *Journal of ethnopharmacology*, 98(1-2), 195-200 .
- Schneider, H., Pryer, K. M., Cranfill, R., Smith, A., & Wolf, P. (2002). Evolution of vascular plant body plans: a phylogenetic perspective. *Developmental genetics and plant evolution*, 330-364 .
- Schneider, H., Smith, A. R., & Pryer, K. M. (2009). Is morphology really at odds with molecules in estimating fern phylogeny? *Systematic Botany*, 34(3), 455-475 .
- Soltis, D. E., & Soltis, P. S. (2003). The role of phylogenetics in comparative genetics. *Plant physiology*, 132(4), 1790-1800 .
- Soltis, E. D., & Soltis, P. S. (2000).(Contributions of plant molecular systematics to studies of molecular evolution. *Plant Molecular Biology*, 42(1), 45-75 .
- Soreng, R. J., Peterson, P. M., Romaschenko, K., Davidse, G., Teisher, J. K., Clark, L. G., Barberá, P., Gillespie, L. J., & Zuloaga ,F. O. (2017). A worldwide phylogenetic classification of the Poaceae (Gramineae) II: An update and a comparison of two 2015 classifications. *Journal of Systematics and evolution*, 55(4), 259-290 .
- Wiens, J. J. (2004). The role of morphological data in phylogeny reconstruction. *Systematic Biology*, 53(4), 653-661 .
- Wodniok, S., Brinkmann, H., Glöckner, G., Heidel, A. J., Philippe, H., Melkonian, M., & Becker, B. (2011). Origin of land plants: do conjugating green algae hold the key? *BMC Evolutionary Biology*, 11(1), 1-10 .
- Zhi-Qin, Z., & Pankai-Yu, H.-Y. (2003). Phylogenetic analyses of Paeonia section Moutan (treepeonies, Paeoniaceae) based on morphological data. *Journal of Systematics and evolution*, 41(5), 436 .