

Anatomical Studies on Subfamily *Nepetoideae* Species (*Lamiaceae*) in West Azerbaijan in Iran

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Abstract: This study deals, with epidermal and internal anatomy of the leaves, stem and petiole of the 12 species (6 genera) of the subfamily *Nepetoideae*. In agreement with classical systematics, all taxa studied cluster within the *Nepetoideae* and are clearly distinguished from members of the subfamily *Lamioideae*. A number of distinctive clades are apparent within the *Nepetoideae*: I-*Satureja*, *Dracocephalum*, II-*Nepeta*, III-*Mentha*, *Ziziphora*, IV- *Thymus*. The tribe *Mentheae* needs to be subdivide in to at least 2 groups (clades I-II and III-IV).

Key words: Leaf, stem, petiole, anatomy, *Nepetoideae*, mentheae

INTRODUCTION

The taxonomic value of the indumentum and its importance in systematic and phylogenetic relationships is well known in *Lamiaceae* and such related families as *Verbenaceae* and *Scrophulariaceae* (Abu-Assab and Cantino, 1987; Cantino, 1990; Metcalfe and Chalk, 1950).

The family *Lamiaceae* covers >4000 species, which are grouped in approximately 220 genera (Hedge, 1992). It is considered to be one of the most highly evolved plant families with regard to floral structures. Many *Lamiaceae* species produce essential oils and are therefore, used by man as perfumes, flavourings, foods and medicinal drugs.

The classification of the *Lamiaceae* is still a matter of debate and Johan Lindley (Professor of Botany at University College, London) already had pointed out in 1829. We are indepted to our friend Mr. Bentham, by whom *Lamiaceae* have been made a particular study and to whom we confidently look for rescuing them from a state of confusion, that has gradually been increasing since the days of Linnaeus, until it has become the disgrace of Botany.

It became evident at least that the *Lamiaceae* are closely related to the paraphyletic *Verbenaceae* (Cantino, 1992; Cantino *et al.*, 1992; Cantino and Sanders, 1986; Olmstead *et al.*, 1992). Erdtman (1945) subdivided them in to 2 major groupings: the *Lamioideae* and *Nepetoideae*.

Although, many chemical, morphological and biological characters have been determined for the taxa within the subfamily *Nepetoideae*, the phylogenetic

relationships between tribes, subtribes and genera are still for from being clear and unambiguous (Hedge, 1992).

MATERIALS AND METHODS

Plants were collected from different localities in West Azerbaijan. Specimens from the herbarium of Urmia university were also examined.

In this study, specimens were fixed in alcohol: Glycerin in ratio 1 : 1. Anatomical characters of leaf, stem and petiole were studied and measured in laboratory by light microscope. Light microscope fitted with a digital camera lucida was used. All anatomical drawings and slides are deposited in the Biology Department of Urmia University, Urmia for further reference. The length, width and frequency of the stomata were measured with an ocular micrometer using the surface section from adaxial and abaxial parts of the leaf epidermis.

RESULTS AND DISCUSSION

Leaf: There is a single layered epidermis on the adaxial and abaxial surface of the leaf. Adaxial epidermis cells are larger than abaxial ones.

Stomata present on both epidermis of intercostal regions and leaves are amphistomatic (stomata on the adaxial and abaxial surface) except *Ballota nigra* subsp *curdica*, *Mentha aquatica* and *Mentha longifolia* and absent at the veins of the leaves (Table 1). Covering trichomes consist of unicellular and multicellular. Multicellular hairs more abundant.

Table 1: Over view of leaf anatomical characters of species under investigation

Species	Lamina thinnest (µm)	Stomata type		Leaf type	Stomata frequency(mm ²)	
		Adaxial	Abaxial		Adaxial	Abaxial
<i>Dracocephalum moldavica</i>	200	Anomocytic	Anomocytic	Dorsiventral	54±1	92±1
<i>Nepeta cataria</i>	210	Diacytic	Diacytic	Dorsiventral	38±1	50±1
<i>Nepeta fissa C.A.Mey</i>	310	Diacytic	Anomocytic	Dorsiventral	52±1	78±1
<i>Nepeta leucostegia boiss</i>	197	Diacytic	Diacytic	Dorsiventral	33±1	67±1
<i>Nepeta meyeri bent</i>	375	Diacytic	Anomocytic	Dorsiventral	57±1	81±1
<i>Nepeta racemosa</i>	255	Diacytic	Diacytic	Dorsiventral	48±1	65±1
<i>Mentha aquatica</i>	190	-	Diacytic	Dorsiventral	-	43±1
<i>Mentha longifolia</i>	150	-	Diacytic	Dorsiventral	-	46±1
<i>Satureja hortensio. L</i>	390	Anomocytic	Diacytic	Isobilateral	28±1	58±1
<i>Thymus migricus</i>	220	Diacytic	Anomocytic	Dorsiventral	27±1	55±1
<i>Ziziphora clinopodioides</i>	216	Diacytic	Anomocytic	Isobilateral	59±1	68±1
<i>Ziziphora tenuior</i>	205	Diacytic	Diacytic	Isobilateral	52±1	62±1

Table 2: Over view of stem anatomical characters of species under investigation

Species	A number of parenchyma layers	A number of collenchyma layers		A number of vascular bundle
		Cornera	Between of corners	
<i>Dracocephalum moldavica</i>	5-6	6-7	1-2	9-10
<i>Nepeta cataria</i>	3-4	10-11	3-4	13-14
<i>Nepeta fissa C.A.Mey</i>	8	3	1	17
<i>Nepeta leucostegia boiss</i>	6-7	6-7	1	9-10
<i>Nepeta meyeri bent</i>	5-6	5-6	2	14-15
<i>Nepeta racemosa</i>	4-5	5-6	2	10-12
<i>Mentha aquatica</i>	4-5	9-10	1-2	11
<i>Mentha longifolia</i>	6	4-5	1-2	12-13
<i>Satureja hortensio. L</i>	6-7	4-5	1-2	8-9
<i>Thymus migricus</i>	8-10	1-2	1-2	7
<i>Ziziphora clinopodioides</i>	4-5	6-7	3	8-9
<i>Ziziphora tenuior</i>	5-6	7-8	4	9-10

Table 3: Over view of petiole anatomical characters of species under investigation

Species	A number of parenchyma layers	A number of collenchyma layers	A number of vascular bundle	
			Median region	Sides
<i>Dracocephalum moldavica</i>	1-2	4-5	2	2
<i>Nepeta cataria</i>	6-8	3-4	1-2	2
<i>Nepeta fissa C.A.Mey</i>	6-8	2	2	2
<i>Nepeta leucostegia boiss</i>	8-10	3	2	2
<i>Nepeta meyeri bent</i>	8-9	3-4	2	2
<i>Nepeta racemosa</i>	7-8	4	2	2
<i>Mentha aquatica</i>	4-5	2-3	2	2
<i>Mentha longifolia</i>	4-5	3-4	1	4
<i>Satureja hortensio. L</i>	5-6	2-3	3	4
<i>Thymus migricus</i>	3-4	2-3	1	2
<i>Ziziphora clinopodioids</i>	2-3	1-2	1	3
<i>Ziziphora tenuior</i>	3-4	2-3	1	4

The stomata were diacytic and anomocytic in species, as recorded in other genera of *Lamiaceae* by Inamder and Bhatt (1972) and occur more frequently on the abaxial surface. Number of stomata range from 92±1 to 27±1 mm⁻². The stomata are the highest abaxial surface of *Dracocephalum moldavica* with 92±1 mm⁻² and the least in adaxial surface of *Thymus migricus* with 27±1 mm⁻² (Table 1).

The taxa exhibit obvious differences in lamina thickness, which ranges from 150-390 µm. The lamina were thinnest in *Mentha Longifolia* (150 µm) and the thickest in *Satureja hortensio. L* (390 µm). In all the taxa

investigated, leaves were dorsiventral except *Scutellaria pinnatifida* subsp *pichleri*, *Ziziphora clinopodioides* and *Satureja hortensio. L* that were isobilateral (Table 1). Type of vascular bundle in all taxa was collateral.

Stem: Epidermis in all species were single layered. Collenchyma tissue was located under the epidermis. Collenchyma was differentiated in to a usually 12-13 layered in *Ballota nigra* subsp *curdia* to 1-2 layered in *Thymus migricus* on corners and 4 layered in *Ziziphora tenuior* to 1 layered in *Nepeta fissa C. A. Mey* and *Nepeta leucostegia Boiss* in between the corners (Table 2). Also,

the taxa exhibit obvious differences in number of layers on parenchyma tissue and number of vascular bundle (Table 2). A number of vascular bundle in *Nepeta fissa* C.A. Mey (17 vascular bundle) and *Thymus migricus* (7 vascular bundle) were the highest and the least, respectively (Table 2).

Petiole: Metcalfe and Chalk (1979) pointed out in the *Lamiaceae* family, the structure of the petiole is important in terms of taxonomy.

Epidermis was single layered. All taxa exhibit differences in number of collenchyma and parenchyma layer and also in number of large vascular bundle in the median region and small vascular bundle in both sides of the petiole (Table 3).

CONCLUSION

In agreement with classical systematics all taxa studied cluster within the *Nepetoideae* and are clearly distinguished from members of the subfamily *Lamioideae*. A number of distinctive clades are apparent within the *Nepetoideae*: I-*Satureja*, *Dracocephalum*, II-*Nepeta*, III-*Mentha*, *Ziziphora*, IV-*Thymus*. The tribe *Mentheae* needs to be subdivide in to at least two groups (clades I-II and III-IV).

J. Lindley might still suggest that the classification of the *Lamiaceae* is the disgrace of Botany, but we are sure that the degree of disgrace has been diminished due to the recent progress by Cantino (1992), Cantino *et al.* (1992), Cantino and Sanders (1986), Harley and Reynolds (1992) and Olmstead *et al.* (1992). But much work needs still to be done to understand probably complicated evolution and history of this particular plant family.

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