Cytological Studies on Some Ecotypes of *Nigella sativa* L. in Iran

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Summary The karyotype of six Iranian ecotypes of *Nigella sativa*, which is a member of the Ranunculaceae family, was studied. The results showed that all studied ecotypes contained 12 chromosomes ($2n=2x=12$). The karyotype formulas for the ecotypes are: G1: $5m+1T$, G2: $4m+1sm+1st$, G3: $5m+1st$, G4: $5m+1T$, G5: $4m+1sm+1T$ and G6: $3m+2st+1sm$. The total form percentages (TF%) of the ecotypes observed were as follows: G1 40.64, G2 41.04, G3 43.83, G4 41.36, G5 39.60, G6 38.94. The largest total haploid length was detected in G6 ($51.76\mu m$) while G4 demonstrated the least ($32.56\mu m$). The first two principal component analysis (PCA) justified over 89% of the total variations of cytological characteristics. UPGMA cluster analysis was carried out for chromosomal parameters which classified the studied ecotypes into five classes.

Key words Black seed, Karyotype, Chromosomal study, Iran.

Nigella sativa L. is an annual flowering plant which is a member of the Ranunculaceae family, native to south and southwest Asia. Seeds of this plant have been employed for thousands of years as a spice and food preservative. *Nigella sativa* L. is also known by different names such as black cumin, black seeds or small fennel and has great potential for cultivation, propagation and production in the Middle East region. This plant has a wide range of medicinal uses. In traditional medicine, seeds of black cumin are used against cough, bronchitis, asthma, chronic headache, migraine, dizziness, chest congestion, dysmenorrheal, obesity, diabetes, paralysis, hemiplegia, back pain, infection, inflammation, rheumatism, hypertension, and gastrointestinal problems such as dyspepsia, flatulence, dysentery, and diarrhea (Datta *et al.* 2012). Major seed components include 20.85% protein, 38.20% fats, 4.64% moisture, 4.37% ash, 7.94% crude fiber, and 31.94% carbohydrate. Potassium, phosphorous, sodium and iron are also predominant elements; it also contains cholesterol, campesterol, stigmasterol, and betasitosterol (Riaz *et al.* 1996, Salma *et al.* 2007, Jabeen *et al.* 2012). Experiments showed *N. sativa* oil improves glycemic control in type II diabetic patients without any side effects (Hosseini *et al.* 2013). Datta *et al.* (2012) reported that this species possesses antimicrobial, anti-malarial, antioxidant, anti-inflammatory, anticancerous, antitumorigenic, anti-hypertensive, antiviral, anti-asthmatic, anti-allergic, anti-diabetic, anti-lipidemic, antiobesity, anticonvulsant, hematological and many other properties.

Members of the family Ranunculaceae have been karyotyped manually and the ecotypes were considered to be good materials for cytological studies as they have relatively low numbers of chromosomes $2n=12$ of suitable sizes with good stainability and a marker telocentric (Datta and Biswas 1983, Saha and Datta 2002, Ghosh and Datta 2006). Datta *et al.* (2012) explained major chromosomal studies of *Nigella sativa* L. which have been reported in their review article. They reported Gregory was the pioneer to enumerate the number of chromosomes ($2n=12$) in somatic complement of *N. sativa* (Gregory 1941, Datta *et al.* 2012). Also, other reports explained 12 chromosomes ($2n=12$) for this plant (Bhattacharyaya 1958, Saha and Datta 2002, Ghosh and Datta 2006). Ghosh and Datta (2006) studied karyotyping of *Nigella sativa* L. (black cumin) and *Nigella damascena* L. (love-in-a-mist) by Image Analyzing System. Their results revealed that both studied ecotypes have 12 chromosomes ($2n=12$). They reported that metacentric chromosomes are prevalent, while a telocentric pair has been marked in both ecotypes. Also, two pairs of submetacentric chromosomes were noted in the somatic complement of *N. sativa*. The somatic chromosome complements in both ecotypes were graded and symmetric in nature. However, the absolute chromosome length in the ecotypes indicated that the chromosomes were relatively longer in *N. sativa* than in *N. damascena*. The total haploid chromatin length was assessed to be $78.622\mu m$ (77.953 to $79.041\mu m$) in *N. sativa* and $70.136\mu m$ (69.507 to $71.014\mu m$) in *N. damascena* (Ghosh and Datta 2006). The objective of this experiment was to study the chromosomal diversity to prepare a standard karyotype for existing ecotypes of *Nigella*

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Fig. 1. Karyotypes and ideograms of studied ecotypes of *Nigella sativa* L. Arrow points to satellited chromosomes. Scale bar=3 μ m.

sativa L. in Iran which will be used to develop improving programs.

Materials and methods

For karyotype analysis, seeds of six ecotypes of *Nigella sativa* L. were presoaked in distilled water for 12h and allowed to germinate ($18 \pm 1^\circ\text{C}$) in plates covered with moist filter papers. Germinated seeds remained in plates on moist filter paper until the long tips became 1–2 cm (collected at 8 to 8:30 a.m.). Suitable tips were cut. Root tips of germinated seeds were treated in 0.1% colchicine solution for 110 min. The root tips were transferred to freshly prepared fixative (3:1 absolute ethyl alcohol and glacial acetic acid, respectively) at room temperature for 24 h. For preservation, the experimental material was preserved in 70% ethanol and placed in a refrigerator for further studies. Root tips were washed in distilled water in order to remove the different additional particles and then hydrolyzed in 1N hydrochloric acid at 60°C for 19 min and then washed in tap water. For staining, 1% aceto-orcein for more than 48 h at 4°C was used. The root tips were squashed in 45% acetic acid. Fully spread metaphase chromosomes in root tips were used for cytological studies. The standard karyotype was prepared for the ecotypes separately, and the parameters of the chromosomes including long length, short length, total length (TL), arm ratio, centromere index and the total form percentage (TF%) were calculated. Chromosome form was expressed according to the nomenclature of Levan *et al.* (1964).

Results and discussion

All six studied ecotypes were diploid ($2n=2x=12$). Fig. 1 shows the karyotypes and ideograms of the *Nigella sativa* L. which represent centromere location,

Table 1. Karyotypic profile of ecotypes.

Ecotype	Chromosome number	Karyotype formula
1	$2n=12$	$5m+1T$
2	$2n=12$	$4m+1sm+1st$
3	$2n=12$	$5m+1st$
4	$2n=12$	$5m+1T$
5	$2n=12$	$4m+1sm+1T$
6	$2n=12$	$3m+2st+1sm$

chromosome length and the number of satellites.

Ecotype 1 has a pair of satellites located at the end of short arms of chromosome number 3. Karyotypes in all ecotypes were nearly symmetrical and more than two pairs of chromosomes in all ecotypes was identified as metacentric (Table 1).

The total form percentage (TF%) of the ecotypes were observed as follows: G1 40.64, G2 41.04, G3 43.83, G4 41.36, G5 39.60, G6 38.94. The total length of the chromosomes was measured between $5.42 \mu\text{m}$ (G4) and $8.62 \mu\text{m}$ (G6). The largest total haploid length (genome size) was detected in G6 ($51.76 \mu\text{m}$) while G4 demonstrated the least ($32.56 \mu\text{m}$). Also, the minimum length of chromosome was observed in ecotype 4 with $2.79 \mu\text{m}$, and the maximum length of chromosome belonged to ecotype 6 with $11.03 \mu\text{m}$. The average of total chromosome length of the studied ecotypes was $7.38 \mu\text{m}$ (Table 2).

Principle Component Analysis (PCA) identified that the first component with 54.52% and the second component with 34.54% justified over 89% of the total variations of cytological characteristics. In the first component, the total length of chromosome and short arm have the most differences, and the second component showed variation in centromere index and arm ratio. These two components can be used for the study of ecotype distribution. Thus, both components were used

Table 2. Averages of measured chromosomal traits in studied ecotypes.

Ecotype	Total length (μm)	Long arm (μm)	Short arm (μm)	Total chromosome length (genome size) (μm)	Arm ratio	Centromere index
1	7.51	4.46	3.05	45.11989	1.84	0.33
2	6.32	3.72	2.59	37.93759	1.62	0.32
3	8.42	4.73	3.69	50.55425	1.51	0.38
4	5.42	3.18	2.24	32.56188	1.91	0.36
5	7.98	4.82	3.16	47.89196	1.88	0.32
6	8.62	5.26	3.36	51.76703	2.24	0.36
Average	7.38	4.36	3.01	45.11989	1.83	0.35

Fig. 2. Ecotype distribution biplot resulting from the first two components of PCA.

for charting the distribution of ecotypes (Fig. 2). Cluster analysis with UPGMA method was used for all chromosomal traits for categorizing the studied ecotypes. Cluster analysis resulted in a dendrogram which divided the ecotypes into five groups (Fig. 3). The first group includes ecotypes 1 and 5, the second group includes ecotype 6, the third group contains ecotype 2, ecotype 3 is categorized into group 4, and ecotype 4 was located in the fifth group.

The results of the present study were similar to other reports that showed *Nigella sativa* L. contains 12 ($2n=2x=12$) chromosomes (Gregory 1941, Bhattacharyaya 1958, Datta and Biswas 1983, Saha and Datta 2002, Datta *et al.* 2012). Jabeen *et al.* (2012) studied the karyotype of three cultivars of *Nigella sativa* L. by using the Feulgen stain method to determine the chromosomal position. They reported that the diploid chromosomes were 12 in number, characterized into six pairs of chromosomes and all cultivars had similar chromosomal formula ($2n=12=10m+2T$). Also, the total lengths of chromosomes among all studied cultivars were reported between $4.50\mu\text{m}$ to $10.84\mu\text{m}$. They explained that the observed variation in centromere position and chromosomal length among these cultivars may be attributed to genetic recombination. Also, Ghosh and Datta (2006) reported metacentric chromosomes are prevalent in the studied karyotype of *Nigella sativa* L. Findings of Ghosh and Datta (2006) also confirm the number of chromosomes ($2n=12$) in *N. sativa*. The present investigation showed that all of the investigated ecotypes had 12 chro-

Fig. 3. UPGMA cluster dendrogram based on all studied chromosomal traits.

mosomes. The results of this study also identified significant cytogenetic diversity between the studied ecotypes of *Nigella sativa* L. in Iran which could be used to develop breeding programs for this plant.

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