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Karyology, morphology and anatomy studies of medicinal plant *Salvia nemorosa* in the North East of Iran

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ABSTRACT

In this study, the karyotype, morphology and anatomy of the petiole and stem the herb Salvia nemorosa was discussed. After collecting and identifying plant , it conducted qualitative and quantitative traits, then, Very thin slices prepared and the samples were stained. To prepare a karyotype, three methods were used which method use of colchicine was more effective. S. nemorosa covered with soft short glandular trichome with low-density and dense non glandular trichome. The general shape of petiole, crescent-shaped, 5-bundle sheath of the center and 2 bundle sheath on either side there. Circular stem epidermis cells, Pith parenchyma cell with 40 to 50 layers of cells polygonal, karyological results showed that the chromosome number of S. nemorosa (2n = 14) with karyotype formula is 4 sm + 3m.

Key words: *Salvia nemorosa*, anatomy, morphology, karyotype, polygonal

INTRODUCTION

Salvia L. has about 900 species in the world and is the largest genus of the family Lamiaceae, This plant in Iran, Europe and the Mediteranean areas can be seen, this plant is used for the production of herbal medicines. Its main use is as a tea, Two compounds in extracts and it contains camphor and essential oil Thujene Alpha used for sanitary materials, This herb has anti-cancer properties [1-2-3]. Mousavi and his colleagues in 2014 to study the micro-anatomy and morphology of leaves of *Salvia* species in the North East of Iran's [4]. Kharazian in 2011 to study the karyotype of *Salvia* [5]. Sheydaei and colleagues in 2010 genus *Salvia* cytologyin Iran were evaluated [6]. Given the importance of *Salvia* plant in the pharmaceutical industry, anatomy, morphology and karyological been low studied. So in this study, to investigate the karyotype of *S. nemorosa* plant then the plant internal structure studied. which structural changes determined and determine if these changes to identify *Salvia* species, which are similar in appearance, to help.

MATERIALS AND METHODS

Morphological study

The study of the morphology, number of samples of the species *Salvia nemorosa* in North Khorasan Province, East south Boujnord, Esfidan, 1561m, Joharchi& Zanghouie, 40219, (FUMH); Quchan, Oghazkohneh, 1800m, Faghihnia & Zangoure, 29451, (FUMH), collected and in addition to new study, some herbarium specimens Institute of Plant Sciences, Ferdowsi University of Mashhad (FUMH) also assessed. Various sectors such as leaf shape, bracelet, the trichomes density on leaves, petioles and stems, inflorescence shape, shaped calyx, surface pedicellus, the outer and inner surfaces of the corolla, etc were studied

Anatomical study

In order to study plant anatomy *S.nemorosa*, example of subject in Formalin: Acetic acid: Alcohol ethylic (FAA) solution was selected. Then, manual cross sections were prepared and stained by green methyl and carmine. Later, the sections were photographed by different magnifications of light microscope Labomed model CZ500 and Dino capture camera [4].

Karyological study

Firstly, collected nutlet in plates cultured. Time sprouting of roots in different species is between 4 to 10 days. After germination, one centimeter from the root tip was separated. Isolation roots in 8-10 hours in the morning which a greater number of cells in mitosis are living, was carried out.For pre-treated to stop mitosis in root tip meristem, three methods were used.

The first method:

1. Put the cut roots in the pre-treatment solution 8-hydroxy quinoline 0/002 molar at room temperature (about 27 ° C) for 3-4 hours.
2. Fixation with Carnoy solution (ethanol: acetic acid net ratio of 1: 3) at 4 ° C for 14 to 32 hours.
3. Puting the roots in a normal HCl solution in 60 ° C water bath for 6-7 minutes.
4-stained with astoursin 2% at room temperature for 1-2 hours (between switching solutions, samples should be washed with distilled water).
5. Squashing (crushing) and observe under a microscope lens, 1000x [7].

The second method

1. Puting the roots cut off at 0/05 percent colchicine pretreatment solution at room temperature for 3 hours.
2. Fixation with Carnoy solution at 4 ° C for 30 minutes.
3. Puting the roots in a normal HCl solution in 60 ° C water bath for 10 minutes (after each wash with distilled water)
4. stained with astoursin 2% for 10-5 minutes.
5. Squashing (crushing) and observe under a microscope with lens 1000 [8].

The third method

1. Stopping the division metaphase roots in ice water 4 ° C for 18 hours.
2. Carnot and the solution fixation in 4 ° C in ice water for 24 hours.
3. Puting the roots in a normal HCl solution and heated in a water bath heated to a temperature of 60 ° C for 5 minutes (after each wash with distilled water).
4. Painting with astoursin 2% at room temperature for 5-2 minutes.
5. Squash (crushing) and observe under a microscope with lens 1000 [5]. In each of the above methods of each slide 7 cells were studied.

RESULTS

Morphology

Perennial plant, 80-50 cm stem length, standing, green, hard and thick, with short-spread glandular trichome, trichome down on. Lower leaves of stem have petiolate length of 1.5-2 cm, upper leaves sessile. The leaves 3-9 in length and 1-4 cm width, Green, ovate to almost bayonets wide, at the base of the heart, on canes scattered, covered with soft short glandular trichome and non glandular trichome dense low-density, white dorsal surface, and covered with crack, leaves margin as small round serrated mostly congregations. Crenate inflorescence leaf, green, non-sharp tip, pistil Inflorescence flower, compressed cycles to 10-6 flowers, Purple - purple, low-branching. Bracts in 8 mm length and width 4-3 mm, Oval, trichome in the front and rear, small bracts, short, Rostral, purple - purple. without peduncle or a lengthy of 3-2 mm, less crack. Calyx at the time of flowering 6mm in length and fruiting time 8 mm in length, Tubular - Bluebell, with 11 lanes, have non-glandular trichome, and often long trichome and glandular trichome without base, the upper lobe, short, curved and three teeth and lower lobe has two sharp teeth. Corolla in length 16-13 mm, Sickle upper lobe, Purplish-violet. Corolla tubein length 10.8 mm, Lack trichome ring.fruit nutlet,1.93 in length and 1.39 width mm, ovate, black.

Anatomy results

Petiole anatomy: general shape of crescent-shaped , A layer of the epidermis elliptical, 4 to 6 Collenchyma layers, 7 to 12 layers at the top Sclerid parenchyma, 5 bundle sheath and double sheath bundle on either side of the center. Glandular and non glandulartrichomes 1 to 4 cell (Figure 1, A, B).

Stem anatomy: general squareshape,The epidermis circular layer after cuticle layer. Collenchyma tissue 6-8 layer was observed at the corners. 5 rows of skin parenchyma tissue, sclerenchyma tissue 10 layer on top 3 phloem layer is placed. Pith parenchyma cell 40- 50 layer and in center are larger with 6 polygonal regular observed (Fig-1: C, D).

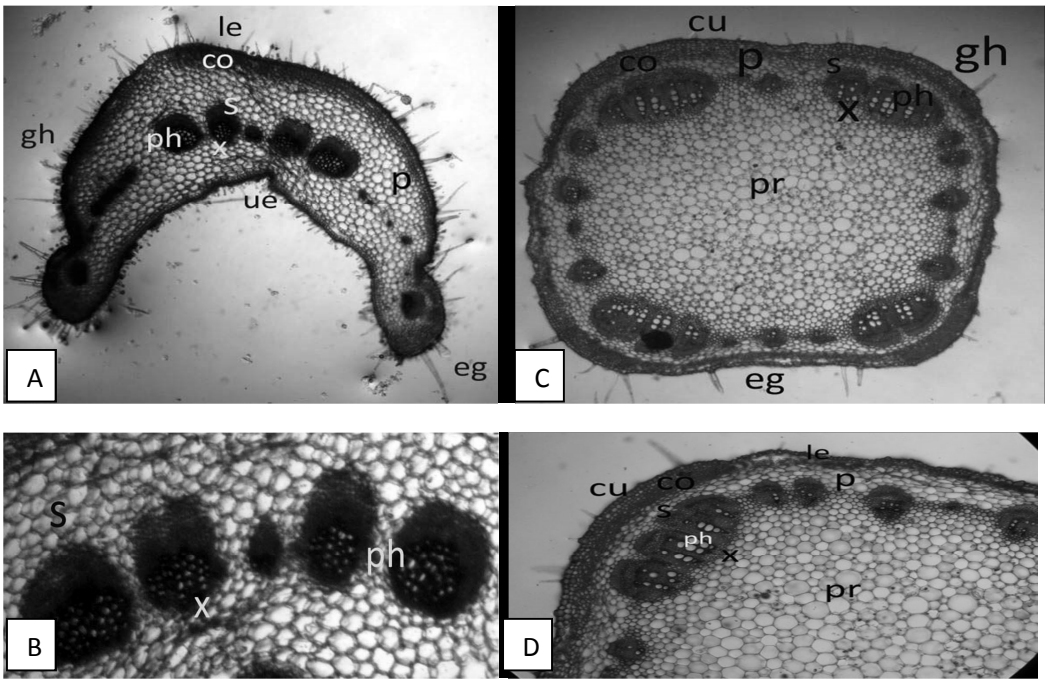

Fig. 1: petiole and stem anatomy of *Salvia nemorosa* (× 40, 100)

Ue: upper epidermis; Le: lower epidermis; Co: collenchyma; P: parenchymatic cell; S: sclerenchyma; X: xylem; Ph: phloem; cu: cuticule; e: epidermis; p: parenchyma; Pr: pith parenchyma.

Table 1: specifications of karyotype *S.nemorosa*

Ch. No	Ch. Total length	Short arm length	Long arm Length	r- value	d- value	R. L.	V	Ch. Type
1	2.06	0.56	1.5	2.06	0.94	0.100	4014	sm
2	1.9	0.5	1.4	2.80	0.9	0.092	3.54	sm
3	1.8	0.89	0.91	1.02	0.02	0.086	3.01	m
4	1.7	0.8	0.9	1.13	0.1	0.083	2.62	m
5	1.56	0.45	1.11	2.47	0.66	0.076	1.94	sm
6	1.5	0.7	0.8	1.14	0.1	0.073	1.87	m
7	1.49	0.69	0.8	1.16	0.11	0.072	2.70	m
8	1.4	0.61	0.79	1.30	0.18	0.068	2.54	sm
9	1.37	0.6	0.77	1.28	0.17	0.067	2.23	sm
10	1.31	0.56	0.75	1.34	0.19	0.064	2.13	sm
11	1.28	0.55	0.73	1.33	0.18	0.062	1.91	sm
12	1.24	0.53	0.71	1.34	0.18	0.060	1.85	sm
13	1.07	0.42	0.65	1.55	0.23	0.052	1.42	m
14	0.9	0.36	0.54	1.50	0.18	0.044	1.19	m

short arm length / long arm length = (arm index) r- value [9]
Short arm's length - long arm length of d- value [10]
Total chromosome length / length a chromosome= relative length R.L
Volume (V)=$r^2 h \pi$ [11].

Karyological results

Three methods were used to study karyology, and the best method, the use of colchicine 0.05 percent as a pretreatment, the results showed that the chromosome number of *S. nemorosa* (2n = 14), (Figure 2). Karyotype formula is 4 sm + 3m (Table 2), Given the clarity of the centromere and arms of chromosomes in the species *S. nemorosa*, In addition to drawing karyotype, information provided (Table 1).

Table 2: Specifications karyotype in *S. nemorosa*

Species name	TL	S	L	T(L/S)	T.V.	D.R.L.%	S%	TF%	2n	class	K. F.
S.nemorosa	20.58	8.22	12.36	2.28	32.09	5.6	43.68	39.94	14	2B	**4sm+3m**

TL: Total length of the chromosome, S: Total small arms, L: Total long arms, L / S: length longest to the shortest chromosomes, TV: The total volume of chromosomes, D.R.L: the difference the relative length of chromosomes, length the longest chromosome / length the shortest chromosome = relative length of the shortest chromosome (S%)× 100 [12].

The total length of all chromosomes / = total length of the short arms =karyotype overall shape (TF%) [11]. (Figure 2)

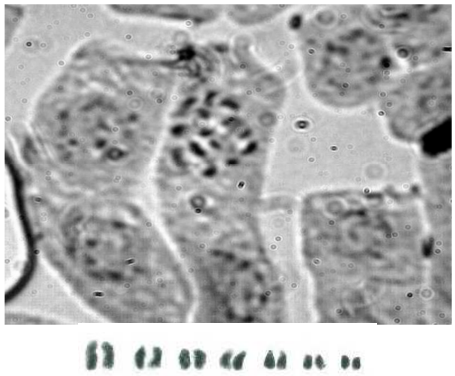

Figure 2: Chromosomes and karyotype *S.nemorosa*, magnification 1000x

DISCUSSION

In this study. In addition to morphology, anatomy of petiole and stem, karyotype drug species *Salvia nemorosa* assesed. The plant is 80-50 cm in length, standing, green, hard and thick, with scattered short glandular trichome. Petiole in *S. nemorosa*, the overall shape of crescent-shaped and recessed upper part, 5 bundle sheath in the center and double bundle sheath on either side (fig1, A,B). In a report provided by Kahraman et al., The *Salvia chrysophylla* petiole have 3 (long vascular bandle) at the center and 2 to 4 small subsidiary bundles on both sides [13]. In *S.nemorosa*, circular stem epidermis cells, collenchyma with 6 to 8 layers was seen in the corners. In *S.indica*, epidermal stem have oval or rectangular cells, Collenchyma tissue composed of regular cells [14]. Burcu and colleagues reported that *S. nemorosa* has a Dorsey ventral leaves, glandular and non glandular trichomes in the different structure, shape and size [15]. Mousavi and his colleagues in 2014 found that the main vein in *S.nemorosa* is elliptical and ventral Dorsey Mesophile [4]. Soy and Ozkan pointed out that the leaves on the *S. blepharoclaena* has a single layer epidermis with glandular and non-glandular and Stomata type is diacytic [16]. In karyological studies, the current study has shown that the number of chromosomes in the species *S. nemorosa*, 2n = 14, karyotype formula 4sm + 3m and chromosome size number between 0/9 – 2/06 micron. In a study Sheidai in 2010 in the areas of Alborz and Kharazian in 2011 on *Salvia* species in the Shahrkord, *S. nemorosa* chromosome number 2n = 14 has been announced [5-6]. If the Kharazian reports that karyotype formula as 4 t + 2m + 1sm and chromosome size between 0.38-1.5 micron [5].

CONCLUSION

In this study, after a detailed review found that to identify plants, karyotype formula and morphology compared to anatomy have the more prominent role.

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