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A COMPARATIVE CHROMOSOMAL COUNT AND MORPHOLOGICAL KARYOTYPING OF THREE INDIGENOUS CULTIVARS OF KALONGI (*NIGELLA SATIVA* L.)

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Abstract

In the present study karyotypic analysis of three cultivars of *Nigella sativa* L., has been carried out to determine chromosomal position by using "Feulgen stain". The diploid chromosomes were twelve in numbers, characterized in six pair of chromosomes. All the cultivars had similar chromosomal formula 2n = 12 = 10 m + 2T. The total length of chromosomes among the land races of Kohat, (10.84µm to 4.50 µm), Faisalabad (10.00µm to 4.50µm) and Kashmir (4.66 µm to 8.16 µm) were calculated. Based on the centromeric position, there is a slight variation in the total length of the chromosome complements of Kohat and Faisalabad, whereas cultivars of Kashmir was found with much smaller chromosomal length. The variation in centromeric position and chromosomal length among these cultivars may be attributed to genetic recombinations.

Introduction

Medicinal plants are being used by humans since civilization. *Nigella sativa* L. (Kalonji) a member of Ranunculaceae family, also known as the black cumin seeds or small fennel have tremendous potential for cultivation, propagation and production in Pakistan (Iqbal *et al.,* 2010; Rabbani *et al.*, 2011). It is an annual aromatic plant and its cultivation traced back more than 3000 to the kingdom of the Assyrians and ancient Egyptians (Khan, 2009). The seeds have the anthelmintic, insecticidal, antimalarial, antibacterial, antifungal and antitumor activates. The seeds are also reported to have antispasmodia, diuretic, carminative, digestive and antiseptic properties (Burits & Burcar, 2000; Morsi, 2000; Teicher, 2002; Ali & Blunden, 2003; Marsik *et al*., 2005; Saleh, 2006; Abdulelah & Abidin 2007; Ahmad & Ghafoor, 2007; Ali *et al*., 2008).

Major constituents of seeds include 20.85% protein, 38.20% fats, 4.64%, moisture 4.37%, ash 7.94%, crude fiber 31.94% carbohydrate. Potassium, phosphorous, sodium and iron were also predominant elements, it also contains cholesterol, campestrol, stigmastrol, betasitosterol (Riaz *et al.,* 1996; Salma *et al.,* 2006). Being the important source of health food, nigella oil produced a large amount of meal which is reported as very important protein source. The *Nigella* meal is a cheaper source of food as compared to other traditional meals like cotton, sun flower and soybean meal (Chaudhry & Tariq, 2008). However, the *nigella* meal proved to be a good additive in the poultry and fish feed (Homidan *et al.,* 2002; Denli *et al.,* 2004; Hernandez *et al.,* 2004; Nair *et al.,* 2005; Hosseini *et al*., 2012).

The karyotype concept originated by the Russian school of cytogenetics. Karyotype provides basic information about morphology of an individual chromosome, number, chromosomal homology and ploidy level in plants. Karyotypic studies of various crops such as *Allium cepa*, *Lens culinaris*, *Helianthus annus* L., barely have been done by previous workers (Lee *et al*., 2003; Yuzbasioglu & Unal, 2004; Muhtasib *et al.,* 2004; Hussain, 2005; Fukui & Kaakeda, 2005; Fregonezi *et al.,* 2006 and Chengqi, 2008).

The objective of the present study was to prepare a standard karyotype and develop a chromosomal formula to determine phylogenetic relationship among local cultivars of the *Nigella sativa* L.

Material and Methods

Karyological study: The seeds of three commercial cultivars of *Nigella Sativa* L., were collected from the Kohat, Faisalabad and Azad Kashmir which were germinated at 25°C by employing blotting paper method.

Method of fixation of material of karyotype study: For fixation of material for karyotypic study following procedure was adopted:

Collection of root tips: Seeds were germinated in petridishes on moist filter paper about 1-2cm long tips were excised.

Pre treatment of root tips: Root tips of germinated seeds were treated with 0.05% colchicine solution for 1h.

Fixation: The root tips were transferred to freshly prepared fixative 3 part absolute alcohol and one part glacial acetic acid (3:1) in specified vials for fixation.

Preservation: Experimental material was preserved in 70% ethanol and placed in cool place for further studies.

Chromosomal preparation: It includes following steps.

1. Hydrolysis: Root tips washed in distilled water with the help of fine brush in order to remove the different reagent particles and then hydrolyzed in 1N HCl for about 8 minutes.

2. Staining: Before staining, root tips are again washed in the distilled water and then placed in Feulgen reagent for about 1h.

3. Slide preparation: For slide preparation 1 to 2 mm pieces from the stained root tips were excised and placed on slide. It was added with one drop of acetoorcein and tapped with flat headed needle so as to achieve the cell suspension. The cover slip was placed on it and heated on spirit lamp repeatedly to avoid boiling of the cell suspension. After heating, the slides were placed on a leveled surface of table and pressed firmly to get a good spread of chromosomes. The slides were then sealed and observed under microscope under oil immersion lens (Anamthawat, 2003).

4. Chromosome analysis: The diploid chromosome number of each cultivar was determined by examining photographs of 10 well spread metaphases. Metaphase chromosomes of a species arranged in a decreasing order of size and keeping in view the centromeric placement that constitutes a karyotype. During preparation of karyotype, the homologous chromosomes were tentatively paired on the basis of their morphology and size. In the karyotype, each chromosome carries a specific number written below it, in small letters. The numbers ranges from 1 to 12 and number 1 stands for the largest chromosome and the number 12 stands for the smallest one. The ideograms were constructed following the procedure by Borsa (1990). Each chromosome was reconstructed from the mean relative length and mean centromeric index values. Ideogram was constructed representing all the 6 pairs, arranged in a serial manner, and according to their morphological classes.

Measurement of chromosome: In order to prepare the karyotype and ideogram 10 best photographs of metaphase spreads of each variety with a magnification of 100x were selected for each *Nigella sativa* variety each chromosome was cut from the photograph and measured in millimeters (mm), and can be divided by magnification factor to get original length of the chromosome in micrometer (μm). The mean value of 10 metaphase was recorded to proceed further (Anamthawat, 2003).

Centromeric index: Centromeric index was calculated by dividing the length of the shorter of the two chromosome arm by the length of the whole chromosome and expressing it as percentage.

Mean centromeric index: The centromeric index values of the homologous chromosome were found different, mean of the centromeric index values of both of homologous chromosomes was calculated to represent the centromeric index value of the particular chromosome pair.

Arm ratio: The arm ratio was calculated by dividing the length of the larger arm of the chromosome by the length of its shorter arm.

Relative length: It is considered as the length of a particular chromosome divided by the total length of the chromosome in the haploid set, including the one being measured, multiplied by 100 and expressed as a percentage. The relative length of particular chromosome can be used as a rough estimate of the proportion of the genome that a given chromosome represents.

Mean relative length: Apparently wherever the homologous chromosome differ in size it may be an artifact due to the bending of the arm of one of the 2 homologous chromosomes. Mean of relative length of both the chromosome of the homologous pair was calculated to represent the relative length of a particular chromosome pair.

Chromosome classification: Individual chromosomes were classified with the chromosome pair by their centromeric index values. . However, irrespective of the criterion used, the classified type of a particular chromosome or homologous pair comes out to be the same. According to this procedure the terms median and terminal for the centromere position are used in two as exact point and regions. For the exact point *i.e.* if the centromere is located exactly in the middle or at the terminal end of the chromosome, it is designated with capital letters M & T respectively. Whereas, if the centromere is situated in the median end terminal regions, it is represented by small letters i.e., M & T, respectively. Sub median and sub terminal position of the centromere always denote region, and are designated by small letters i.e., Sm and St, respectively. The system of classification of individual chromosomes based on their arm ratio with reference to centromeric position, as given above, has been summarized as under (Table 1).

Results and Discussion

The basic chromosomes number of X = 6 have been found in all cultivars of *Nigella sativa* L. The diploid chromosome complement was 2n =12. In some cultivars, X = 5 is also reported (Datta & Biswas 1984). The classification of chromosomes on the basis of centermeric index in Kohat cultivar, out of 12 chromosomes 10 were found to be metacentric and 2 were found to be teleocentric, 10 metacentric chromosomes have centromere at median region (Table 2, Fig 1 & 2). The mean relative lengths (MRLs) of the chromosomes of this variety ranged from 19.82 μm to 8.92 μm from largest to the shortest chromosomes pairs (Tables 2 & 3). The maximum difference in mean relative length between the chromosome pair 1 and 2 i.e., 0.74% followed by the difference between 2 and 3. The remaining consecutive chromosome pairs are very small, chromosomes pair 1 and 2 are larger than all other chromosomes pairs and 6th one is the smallest. The group of metacentric chromosomes as a whole consists of relatively larger chromosomes as compared to other larger chromosomes of this variety. The chromosome pair 6 is the only telocentric pair with centromere at the terminal point. While in Kashmir and Faisalabad cultivars of *Nigella sativa*, out of total 12 chromosomes 10 was found to be metacentric and 2 were telocentric (Tables 4 & 6).

Table 1. System of classification of individual chromosomes based on their arm ratio and centromeric position.

Diagram	Arm Ratio	Centromeric Position	Chromosome Terminology
0	1.0	Median point	M (metacentric)
0.	1.0 – 1.7	Median point	M (metacentric)
.0	1.7 – 3.0	Submedian region	Sm (submetacentric)
.0	3.0 – 7.0	Subterminal range	St (subacrocentric)
0	7.0 – ∞	Terminal region	t (acrocentric)
.0	∞	Terminal point	T (telocentric)

Table 2. Measurements and classification of the chromosome (Kohat cultivar).

Chromosome number	Long arm (L) µm	Short arm (S) µm	Total length (L+S)=T	Arm ratio L/S	Relative length T/Hx100	Centromeric Index S/Tx100	Chromosome Morphology
1	5.50 ± 0.370	4.50 ± 0.400	10.00 ± 0.447	1.22	19.82	45.00	(metacentric)
2	5.50 ± 0.415	4.50 ± 0.372	10.00 ± 0.479	1.22	19.82	45.00	m
3	5.30 ± 0.379	5.00 ± 0.371	10.30 ± 0.401	1.06	20.42	48.54	m
4	5.30 ± 0.371	4.50 ± 0.370	9.80 ± 0.391	1.17	20.42	48.54	m
5	4.8 ± 0.370	4.5 ± 0.370	9.3 ± 0.390	1.06	19.43	48.23	m
6	4.50 ± 0.370	4.30 ± 0.370	8.83 ± 0.391	1.03	18.50	49.03	m
7	4.5 ± 0.376	4.3 ± 0.375	8.8 ± 0.421	1.04	17.50	48.86	m
8	4.50 ± 0.373	4.3 ± 0.372	8.8 ± 0.407	1.04	17.44	48.86	m
9	4.16 ± 0.297	3.88 ± 0.493	7.96 ± 0.493	1.09	15.86	47.05	m
10	4.16 ± 0.403	3.8 ± 0.395	7.96 ± 0.523	1.09	15.86	47.05	m
11	4.5 ± 0.325	0	4.50 ± 0.507	0	8.92	0	T
12	4.5 ± 0.290	0	4.50 ± 0.507	0	8.92	0	T

Table 3. Data on homologous chromosomes pairs of Kohat cultivars.

Chromosome pairs	Mean relative length (MRL)	Mean centromeric index (MCI)	MCI x MRL/ 100 = X	MRL - X	Chromosome morphology
1	19.82	45.00	8.81	10.91	(metacentric)
2	19.92	48.54	9.66	10.26	m
3	18.00	48.63	8.75	9.25	m
4	17.44	48.86	8.52	8.92	m
5	15.86	47.05	7.46	8.04	m
6	8.92	0	0	8.92	T (Telocentric)

Table 4. Measurements and classification of the chromosome (Kashmir cultivar).

Chromosome number	Long arm (L) µm	Short arm (S) µm	Total length (L+S)=T	Arm ratio L/S	Relative length T/Hx100	Centromeric Index S/Tx100	Chromosome Morphology
1	4.16 ± 0.379	4.00 ± 0.395	8.16 ± 0.471	1.04	20.37	49.01	(metacentric)
2	4.16 ± 0.398	4.00 ± 0.373	8.16 ± 0.451	1.04	20.37	49.01	m
3	4.00 ± 0.378	3.50 ± 0.408	7.50 ± 0.491	1.14	18.72	46.66	m
4	4.00 ± 0.377	3.50 ± 0.378	7.50 ± 0.420	1.14	18.72	46.66	m
5	4.16 ± 0.393	2.60 ± 0.397	6.76 ± 0.509	1.56	16.87	38.46	m
6	4.16 ± 0.408	2.60 ± 0.373	6.76 ± 0.471	1.56	16.87	38.46	m
7	4.16 ±0.373	2.50 ± 0.390	6.66 ± 0.442	1.66	16.63	37.53	m
8	4.16 ± 0.370	2.50 ± 0.372	6.66 ± 0.396	1.66	16.63	37.17	m
9	4.16 ± 0.391	2.16 ± 0.372	6.32 ± 0.440	1.92	15.78	34.17	m
10	4.16 ± 0.373	2.16 ± 0.373	6.32 ± 0.409	1.92	15.78	34.17	m
11	4.66 ± 0.374	0 ± 0.375	4.66 ± 0.417	0	11.63	0	T
12	4.66 ± 0.377	0	4.66	0	0	0	T

Fig. 1. Metaphase chromosomal spread in root tip cell of *Nigella sativa* (Kohat cultivar 2500X).

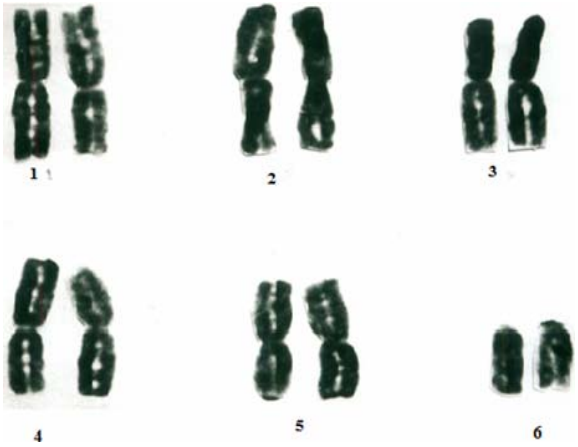

Fig. 2. Chromosomal pairs of cultivar (Kohat) arranged in serial manner.

Table 5. Data on homologous chromosomes pairs of Kashmir cultivars.

Chromosome pairs	Mean relative length (MRL)	Mean centromeric index (MCI)	MCI x MRL/100 = X	MRL - X	Chromosome Morphology
1	20.37	49.01	9.98	10.39	(metacentric)
2	18.72	46.66	8.73	9.99	m
3	16.87	38.46	6.48	10.39	m
4	16.63	37.53	6.24	10.39	m
5	15.78	34.17	5.39	10.39	m
6	11.63	0	0	11.63	T

Table 6. Measurements and classification of the chromosome (Faisalabad cultivar).

Chromosome number	Long arm (L) µm	Short arm (S) µm	Total length (L+S)=T	Arm Ratio L/S	Relative length T/Hx100	Centromeric Index S/Tx100	Chromosome morphology
1	5.17 ± 0.370	4.83 ± 0.377	10.00 ± 0.405	1.07	20.25	48.3	(metacentric)
2	5.17 ± 0.371	4.83 ± 0.371	10.00 ± 0.395	1.07	20.25	48.3	m
3	5.33 ± 0.372	4.16 ± 0.372	9.49 ± 0.400	1.28	19.21	43.83	m
4	5.33 ± 0.373	4.16 ± 0.396	9.49 ± 0.453	1.28	19.21	43.83	m
5	4.33 ± 0.290	4.17 ± 0.397	8.5 ± 0.507	1.03	17.20	49.05	m
6	4.33 ± 0.391	4.17 ± 0.384	8.50 ± 0.477	1.03	17.20	49.05	m
7	4.66 ± 0.433	3.84 ± 0.419	8.50 ± 0.616	1.20	17.20	45.17	m
8	4.66 ± 0.376	3.84 ± 0.370	8.50 ± 0.417	1.20	17.20	45.17	m
9	4.5 ± 0.374	4.00 ± 0.404	8.50 ± 0.470	1.17	17.20	45.97	m
10	4.5 ± 0.371	0 ± 0.305	4.50 ± 0.445	1.12	16.86	47.05	m
11	4.50 ± 0.371	0	4.50 ± 0.445	0	9.11	0	T
12	4.50 ± 0.285	0	4.50 ± 0.467	0	9.11	0	T (telocentric)

The mean relative length (MRLs) of the chromosomes of these cultivars ranged from 20.37 to 11.63 in Kashmir and 20.25 to 9.11 Faisalabad from the largest to shortest chromosome pairs (Tables 5 & 7). The maximum difference in mean relative lengths between chromosomes pair 1 and 2 found to be 0.74%, followed by the difference between 2 and 3. The difference between the remaining consecutive chromosomes pairs are very small. The chromosomes pair 1 and 2 are larger and the chromosome pair 6 was smaller in size, remaining chromosomes pairs gradually decrease in size (Figs. 3 & 4). The group of metacentric chromosomes as a whole consists of relatively larger chromosomes as compared to other group of chromosomes of their variety. Pair 6 of the chromosome is the only teleocentric one (Figs. 5 & 6). Difference in chromosomes size also studied in different plant species as in lential, wheat, *Helianthus annus* (Ahmad *et al.,* 1992; Hussain 2005; Fregonezi *et al.,* 2006; Chengqi, 2008; Fernandes *et al.,* 2009).

Table 7. Data on homologous chromosomes pairs of Faisalabad cultivars.

Chromosome pairs	Mean relative length (MRL)	Mean centromeric index (MCI)	MCI x MRL/100 = X	MRL - X	Chromosome Morphology
1	20.25	48.30	9.78	10.47	(metacentric)
2	19.21	43.83	8.41	10.80	m
3	17.20	49.05	8.35	8.68	m
4	17.20	45.17	7.76	9.44	m
5	17.03	45.97	7.75	9.28	m
6	9.11	0	0	9.11	T

Fig. 3. Metaphase chromosomal spread in root tip cell of *Nigella sativa* (Kashmir cultivar 2500X).

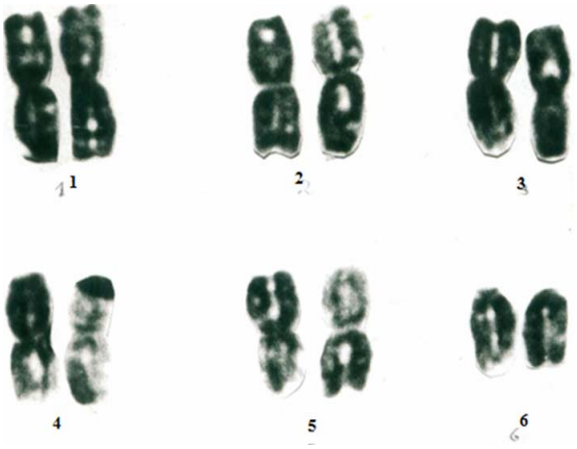

Fig. 4. Chromosomal pairs of cultivar (Kashmir arranged in serial manner.

Fig. 5. Metaphase chromosomal spread in root tip cell of *Nigella sativa* (Faisalabad cultivar 2500X).

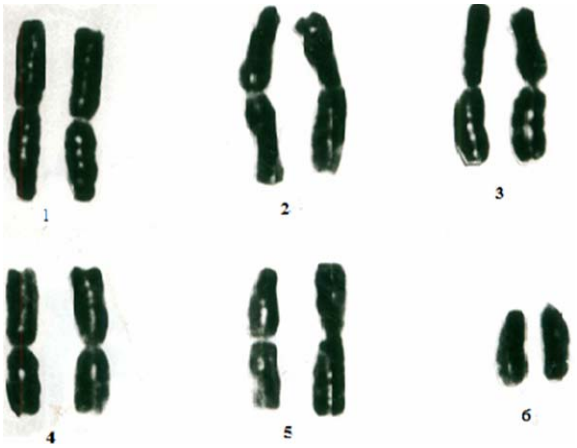

Fig. 6. Chromosomes pairs of cultivar (Faisalabad) arranged in serial manner.

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