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CYTOGENETIC STUDY OF *CUMINUM SETIFOLIUM* (BOISS) KOS-POL AS A MEDICINAL PLANT

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ABSTRACT
Medicinal plants are the most important source of medicine. *Cuminum setifolium* (Boiss.) Kos.- Pol (1916) with common name of white cumin, a member of the Apiaceae family, growing wild on mountains was investigated as a species having capability for cultivation and crop breeding. Generally, there is not information about *Cuminum setifolium*. For studying of chromosomes morphology in *Cuminum setifolium* 100 slide samples were prepared and studied using their root meristems after fixation, hydrolysis and staining stages. Cytological study showed that *Cuminum setifolium* had $2n = 14$ (X=7) chromosomes with karyotypic formula of 2Sm+1M+St(Ac) in which chromatin lengths were 229.12 and 29% was submetacentric, 14% metacentric and 57% subtelocentric. Maximum and minimum chromosomes lengths were 10.88µ and 4.4µ respectively.

KEYWORDS*: Cuminum setifolium*, botany, cytogenetic, medicinal plant, karyology.

INTRODUCTION

Cuminum setifolium plant is an annual herb, a member of the Apiaceae family (14, 21), the Apiaceae contains about 300 genera and 2500 to 3000 species (21). Cytogenetic is hibritic science of genetic and cytology which studies cellular events specially chromosomic events and genetic phenomena, on the basis of the fact that inheritance substances one exist systematically in one or more chromosome (32). The importance of cytological studies in plant species and their population specifically wild and endemic species is significant, because it can be explain genetical variation, variation in their shapes and the size of chromosome in the mitosis division and the behavior of chromosomes in the meiotic division. Since it has proved that different variety of population of species shows its one special adaptation environment. Thus one of the essential steps in the study of biosystematic, genetic , modification and also for the completion of classification have been done, study of the particular of nucleus and specially the study of the structures of nuclear genome within approach karyotaxonomy (31,36). The difference in the numbers of chromosomes cause of characteristic creation and separation and karyotypic evolution is remarkable important. The plant genetical and cytogenetical information can be a big help for modifier so that he or she can apply the appropriate methods of plants modification for the genetic improvement (2). The modifier with make use of the acquired information about the genome of species and possible recognition of various population of one species, will be able to make the appropriate decision about parents for interaction of species and even the transportation of gene in to agricultural species through hybridization. These studies can help to determine the difference existing variety within individual a group will show the process of evolution of change in chromosomes of composition genome, it is because the different population of one species shows particular adaptation within its one environment (29). In this way by increase in the difference between plant population which are grows in different environment at the result of phenomenon of the adaptation with the environment, may be introduce various or different species. Such adaptation has been taken in to consider in the level of genome. So there is possibility of structural and behavior changes in the level of species chromosomes extremity result in to genetical variety within their population. Such variety may bring about the possibility of modify genotype in its best possible way (37). Since chromosomes carried genes and the particular of plants are under the control of genetical substances of nucleus, thus one of the approaches of studying the variety between figures and difference plant of species is studying cytogenetic. The species which are similar for purposes of cytogenetic parameters and karyotypic particulars have close connection between species. In the case of having desired attributes in this species will be possible between species junction for collecting of desired genes in the plants (11). Karyotype is category of chromosomes of an individual in the basis of the number, size, shape and other typical chromosomal particulars (9). It is possible to distinction individual chromosomes within the tissue in which cellular division achievable in high rate, these tissues are; root tip meristem, very young leaf, stem tip, high rate growing callus tissues or cellular cultures. Developing leaf and root tip are the best source for dividing active cells (30). The size, the number and the shape of chromosomes are important factors for studying the evolution. The existence of expressive differences within species of genus from the length of chromosomes shows the role of DNA quantitative changes in the process of generating species. The existence of expressive differences this parameter among different population of species can be at the result of adaptability changes in regard to environment. In most cases the numbers of chromosomes are related with the degree of complexity of species, as species with high degree complexity have

smaller chromosome and fewer numbers of chromosomes (32). The difference in the shape of chromosomes and in other words subject of karyotype symmetry are important factors in the study of the evolution of karyotype upon which three type karyotype are defined as follows (23):

A) Symmetrical karyotype: symmetrical karyotype is status in which chromosomes are the same size and have mid Centro mere or approximately middle.
B) Non karyotype: non karyotype is status in which the sizes of chromosomes are different from each other.
C) Distinct karyotype: non symmetrical karyotype is combined from two types of chromosomes with different sizes.

Since the mitosis studies are taken place in metaphase stage which have the shortest length and the best conditions for studying, thus it is necessary that the tissue under research has been prepared in way that high percentage of its cells are in the mitosis metaphase stage. The pretreatment solution is used a mixture colchicine, 8-hydroxyquinoline, PDB and DMSO (1, 18, 19, 31). For chromosome counting of *Artemisia spp* is used of 0.05% colchicines solution for 2 h (6). The roots of *Swainsona formosa* were soaked in 0.1% colchicines solution for 4 h at room temperature to prevent spindle elongation (39). Palmer and Hollys are carried out different pretreatment solution for chromosome numbering of soybean containing MBN, 1% - 5% colchicines, 0.001 – 0.004 M 8- hydroxyquinoline, PDB solution and different temperature (22, 38). For the karyotype studying of Nerfolk plant is used from PDB solution for 18 h at 4 ^{0}C temperatures (5). For the most of plants especially aqueous plants and wheat chromosomes is effective PDB solution, treatment time is recommended for 4 h at low temperature (28). In the Wollemi (*Wollemia nobiltis*) for studying chromosome is used from αBN solution for 2 h at 4 ^{0}C temperature (10). For early time, Tjio and Levan are used of 8 – hydroxyquinoline solution for shorten the length of chromosome (33). Wolff and Luippold for shorten the length of chromosome are used of colchicines solution (37). Fixation is carried out for purposes of sudden death cells of one tissue so that the structure and internal parts of cells to keep in its original status (28). The fixing substances quietly and slowly penetrated in wax tissues and in the plants with epidemic tissue or cuticule layer stops watery substances penetrating and so using alcohol before fixation or evacuating air while it is inside fixation substance can help fixation substance to penetrate(30). For purposes of cell cycle studies, the studying by Karimzadeh et al. was carried at wheat root that it is kept for long period time in fixation solution (15). To keep the samples for long period time for purposes of study them in suitable time offer outering them of the fixation solution the must be washed with distilled water or alcohol and there are placed in alcohol at 4^{0}C temperature in refrigerator It is clear, in the case were not necessary for keeping roots, it is enough to wash them by distilled water or alcohol (19, 29, 38). Since 1920 different colors such as acetocarmine, acetoorcein and fulgent have been used for staining of chromosomes (11, 19, 28). In the most studies of *Cuminum cyminum* karyotype, chromosomes number was 2x=14(14). In the study by Jha and Roy on the tissue culture of *Cuminum cyminum* was determined that callus; roots and buds were diploid and including 14 chromosome numbers (13). Sharma and Chattopadhyay to determine that somatic chromosomes number of *Cuminum cyminum* were 2x=14.The most chromosomes were including middle half Centro mere (4, 28).

Regarding to great importance of the plant species as a conservator against soil erosion and windbreak in desertification projects and rehabilitation of sandy desert areas of Iran, it is appeared necessarily to pay more attention to study on such plants and increasing data about their different characteristics of karyology, cytology, phonology, physiology, morphology, etc. as well as edaphic and climatic condition for cultivation and plant breeding programs and improve certain characteristics such as root length, tolerance to drought and salinity etc. for better utilization of natural resources and reaching to sustainable development.

The purpose of this study was to introduce plant species of *Cuminum setifolium* Bunge ex Boiss. and to investigate the karyotypic characteristics, finally using this data, the plant karyotype was drawn as an ideogram.

MATERIALS AND METHODS

In the present study, 100 seeds of the *Cuminum setifolium* plant were treated frequently in Petri dishes (Fig. 1). Considering hard cover of the seeds, scratching method was made to accelerate seed germination before cultivating then, the scratched seeds incubated in 4°C degree centigrade temperature. The treated seeds germinated after 10 days. The mitotic study of caryotype, morphological characteristic of chromosomes of species of *Cuminum setifolium* was done on the meristematic cells of root tips, from seed germination. The root tips were pretreated with 8-hydroxyquinoline and then were fixed in carnoy (3:1 , ethanol: acetic acid), then root tips were hydrolyzed in hydraulic acid 1N at the temperature condition of 60 ^{0}C Staining was carried out with aceto – orsein and then squashed in 45% acetic-acid. Five metaphase cells were studied for several karyotypic parameters. The chromosome studying including to determine of chromosomes number, ploidy level and to provide of karyotype was been carried. For the karyotype studying were been determined total length of chromosome (TL)(35), long arm length (LA), short arm length(SA), relative length of chromosome(RL%)(24), r-value(10,17), d-value, arm ratio(L/S), total length of chromatin in karyotype(X), difference of range of relative length(DRL)(8,12),total form percentage(TF%)(7,12), form percentage (F%)(8,12), symmetry index(S%)(29), dispersion index (DI) (16), centromeric index(CI)(16), coefficient variation(CV)(29). The chromosomes type was determined by Levania et al. table (16). To determine evolution status and symmetrical studying is used of Stebbins table (31).

RESULTS AND DISCUSSION

The chromosome number of *Cuminum setifolium* was reported for the first time in this study and it was diploid

and that's chromosome number was 2x=14 (X=7) (Fig.3, Table 2). Chromosome number of *Cuminum cyminum* was also determined to be 2x=14 (3). Chromosome information is used in plant classification as two distinct methods including karyotype studies and chromosome pairing in meiosis division (26, 27). Huziwara (1962) used from general karyotype form as a classification factor for expression of symmetry conditions.

Car**yotypic** formula of *Cuminum setifolium* was 2Sm+ 1 M +4St (Ac) (Fig. 4, Table 3) including 57% subtelocentric,29% submetacentric and 14% metacentric (Fig.4.). The total length of chromatin was 229.12 and length of the tallest and the shortest chromosomes were estimated 10.88 and 4.4 µ respectively. According to most researches made on plant karyology, best part of a plant for studying mitosis division and identification of chromosomes arrangement and preparation of karyotype is apical meristems of roots, because root induction is occurred rapidly, mitosis division is very fast in this area and lacking chlorophyll in the root cause to study easily the cytogenetic characteristic (25). The comparison of the karyotypes using the Stebbins method have showed different degree three on the basis ratio of the tallest chromosome to the shortest chromosome and degree four on the basis ratio of the acrocentric or telocentric chromosomes. In Stebbins table have twelve groups in which symmetrical karyotype decrease from left to right and from upper to down. So that karyotype of 1A group will be symmetric lest karyotype and the karyotype of 4A group will be non- symmetric lest karyotype (10, 20) (Table1). The chromosomes of *Cuminum setifolium* on the basis Stebbins table (31), to take place in the 3A class (Table 3).

TABLE 1. Karyotype classification by Stebbins method

Shortest chromosome length to tallest chromosome length	0.0	0.01 -0.5	0.5- 0.99	1.0
<2:1	1A	2A	3A	4A
2:1-4:1	1B	2B	3B	4B
>4:1	1C	2C	3C	4C

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FIGURE 1. *Cuminum setifolium* seeds

FIGURE 2. Germination of *Cuminum setifolium* seeds

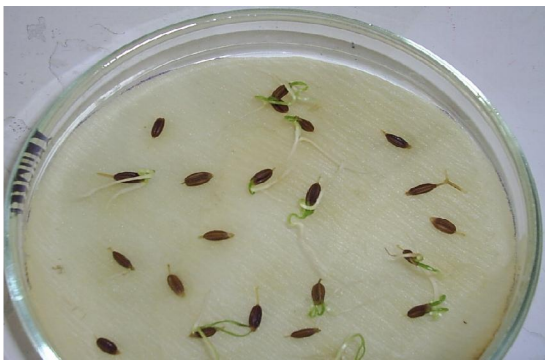

FIGURE 3. Chromosomes of *Cuminum setifolium* with 2x=14

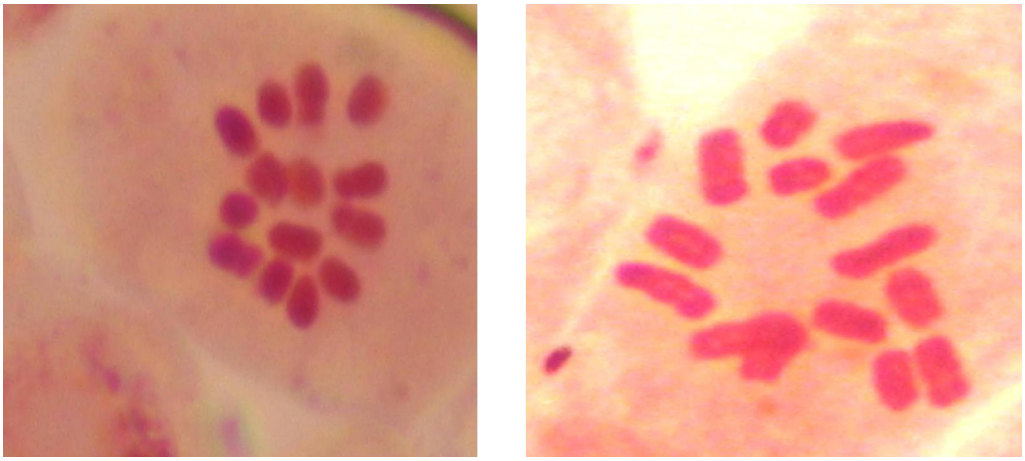

FIGURE 4. Percentage of chromosomes of *Cuminum setifolium*

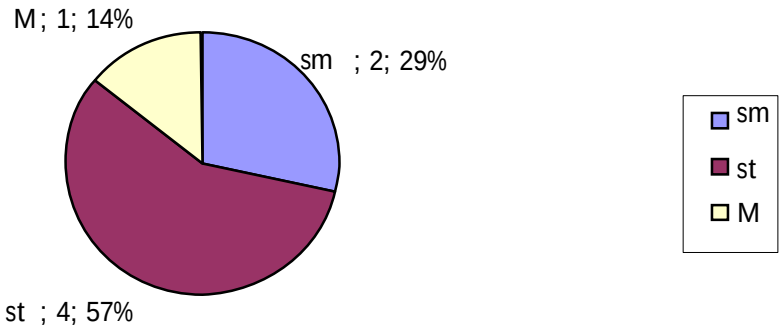

TABLE 2. Characteristics of *Cuminum setifolium* chromosomes

No.	SA	LA	TL	r-value	d-value	RL%	F%	CI	Chromosome type
1	2.56	8.32	10.88	3.25	5.76	9.497	2.23	0.23	st
2	2.56	8.32	10.88	3.25	5.76	9.497	2.23	0.23	st
3	3.84	7.04	10.88	1.83	3.20	9.497	3.35	0.35	sm
4	3.20	7.04	10.24	2.20	3.84	8.938	2.79	0.31	sm
5	3.20	7.04	10.24	2.20	3.84	8.938	2.79	0.31	sm
6	3.20	7.04	10.24	2.20	3.84	8.938	2.79	0.31	sm
7	1.28	5.12	6.40	4.00	3.84	5.586	1.11	0.20	st
8	1.28	5.12	6.40	4.00	3.84	5.586	1.11	0.20	st
9	1.28	5.12	6.40	4.00	3.84	5.586	1.11	0.20	st
10	1.28	5.12	6.40	4.00	3.84	5.586	1.11	0.20	st
11	1.28	5.12	6.40	4.00	3.84	5.586	1.11	0.20	st
12	1.28	5.12	6.40	4.00	3.84	5.586	1.11	0.20	st
13	3.20	3.20	6.40	1.00	0.00	5.586	2.79	0.50	M
14	3.20	3.20	6.40	1.00	0.00	5.586	2.79	0.50	M

TABLE 3. Characteristics of *Cuminum setifolium* chromosomes

S%	TF%	DRL%	CV%	DI	S.Class	A2	A1	X	∑TL	TLA/TSA	Karyotype formulae	2n	species
58.82	28.49	3.41	26.16	0.01	3A	0.2624	0.139	229.12	114.56	2.5	2sm+M+4st	14	*Cuminum setifolium*