

RESEARCH ARTICLE

Karyotype analysis in Asiatic *Lilium* cultivar Tresor from Maharashtra (India)

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Abstract

Lilium of family liliaceae make up of approximately 110–115 species and many cultivars. It is among the most significant genera in horticulture for the production of cut flowers and pot plants. Asiatic *Lilium* cultivar Tresor is autotetraploid. The observed number of chromosomes was $2n=4x=48$. The formula for the karyotype was $45m+36st+8t$. It was noticed that the karyotype was asymmetrical and belonged to the Stebbins 3A category. In India the karyomorphological investigations is conducted for the first time.

Keywords: *Lilium*, Asiatic group, Cultivar, Tresor, Karyotype.

Introduction

The *Lilium* family, which includes about 110–115 species, is found in the temperate and frigid parts of the Northern Hemisphere (McCrae, 1998) especially in East Asia, Europe, and North America (Woodcock and Stearn, 1950; Wang *et al.* 2015). The north and southwest of China are home to about 55 species of wild lilies and are regarded as the global centre of variety for wild lilies (De Jong, 1974). More than 10,000 cultivars of lilies have been created to date and for over 200 years, lilies have been selectively bred and cultivated (Peng, 2002). The genus *Lilium* has developed with significant phenotypic variety despite genetic similarities, which is of great evolutionary relevance (Patterson & Givnish, 2002). The history of lilies is vast as an ornamental plant. Asiatic lilies

were produced through hybridization and/or chromosome duplication in species of the section of Sinomartagon (Van Tuyl *et al.* 2011). Tresor lilies is the Asiatic lily which have yellow, orange, or sometimes a combination of the two colors in their flowers. Additionally, the petals have spots at the base and the buds are full. The majority of its variants are non-fragrant. The stems of flowers are straight, not curled. Lilies, particularly those found in Europe, North America, East Asia, and other temperate regions of the Northern Hemisphere, are well-known for their strong resistance and durability. The Tresor variety of lilies is especially noted for its remarkable resilience.

Cytological studies have been undertaken on *Lilium* species/ cultivars by several authors (Stewart 1974; Lighly 1960). But comparatively meagre work is available on *Lilium* in India. Hence the present work is undertaken with focus on karyomorphology in *Lilium* cultivar Tresor that shows large variations.

Materials and methods

Tresor bulbs, were collected for this study from the Council of Scientific and Industrial Research-Institute of Himalayan Bioresource Technology in Palampur, Himachal Pradesh, India. The plants were then grown in the Botanical Garden of the Department of Botany at Sant Gadge Baba Amravati University in Amravati. The tips of the roots were used to prepare the mitotic material. After pre-treating root tips

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for five hours with 0.5% colchicine, they were cleaned with distilled water and left overnight in Carnoy's solution (3:1 absolute ethanol: glacial acetic acid). After hydrolyzing the root tips in 1 N HCl for 5 minutes at 60°C, they were cleaned. After that root tips were crushed with, a drop of 1% aceto-orcein. Trinocular Fluorescence Microscope (Aziostar Plus, M/S Carl Zeiss, Germany) was used for the photography at Department of Botany, Sant Gadge baba Amravati University, Amravati.

The degree of karyotype asymmetry was assessed using Stebbins' categories (Stebbin, 1971). The coefficient of variation of chromosomal length (CVCL) was determined following the method described by (Peruzzi and Eróglu 2013) and chromosomal nomenclature adhered to the system established by Levan (Levan *et al.* 1964).

Results

The somatic chromosome count of *Lilium* cv. Tresor was found to be $2n=4x=48$ (Fig. 1) A somatic chromosome showed 12 pairs of chromosomes represented as an idiogram (Fig. 2). The length of chromosome varied from 11.25 to 23.5 μm . Long arm length ranged from 9.75 to 16.25 μm while short arm length varied from 1.5 to 8.5 μm . Chromosome pair I showed highest absolute length 23.5 μm with centromere at submedian position. Chromosome pair II measured 19 μm having longest long arm 16.25 μm with centromere at subterminal position. Chromosome pair III showed 17 μm absolute length with terminal centromeric position. The chromosome pairs IV, V, VI showed total length 16.25, 15.25, 15 μm respectively having centromere at

subterminal position. Chromosome pair VII had centromere at terminal end with length 14 μm . Chromosome pairs VIII, IX, X, XI, XII showed absolute length 13.75, 13, 13, 12.5, 11.25 μm respectively having subterminal centromeric position. Out of all pairs, 2 pairs had terminal region of centromere while only one pair showed submedian location on chromosome and pair IV, V, VI, VIII, IX, X, XI and XII found to be subterminal in position. Arm ratio (r) varied from 1.76 to 8.71. Similarly, centromeric index (i) showed lowest range 10.29 and highest 36.17. The relative chromosome length (RCL%) varied from 47.87 to 100. The TCL was recorded 183.5 μm (Table 1). The total form percent (TF%) was found to be 18.25 and karyotype asymmetry index (As K%) was 81.74. The centromeric gradient (CG) was 17.24, coefficient of variation (CV) was 21.90 and dispersion index (DI) was 3.77 noted. The karyotype formula suggested for *Lilium* cv. Tresor was $4sm+36st+8t$. The karyotype was asymmetric and 3A type (Table 2).



Figure 1: *Lilium* cultivar Tresor Mitotic metaphase showing $2n=4x=48$

Table 1: Measurements of somatic chromosomes of tetraploid *Lilium* cultivar Tresor ($2n=4x=48$) at Metaphase

Chromosome Pair	Long arm l (μm)	Short arm s (μm)	Total length c (μm)	d	Arm ratio (r)	Centromeric Index (i)	RCL%	Position of Centromere
I	15	8.5	23.5	6.5	1.76	36.17	100	sm
II	16.25	2.75	19	13.5	5.90	14.47	80.85	st
III	15.25	1.75	17	13.5	8.71	10.29	72.34	t
IV	13.5	2.75	16.25	10.75	4.90	16.92	70.49	st
V	13	2.25	15.25	10.75	5.77	14.75	66.16	st
VI	13	2	15	11	6.5	13.33	63.82	st
VII	12.25	1.75	14	10.5	7	12.5	59.57	t
VIII	11	2.75	13.75	8.25	4	20	58.51	st
IX	10	3	13	7	3.33	23.07	55.31	st
X	10.25	2.75	13	7.5	3.72	21.15	55.31	st
XI	10.75	1.75	12.5	9	6.14	14	53.19	st
XII	9.75	1.5	11.25	8.25	6.5	13.33	47.87	st

Table 2: Karyotype table of tetraploid *Lilium* cultivar Tresor ($2n=4x=48$)

Cultivar Name	Chromosome Number	Chromosomal size range (μm)	Total Chromosome length (μm)	Karyotype formula	Karyotype	CG	CV	DI
Tresor	$2n=4x=48$	11.25 – 23.5	183.5	$4sm+36st+8t$	3A	17.24	21.90	3.77

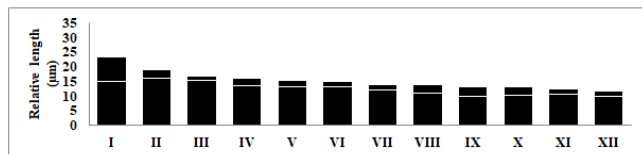


Figure 2: Idiogram of *Lilium* cultivar Tresor

Discussion

The base number reported for the genus *Lilium* is $X=12$ (Stebbins 1971; Son 1977; Yokolev *et al.* 2003; Tahami *et al.* 2014). However while investigating the cytology of *Lilium*, authors has thrown light on the pattern of cytological evaluation which indicate that the chromosomal evolution is through polyploidy associated with structural changes (Tuyl *et al.* 1989). Previous reports have indicated that the somatic chromosome number for various *Lilium* species, including *L. alexandrae*, *L. japonicum*, *L. auratum*, *L. callosum*, *L. maximowiczii*, *L. concolor*, *L. dauricum*, *L. medeoloides*, *L. leichtlinii*, *L. rubellum*, *L. longiflorum*, *L. speciosum*, *L. nobilissimum*, *L. tigrinum* var. *flaviflorum*, *L. candidum*, *L. henryi*, and *L. pumilum*, is $2n=24$. (Asano, 1985; Stewart 1947; Beattie and White 1993, Marasek *et al.* 2004; Zhou *et al.* 2011).

However, the present investigation showed $2n=4x=48$ chromosomes in all the somatic cells analysed which indicate that the cultivar of Asiatic group i.e. Tresor is tetraploid and is in agreement with previous reports from China (Wang *et al.* 2015; Zhang *et al.* 2018; Kwon *et al.* 2017; Zhou *et al.* 2011).

Tresor showed submedian to telomeric centromeric position but as per the analysis of Tang *et al.* (2020) and Wang *et al.* (2015) the centromeric position of chromosome shifted from median to subterminal region. The arm ratio ranges from 1.76- 8.71 in Tresor which showed positive correlation with results i.e.4.08 reported by Tang *et al.* (2020) and Wang *et al.* (2015) along with other cultivars Renoir, Gironde, Navona, Detroit, Lotero and Freya in region of China. Tresor shows high asymmetric karyotype (81.74%) which showed difference i.e. 77.43% in the report of Tang *et al.* (2020) and Wang *et al.* (2015).

The *Lilium* chromosomes are large which increase the likelihood of chromosomal tangling and structural diversity, its karyotype is often stable (Wang *et al.* 2015; Zhang *et al.* 2018). The length of longest chromosomes in all species varies according to their ploidy level. The size of chromosomes varies slightly with respect to the position of the centromere and arm length. Such variation among conspecifics may arise because of difference in the repetitive DNA sequences, which eventually make up the genome size (Schmidt *et al.* 1998).

FISH has proven that the cultivars are closely related to each other because 5SrRNA gene unit organization clarifies the inter-specific relationships among the *Lilium* species (Sultana *et al.* 2010, Ren *et al.* 2012). Asiatic tetraploid lilies are autotetraploid in nature. This is also corroborated by

the simple and productive hybridizations that can occur within Sinomartagon or Asian cultivars (Van Tuyl *et al.* 2011, Zhou *et al.* 2015), suggesting that they could be seen of as a “biological” species complex. According to Stebbins (1971) *Lilium* shows asymmetric karyotype in which large size metacentric chromosomes were observed in one or two pairs. According to previous reports 3A and 3B class of karyotype was observed in *Lilium* (Stebbins 1971 and Gao *et al.* 2011).

All the karyomorphological characters such as chromosomal size, position of centromeres and satellite, centromeric index (i), difference between short arm and long arm (d), arm ratio, total chromosome length (TCL%), relative chromosome length, total form present (TF%), karyotype asymmetry index (AsK%), centromeric gradient (CG), coefficient of variation (CV), dispersion index (DI), Class of asymmetry and Karyotype formula on *Lilium* cultivar Tresor was reported first time in India.

The cultivar Tresor showed the evolution in the *Lilium* cultivars. Though some kind of work has been done but karyomorphology showed variation this may be due to difference in environmental condition.

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