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Karyotypic analysis in three species of *Allium* and their some Varieties

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Abstract

Karyotypic studies were made in 3 three species of Allium genus (Allium cepa L., Allium sativum L. and Allium tuberosum Rottl). All the three varieties of Allium cepa as Nasik, White and Kashmiri and two varieties Allium sativum as Deshi and Kashmiri were diploid with (2n=16) and Allium tuberosum was tetraploid with (2n=32). The chromosome types were detected as mostly metacentrics (M), submetacentrics (Sm) and few subtelocentrics (St). Karyotype formula is 8M+6Sm+2St in all the varieties of Allium cepa and Allium sativum. The sixth pair of subtelocentric chromosomes was nucleolar represented by prominent satellites in Allium sativum. Karyotype formula is 22M+6Sm+4St in Allium tuberosum. All the species have symmetrical karyotype.

Keywords: Karyotype, *Allium* genus.

Introduction

The genus *Allium* is one of the largest in the world flora. About seven hundred *Allium* species in the world[1]. Chromosomes must be regarded as a significant component of the eukaryote genetic system. Chromosome numbers are known for only about one-third of them and detailed cytological data are very limited. Cytological characters, included chromosome number and karyotype analysis have been considered as reliable guides in studies of texonomic and evolutionary reletionships[2]. The chromosome number, size and shape were used to karyotype of plant and define the taxonomic differences between them[3].

The genus *Allium* is karyological interesting in many respects, plants characterized by large genomes bur medium size of chromosomes and polyploidy. Present study deals with the karyomorphology of *Allium* species and their varieties. The main object of the present investigation was to understand chromosomal morphology and evolutionary trend among the species.

Material and Methods

Collection of Plant species: *Allium* species were collected from different localities of Ujjain and Kashmiri varieties were collected form Melahura Kashmir.

Study of Mitosis: Bulbs were sown in the pots. After five days bulbs were geminated. For the chromosomal study, root tips, about 0.5 to 1.0 cm length were collected directly from pots and pretreated with 0.02% colchicine solution for about 3 hours. Washed root tips were fixed in freshly prepared Conroy's fluid (absolute alcohol: acetic acid in 3:1 ratio) for at least 24 hours. Fixed root tips were taken out and were washed and hydrolyzed in 1N HCl at 60^0C for 10 to 15 minutes. After hydrolysis, root tips were washed and transferred to Leucobasic fuchsine (fulgent stain) for 1 hour in dark. After 1 hour, stained root tips were taken out and were washed with water. After washing the tips were squashed in 1% acetocarmine. Slides with well spread cells and clear chromosomes were selected for Photomicrography. The photomicrographs were taken under oil immersion and magnified to X1000.

Preparation of Karyotype: Karyotype analysis was based on at least five high-quality metaphase cell plates. Photo-ideograms were prepared from photomicrographs by cutting out individual chromosomes, arranging them in descending order of their length and matching on the basis of their position of centromere. Following parameters were used for the somatic karyotype analyses. i. length of long arm (LA), short arm (SA) and the whole chromosome (TL), ii. total length of long arms (TLLA), total length of short arms (TLSA) and total length of the whole chromosomes (TLWC), iii. relative chromosomal length (RCL), iv. arm ratio (AR), v. centromeric index (CI), vi. chromosome type (CT), vii. group (G), viii. Karyotypic formula (KF) etc. Kutarekar and Wanjari[4] classification was used for determining various categories of chromosomes.

On the basis of the position of centromere, the chromosomes were classified as metacentric (M), submetacentric (Sm) and subtelocentric (St) having an arm-ratio (short/long) above 0.76, 0.75 to 0.51 and less than 0.50 respectively. Based on their length, the chromosomes were grouped into Long (L) (> 4.5µm), Medium (M) (3.0-4.49µM), Relatively short (S_1)

(2.25-2.99µm) and Short (S_2) (<2.24µm).

Results and Discussion

Cytological Studies: In the present investigation, cytological studies include mitotic and karyotype. Chromosome numbers were studied in mitotic division (table-1). Various types of data were recorded during karyotypic study such as length of chromosome, arm ratio, centromeric index, chromosome type, karyotypic formula etc.

Allium cepa: (2n=16)

Nasik variety: The somatic complement has four pairs with median centromeres, three pairs with submedian centromeres and a pair with subterminal centromeres. The total length of long arms is 14.62 µm, ranging from 1.5 to 2.25µm. Total length of short arms is 10.87 µm, ranging from 0.75µm to 1.87µm. Total length of chromosome ranges from 2.5 to 3.75µm and the total length of the haploid complement is 25.5 µm. The range of relative chromosomal length, arm ratio and centromeric index are 98.03-147.05µm, 0.33-1.0µm and 0.25-0.50µm respectively. The karyotypic formula is 8M+6Sm+2St. (table-1, figures-1,7)

White variety: The karyotype of this variety consists of four pairs of median metacentric, three pairs median submetacentric and a pair median subtelocentric chromosomes. The total length of long arms is 17.6 µm, ranging from 1.8 to 2.60µm. Total length of short arms is 13.2µm, ranging from 1.0 to 2.20µm. The length of chromosomes ranges from 3.20 to 4.40µm and the absolute length of the haploid chromosome compliment is 30.8µm. The range of relative chromosomal length, arm ratio and centromeric index are 103.89-142.85µm, 0.38-1.0µm and 0.27-0.50µm respectively. The karyotypic formula is 8M+6Sm+2St (table-2, figure-2,8).

Kashmiri variety: The somatic complement includes four pairs of metacentric chromosomes, three pairs of submetacentric chromosomes and a pair subtelocentric chromosomes. The total length of long arms is 22.0µm, ranging from 2.0 to 3.25µm. Total length of short arms is 16.25µm, ranging from 1.0 to 2.75µm. The chromosome length ranges from 3.75 to 5.75µm and the absolute length of haploid complement is 38.25µm. The range of relative chromosomal length, arm ratio and centromeric index were 98.03-150.32µm, 0.30-1.0µm and 0.23-0.50µm respectively. The karyotypic formula was 8M+6Sm+2St. (table-3, figures-3,9).

***Allium sativum* (2n=16)*:* Deshi variety:** In this species, there are four pairs of metacentric, three pairs of submetacentric and one pair subtelocentric chromosomes. The sixth pair of subtelocentric chromosomes was nucleolar represented by prominent satellites. The total length of long arms is 20.25µm, ranging from 1.875 to 3.25µm. Total length of short arms is 14.375 µm, ranging from 1.0 to 2.625µm. The chromosome range from 3.25 to 5.255µm with the total length of the haploid complement being 34.625µm. Range of relative chromosomal length, arm ratio and centromeric index were 93.86-151.62µm, 0.33-1.0µm and 0.25-0.50µm respectively. The karyotypic formula was 8M+6Sm+2St (table-4, figures-4,10).

Kashmiri variety: In this variety, the somatic complement consist of four pairs of metacentric, three pairs of submetacentric and a pair subtelocentric chromosomes. The total length of long arms is 21.0µm, ranging from 2.25 to 3.25 µm. and the total length of short arms is 15.25µm, ranging of from 1.5 to 2.50 µm. The length of chromosomes in the complement ranges from 3.75 to 5.255µm. The absolute length of the haploid complement is 36.25µm. The range of relative chromosomal length, arm ratio and centromeric index were 103.44-144.82µm, 0.30-0.90µm and 0.23-0.47µm respectively. The karyotypic formula was 8M+6Sm+2St. The sixth pair of subtelocentric chromosomes was nucleolar represented by prominent satellites (table-5, figures-5,11).

***Allium tuberosum*: (2n=32):** Karyotype in this species consists of eleven pairs of metacentric, three pairs of submetacentric and two pairs of subtelocentric chromosomes. The total length of long arms is 42.375µm, ranging from 1.75 to 3.5µm. Total length of short arms is 31.875µm, ranging from 0.75 to 2.75µm. The length of chromosome ranges from 3.25 to 6.25 µm and the length of the haploid complement being 74.25µm. The range of relative chromosomal length, arm ratio and centromeric index were 43.77-84.17µm, 0.25-1.0µm and 0.20-0.50µm respectively. The karyotypic formula was 22M+6Sm+4St (table-6, figures-6,12).

Discussions: Karyotype analysis has been extensively carried out in plant phylogenetic and diversity studies for more than hundred years. In this era of modern molecular techniques, cytology is still a valuable tool for taxonomy, phylogeny and diversity studies. The information like chromosome number, size and morphology has been of considerable value in understanding interrelations and delimitation of taxa[5.] Cytotaxonomic, chromosome architecture and its behavior are blue prints to adopt proper strategy for the genetic improvement of the plant species.

Several workers have done cytogenetic studies particularly chromosome number, and morphology at mitotic division and, chromosomal association and behavior during meiotic division in the members of the family Liliaceae as in *A. cepa*[6-8], *A. sativum*[9,10]. *tuberosum*[11-13].

In the present investigation, mitotic study was done in three species of *Allium* included their varieties. The chromosomes

numbers in all the varieties of *A. cepa* and *A. sativum* was observed 2n=16 and 2n=32 chromosomes in *A. tuberosum*. The difference in chromosomes complements among the species is characterized by the differences in relative length of haploid complement of chromosomes, arm ratio, centromeric index and chromosome type etc. Karyotypic formula of *A. cepa* was found to be 8M+6Sm+2St in all the varieties. Karyotype of *Allium sativum* was same to *A. cepa* 8M+6Sm+2St but sixth pair of subtelocentric chromosomes is nucleolar, represented by prominent satellites. In the *A. tuberosum* karyotypic formula was 22M+6Sm+4St. Same results were found by Mukherjee A[7] in the above mentioned three species of *Allium*. Polyploidy has been a major factor in the evolution of plants, with the result 70% angiosperms are polyploidy[14]. *A. tuberosum* is natural tetraploid in present investigation. It was observed that tetraploid species showed decreased chromosome size as compared to their diploid counterparts. Such decrease in chromosome size with the increase of the ploidy level has been reported in several genera by the previous workers such as in *Crinum*[15] and *Allium*[7,16]. The polyploids maintain nucleoplasm balance for the adaptation in natural environment[17].

Table-1
Morphology of somatic chromosomes in *Allium cepa* Nasik variety (2n=16)

Chromosome number	LA (µm)	SA (µm)	TL (µm)	RCL (µm)	AR (µm)	CI (µm)	CT	G
I	1.87	1.87	3.75	147.05	1.00	0.50	M	M
II	2.00	1.75	3.75	147.54	0.87	0.46	M	M
III	2.00	1.50	3.50	137.25	0.75	0.42	Sm	M
IV	1.75	1.50	3.25	127.45	0.85	0.46	M	M
V	1.75	1.25	3.00	117.64	0.71	0.41	Sm	M
VI	2.25	0.75	3.00	117.64	0.33	0.25	St	M
VII	1.50	1.25	2.75	107.84	0.83	0.45	M	S^1
VIII	1.50	1.00	2.50	98.03	0.66	0.40	Sm	S^1
	14.62	10.87	25.5					

Table-2
Morphology of somatic chromosomes in *Allium cepa* White variety

Chromosome number	LA (µm)	SA (µm)	TL (µm)	RCL (µm)	AR (µm)	CI (µm)	CT	G
I	2.20	2.20	4.40	142.85	1.00	0.50	M	M
II	2.20	2.00	4.20	136.36	0.90	0.47	M	M
III	2.40	1.80	4.20	136.36	0.75	0.42	Sm	M
IV	2.00	1.80	3.80	123.37	0.90	0.47	M	M
V	2.40	1.40	3.80	123.37	0.58	0.36	Sm	M
VI	2.60	1.00	3.60	116.88	0.38	0.27	St	M
VII	1.80	1.80	3.60	116.88	1.00	0.50	M	M
VIII	2.00	1.20	3.20	103.89	0.60	0.37	Sm	M
	17.6	13.2	30.8					

Table-3
Morphology of somatic chromosomes in *Allium cepa* Kashmiri variety

Chromosome number	LA (µm)	SA (µm)	TL (µm)	RCL (µm)	AR (µm)	CI (µm)	CT	G
I	3.00	2.75	5.75	150.32	0.91	0.47	M	L
II	2.75	2.75	5.50	143.79	1.00	0.50	M	L
III	3.25	2.00	5.25	137.25	0.61	0.38	Sm	L
IV	2.50	2.50	5.00	130.71	1.00	0.50	M	L
V	3.00	1.75	4.75	124.18	0.58	0.36	Sm	L
VI	3.25	1.00	4.25	111.11	0.30	0.23	St	M
VII	2.00	2.00	4.00	104.57	1.00	0.50	M	M
VIII	2.25	1.50	3.75	98.03	0.66	0.40	Sm	M
	22.0	16.25	38.25					

Table-4
Morphology of somatic chromosomes in *Allium sativum* Deshi variety

Chromosome number	LA (µm)	SA (µm)	TL (µm)	RCL (µm)	AR (µm)	CI (µm)	CT	G
I	2.625	2.625	5.250	151.62	1.00	0.50	M	L
II	2.500	2.500	5.000	144.40	1.00	0.50	M	L
III	3.250	1.750	5.000	144.40	0.53	0.35	Sm	L
IV	2.500	2.000	4.500	129.96	0.80	0.44	M	L
V	2.500	1.750	4.250	122.74	0.70	0.41	Sm	M
VI	3.000	1.000	4.000	115.52	0.33	0.25	St^{Sat}	M
VII	1.875	1.500	3.375	97.47	0.80	0.44	M	M
VIII	2.000	1.250	3.250	93.86	0.62	0.38	Sm	M
	20.25	14.375	34.625					

Kang et al.[18] suggested that major karyotypic differences among species are caused by dramatic chromosomal rearrangements, such as translocation, deletion and inversion. On the other hand, the karyotype can also be changed through inter-specific hybridization. In the present study, variation in the number and morphology of chromosomes in the same species may be due to the mutations in the natural populations. In the present investigation also secondary constriction in 5^{th} pair of chromosome was observed. The role of structural alteration of chromosomes in the evolution of races is evidenced by detailed analysis of karyotype. The constancy of the karyotype within the population indicates certain adaptability to the micro environmental condition to which they are subjected[19].

A. sativum have nucleolar organizers exclusively in the form of secondary constrictions represented by satellites. Secondary constriction was present near the centromere of the short arm in the *Allium sativum.* It is present near the telomere of the long arm. Verma and Raina[20] suggested that shifting of nucleolar organizer in the chromosome arm could be brought by deletion, unequal translocation or inversion. Karyotype of all the species of *Allium* are symmetrical. In the symmetrical karyotype, all the

chromosomes are of approximately same size and have median or submedian centromere. Imai et al.[21] suggested that karyotype evolution generally tends towards an increasing number and terminal centromeric chromosomes are advance. The opposite tendency, the reduction of chromosome number and formation of median centromeric chromosomes (metacentric) are primitive.

Table-5
Morphology of somatic chromosomes in *Allium sativum* Kashmiri variety

Chromosome number	LA (µm)	SA (µm)	TL (µm)	RCL (µm)	AR (µm)	CI (µm)	CT	G
I	2.75	2.50	5.25	144.82	0.90	0.47	M	L
II	2.75	2.50	5.25	144.82	0.90	0.47	M	L
III	2.75	2.00	4.75	131.03	0.72	0.42	Sm	L
IV	2.50	2.25	4.75	131.03	0.90	0.47	M	L
V	2.50	1.75	4.25	117.24	0.70	0.41	Sm	M
VI	3.25	1.00	4.25	117.24	0.30	0.23	St^{Sat}	M
VII	2.25	1.75	4.00	110.34	0.77	0.43	M	M
VIII	2.25	1.50	3.75	103.44	0.66	0.40	Sm	M
	21.0	15.25	36.25					

Table-6
Morphology of somatic chromosomes in *Allium tuberosum* (2n=32)

Chromosome number	LA (µm)	SA (µm)	TL (µm)	RCL (µm)	AR (µm)	CI (µm)	CT	G
I	3.50	2.75	6.25	84.17	0.78	0.44	M	L
II	3.25	2.75	6.00	80.80	0.84	0.45	M	L
III	2.75	2.75	5.50	74.07	1.00	0.50	M	L
IV	3.00	2.25	5.25	70.70	0.75	0.42	Sm	L
V	2.75	2.50	5.25	70.70	0.90	0.47	M	L
VI	2.50	2.25	4.75	63.97	0.90	0.47	M	L
VII	2.50	2.25	4.75	63.97	0.90	0.47	M	L
VIII	2.50	2.00	4.50	60.60	0.80	0.44	M	L
IX	2.25	2.00	4.25	57.23	0.88	0.47	M	M
X	2.75	1.50	4.25	57.23	0.54	0.35	Sm	M
XI	2.125	2.125	4.25	57.23	1.00	0.50	M	M
XII	2.25	2.00	4.25	57.23	0.88	0.47	M	M
XIII	2.50	1.50	4.00	53.87	0.60	0.37	Sm	M
XIV	3.00	1.00	4.00	53.87	0.33	0.25	St	M
XV	3.00	0.75	3.75	50.50	0.25	0.20	St	M
XVI	1.75	1.50	3.25	43.77	0.85	0.46	M	M
	42.375	31.875	74.25					

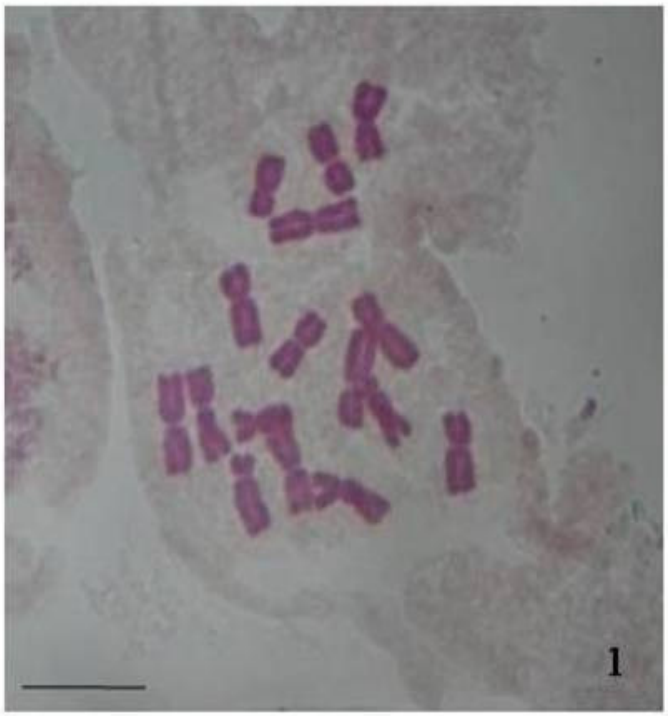

Figure-1
Mitosis of *Allium cepa.,* Nasik variety, 16 chromosomes at metaphase

Figure-3
Mitosis of *Allium cepa.,* Kashmiri variety, 16 chromosomes at metaphase

Figure-2
Mitosis of *Allium cepa.,* White variety, 16 chromosomes at metaphase

Figure-4
Mitosis of *Allium sativum*, Deshi variety, 16 chromosomes at metaphase

Figure-5
Mitosis of *Allium sativum*, Kashmiri variety, 16 chromosomes at metaphase

Figure-6
Mitosis of *Allium sativum*, Mitosis of *A. tuberosum* Rottl, 32 chromosomes at metaphase

Figure-7
Karyotype in *Allium cepa* L., 8M+6Sm+2St in Nasik variety

Figure-8
Karyotype in *Allium cepa* L., 8M+6Sm+2St in White variety

Figure-9
Karyotype in *Allium cepa* L., 8M+6Sm+2St in Kashmiri variety

Figure-10
Karyotype in *Allium sativum* L., 6M+6Sm+St in Deshi variety

Figure-11
Karyotype in *Allium sativum* L., 6M+6Sm+St in Kashmiri variety (6th pair represented predominate satellites in *Allium sativum* L.)

Figure-12
Karyotype in *Allium sativum* L., 22M+6Sm+4St in *Allium tuberosum* Rottl. (Scale bar=4µm)

Conclusion

Karyotypic study of present investigation will be helpful to understand the number, morphology of chromosomes which are useful in cyto-taxonomy and also beneficial for further research in cytogenetics.

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