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Karyotype analysis of *Lilium lancifolium* and four related cultivars

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Abstract. *Lilium lancifolium* is one of the most important species of genus *Lilium*. Besides ornamental value, it also has highly edible and medicinal properties. We investigated the karyotypes of *L. lancifolium* and four related cultivars. The results indicated that the ploidies of four cultivars varied from diploid to tetraploid. Both 'Flore Pleno' and 'Red Velvet' were triploid (2n=3x=36), consistenting with the wild species *L. lancifolium*. 'Sweet Surrender' was diploid (2n=2x=24), and 'Red Life' was tetraploid. All karyotypes of candidates belonged to 3B type except 'Flore Pleno', which belonged to 3A type. Karyotype symmetry analysis revealed that the wild species *L. lancifolium* had a middle value of A1, A2, and TF%, which meant that the cultivars related to *L. lancifolium* had different tendencies to symmetry compared to *L. lancifolium*, but whether they were higher or lower was unclear.

Keywords: *Lilium lancifolium*, cultivars, karyotype, chromosome.

INTRODUCTION

Genus *Lilium* includes approximately 100-115 wild species (Liang and Tamura, 2000). *Lilium lancifolium*, the tiger lily, one of the most important species of genus *Lilium*, is widely distributed in northern and eastern Asia, including Korea, Japan, China and Sakhalin (Feldmaier and McRae, 1982). Besides ornamental value, *L. lancifolium* is also extensively used as both food and a traditional Chinese medicine for many centuries in China due to its health-promoting properties and treatments of bronchitis, pneumonia, chronic gastritis (Chau and Wu, 2006; Luo *et al.*, 2012; Joung *et al.*, 2007; Kwon *et al.*, 2010; Gao *et al.*, 2015). In addition, *L. lancifolium* has been used as a modal plant to study the mechanism of bulbil formation in lilies (Yang *et al.*, 2017, 2018; He et al, 2020), because it is one of the four *Lilium* species which can bear bulbils in leaf axils (McRae, 1998; Liang and Tamura, 2000; Bach and Sochacki, 2012).

Karyomorphological investigations are very important for the views of taxonomical, ecological and cytological studies. Although the karyotype features are generally constant in a group of species and even a genus, the variations in structure and/or number can change the number, size, and position of the centromere on chromosomes, causing genetic variation (Ahn *et al*., 2017). In addition, chromosome number can also complement information of polyploidy and other highly significant genome changes which are invisible by morphological and molecular methods. For more than 40 years, karyotype analysis of many wild *Lilium* species including *L. lancifolium* (Gill *et al*., 1974; Vosa *et al*., 1976; Gao *et al*., 2011) and hybrids (Khan *et al*., 2009; Liu *et al*., 2011) has been carried out. That provides a lot of significant theoretical basis for cytological and taxonomic studies of *Lilium*. It is interesting that all *Lilium* species are diploids (2n=2x=24) except *L. lancifolium*, which consists of ploidy complex with diploids, narrowly distributed, and triploids (2n=3x=36), widely distributed in inlands of China, Korea, Japan and Russia, in nature (Noda 1978, 1986; Kim *et al*., 2006; Hwang *et al*., 2011). Because *L. lancifolium* distributed in China is natural triploid (2n=3x=36) (Noda, 1986), it is highly sterile in cross breeding, neither as male or female parent, which seriously stunts the upgrading of its varieties.

Recent years, several varieties related to *L. lancifolium* have emerged on market, but the karyotype analysis of *L. lancifolium* related cultivars have never been reported. Here, we collected four cultivars related to *L. lancifolium* and investigated the karyotypes of *L. lancifolium* and four related cultivars, providing cytological and genetic foundation for the breeding of *L. lancifolium*.

MATERIALS AND METHODS

Plant materials

L. lancifolium and four related cultivars ('Red Life', 'Sweet Surrender', 'Red Velvet', 'Flore Pleno') were used as materials in this study (Figure 1). *L. lancifolium* bulbs were harvested from our farm (Beijing, China: 116.58°E; 40.07°N) in October 2017. Bulbs of *L. lancifolium* related cultivars were purchased from Licai Garden Co., Ltd (Zhejiang, China). All bulbs were planted in the matrix of peat, vermiculite, perlite with volume ratio of 5:3:1, in a greenhouse at the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, China in March 2018. Root tips were obtained from each population for squashing.

Chromsome preparation and observation

Actively growing root tips of each populations for the length around 0.5 cm were taken between 9 am and 11 am in a clear day, and pretreated with 0.7% cycloheximide solution at 4°C for 10 h, then fixed in Carnoy's I Fluid (methanol: glacial acetic acid = 3:1) for 24 h at room temperature, and finally kept in 70% alcohal until use. The roots were hydrolyzed in 1 mol/L HCl at 60°C for 10 min, then the chopped root tips were stained in Carbol fuchsin stain for 5~6 min at room temperature. The roots must be rinsed in distilled water for 3 to 5 times before each step. The observations of the best metaphase plates were made using an Olympus CX31 (Olympus light microscope, Tokyo, Japan) equipped with a 100 × /1.25 oil objective and a mounted Canon 550D digital camera (Canon, Japan).

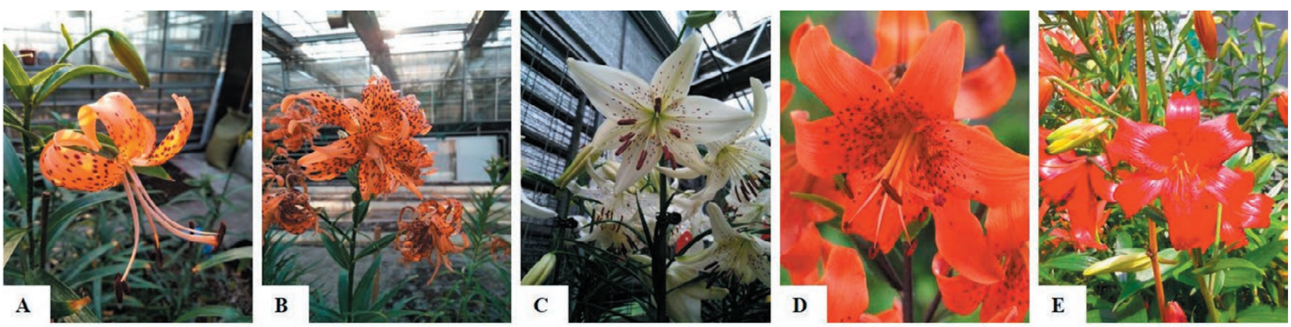

Figure 1. The five materials in this study. A: *L. lancifolium*, B-E: *L. lancifolium* cultivars: 'Flore Pleno', 'Sweet Surrender', 'Red Life', 'Red Velvet', respectively.

Karyological analyses

The measurement of basic parameters such as long arm (LA), short arm (SA) related to every chromosome was performed utilizing Photoshop V.7. Based on which we calculated total length of genome (TLG), AR (arm ratio), CI (centromeric index), LA% (long arm percentage), SA% (short arm percentage), TF% (total form percentage), VRC (value of relative chromatin), A1 (intrachromosome asymmetry index), A2 (interchromosome asymmetry index) and DI (dispersion index). The Karyotype formula was calculated according to the definition of metacentric (m), submetacentric (sm), telocentric (t), and sbtelocentric (st), proposed by Levan *et al.* (1964). The karyotype classification was defined by using the method of Stebbins (1971). Finally, the data analysis was conducted in excel.

RESULTS

This study revealed detailed pictures of mitotic chromosome plates, related karyotypes and karyograms in *L. lancifolium* and its four cultivars (Figure 2). The related parameters and karyotypic formula were summarized in Table 1 and Table 2.

The results showed that, *L. lancifolium* (Figure 2-A) is triploid, which had a chromosome number of 2n=3x=36; while in the four cultivars, only 'Flore Pleno' (Figure 2-B) and 'Red Velvet' (Figure 2-E) were triploid, with the same chromosome number as *L. lancifolium*, 2n=3x=36; 'Sweet Surrender' (Figure 2-C) was diploid, 2n=2x=24; and 'Red life' (Figure 2-D) was tetraploid, 2n=4x=48. In five materials, only *L. lancifolium* had satellites attached to the first two pairs of chromosomes, and 'Flore Pleno'had one group of chromosomes attached with satellites, the other three cultivars had no satellites. According to the karyograms in Figure 2, besides 'Red Velvet' had two groups of telocentric chromosome, *L. lancifolium*, 'Flore Pleno' and 'Sweet Surrender' had one set telocentric chromosome, and 'Red life' has no telocentric chromosome (Figure 2).

The length of shortest chromosome in *L. lancifolium* (Table 1-A) is 10.27 μm, which was obviously longer than its four cultivars'. The total genome length ranged from 117.44 to 191.05 μm between tetraploid 'Red Life' (Table 1-D) and wild species *L. lancifolium* in all populations. Even the karyotype formulas of five materials were complicated, at least four different chromosome-types were contained in each species, most of the five materials had the same karyotype 3B except 'Flore Pleno' (Table 1-B), whose belongs to 3A (Table 1). The

Table 1. Karyotype analysis of *L. lancifolium* and four related cultivars.

Sample	Chromosome number	Chromosome size range (μm)	Total genome length (μm)	Karyotype	Karyotypic formula
A	2n=3x=36	10.27-21.15	191.05	3B	3m(2SAT)+3sm(3SAT)+12st+15t+3T
B	2n=3x=36	7.57-14.80	127.37	3A	3sm(3SAT)+18st+12t+3T
C	2n=2x=24	8.68-19.83	155.29	3B	2m+4sm+4st+12t+2T
D	2n=4x=48	6.52-14.15	117.44	3B	4m+8sm+28st+8t
E	2n=3x=36	7.25-16.54	153.44	3B	3m+9sm+18st+6T

Notes: A: *L. lancifolium*, B-E: *L. lancifolium* cultivars: 'Flore Pleno', 'Sweet Surrender', 'Red Life', 'Red Velvet', respectively.

Table 2. Mean of parameters of chromosomes analysis of *L. lancifolium* and four related cultivars.

Species	TL	LA	SA	AR	CI	LA%	SA%	TF%	VRC	A1	A2	DI
A	15.64	12.98	2.66	4.88	0.17	6.79	1.39	16.70	15.92	0.79	0.20	2.47
B	10.45	8.92	1.53	5.83	0.15	7.00	1.20	14.45	10.61	0.81	0.21	3.01
C	12.94	10.49	2.45	4.28	0.19	6.67	1.58	18.95	12.94	0.77	0.26	3.09
D	9.78	7.58	2.20	3.45	0.22	6.45	1.87	22.50	9.79	0.71	0.22	3.44
E	12.79	10.06	2.73	3.68	0.21	6.56	1.78	21.35	12.79	0.70	0.19	3.43

Notes: A: *L. lancifolium*, B-E: *L. lancifolium* cultivars: 'Flore Pleno', 'Sweet Surrender', 'Red Life', 'Red Velvet', respectively. TL: total length of chromosome, LA: long arm, SA: short arm, AR: arm ratio, CI: centromeric index, LA%: long arm percentage, SA%: short arm percentage, TF%: total form percentage, VRC: value of relative chromatin, A1: intrachromosome asymmetry index, A2: interchromosome asymmetry index, DI: dispersion index.

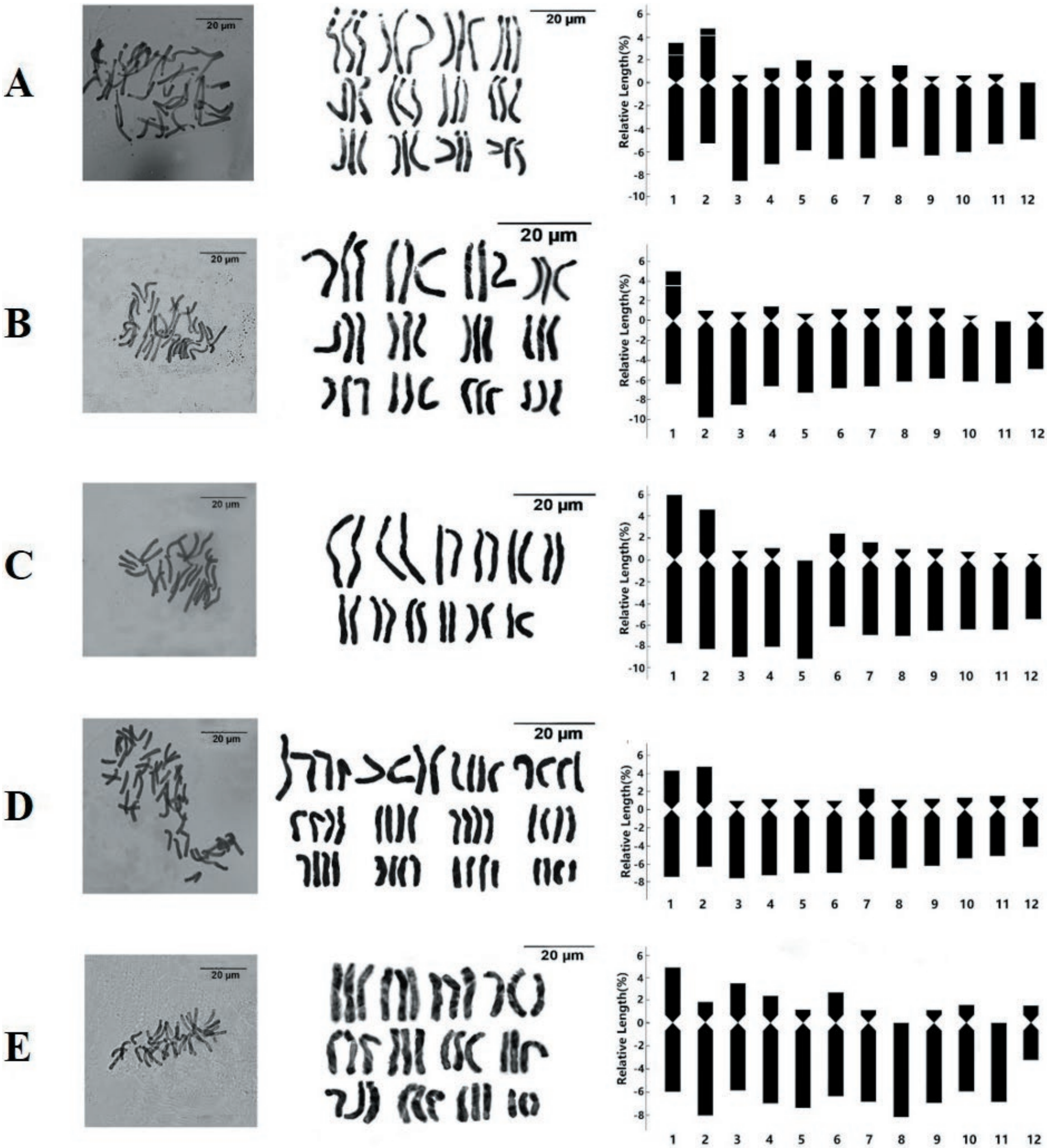

Figure 2. Mitotic chromosome plates, related karyotypes and karyograms of *L. lancifolium* and four related cultivars. A: *L. lancifolium*, B-E: *L. lancifolium* cultivars: 'Flore Pleno', 'Sweet Surrender', 'Red Life', 'Red Velvet', respectively.

mean value of the chromosome long arm varied from 7.58 μm to 12.98 μm in 'Red life' (Table 2-D) and *L. lancifolium* (Table 2-A), respectively. The average of short arm lengths ranged between 1.53 μm and 2.66 μm in 'Flore Pleno' (Table 2-B) and *L. lancifolium*. And the average total length of chromosomes varied from 9.78 μm to 15.64 μm in 'Red life' and *L. lancifolium* (Table 2).

To evaluate the symmetry of karyotype, we calculated AR (arm ratio), CI (centromeric index), LA% (long arm percentage), SA%(short arm percentage), TF% (total form percentage), VRC (value of relative chromatin), A1 (intrachromosome asymmetry index), A2 (interchromosome asymmetry index) and DI (dispersion index) respectively based on TL (total length of chromosome), LA (long arm) and SA (short arm) (Table 2). The results showed that, among the studied populations, the highest TF% value (22.50) was estimated in 'Red Life' and the lowest TF% value (14.45) was estimated in 'Flore Pleno', the TF% valve (16.70) of *L. lancifolium* was the second lowest. The analysis of the intra-chromosome asymmetry (A1) and inter-chromosome asymmetry (A2) revealed that, 'Red Velvet' (with mean value of A1=0.70, A2=0.19) presented the smallest asymmetry. In this study, the wild species *L. lancifolium* had a lowest DI value (2.47), and the DI values four cultivars were much higher (3.01~3.44) (Table 2).

DISCUSSION

The karyomorphological investigations of *Lilium* are very important for the views of taxonomical and ecological studies (Ahn *et al.*, 2017). In this study, the results showed that, *L. lancifolium* (A) was triploid, which had a chromosome number of 2n=3x=36, coinciding with previous report (Gao *et al.*, 2011). While the ploidy of the four cultivars related to *L. lancifolium* (A) varied from diploid to tetraploid. Since the chromosome numbers of F1 hybrids of triploid *L. lancifolium* × diploid *L. leichtlinii* range from 24 to 34 (Suzuki and Yamagishi, 2016), and the chromosome numbers of F1 hybrids of triploid *L. lancifolium* × tetraploid 'Brunello' can reach to 50 (Ma, 2017), these four cultivars we studied might be obtained by hybridization between triploid *L. lancifolium* and other tetraploid lilies. According to previous studies, all the karyotypes of *Lilium* belong to 3B and 3A type (Stebbins, 1971; Gao *et al.*, 2011). In our study, only 'Flore Pleno' belonged to 3A, the other four belonged to 3B. That corroborate that the karyotype of genus *Lilium* is stable.

Both the karyograms and the value of TF% indicate that the karyotypes of five materials are very asymmetric. By using the values of A1, A2 (Zarco, 1986) and TF% (Huziwara, 1962), we can evaluate the symmetry of karyotypes among close classes. The present study revealed that the wild species *L. lancifolium* had a middle value whether of the A1, A2, or TF%, which meant that the cultivars related to *L. lancifolium* had different tendencies to symmetry compared to *L. lancifolium*, but whether they were higher or lower was uncertain.

DI index plays an important role in arranging the species within the same class of karyotype asymmetry in an advancing order of specialization by permitting further gradations, as depicted by species arrangement within sections (Lavania and Srivastava, 1992). We found that the wild species *L. lancifolium* had the lowest DI value compared to its cultivars. This might indicate that the hybrid progenies of *L. lancifolium* tend to have higher DI values.

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