Karyotype Analysis of *Lilium longiflorum* Thunb. cv. Easter Lily Cultivated in Pyin Oo Lwin

Nang Wut Hmon Phoo[1]

Abstract

This research aimed to investigate the karyotype analysis of *Lilium longiflorum* Thunb. cv. Easter Lily. This research was done at the Department of Botany, Lashio University, Northern Shan State, Myanmar. Microsporogenesis showed the chromosome number of n=12 in diakinesis. The karyotypes were determined by analyzing photographs of 10 well-spread metaphase cells, resulting in a karyotype formula of 1m+1sm+3st+7t, with only one satellite chromosome observed in the number 4 subterminal chromosomes. This characteristic suggests that the first two pairs of chromosomes were relatively stable. Furthermore, the degree of asymmetry or symmetry of the karyotypes was estimated by the following Stebbins, (1971). The mean total length of mitotic metaphase chromosomes was 159.78 µm. These results contribute valuable taxonomic information for future studies of *Lilium* and may serve as a resource for breeding research focused on cut flower and potted plant production. Moreover, the comprehensive cytogenetic information contributes to its cytotaxonomy and supports taxonomic studies in *Lilium* of other areas.

Keywords: Karyotypes, Diakinesis, Metaphase, Satellite

Introduction

Lilium is one of the most lovely and famous decorative bulbous flowers. The appearance, beauty, and color of the bloom are very spectacular and attractive. Hybrid lilies are especially beneficial as reduce flower and pot plants (Bowser, 1986). They have been long admires and demanded for their aesthetic quality and have often been depicted as a symbol of purity and regality.

In the plant kingdom, the genome size of Liliaceae such as *Fritillaria*, *Hycinthus*, *Lilium*, and *Tulipa* were the largest. Many cytogeneticists have used lily as a general model plant for chromosome studies. The species of *Lilium* possess a wide range of ornamental traits as well as resistance to fungal and virus diseases. *Lilium* species have attracted much attention in cytological respect since they have almost the biggest genome size in the plant kingdom (Peruzzi *et al*., 2009)

The chromosomes of these species are also exceptionally large and have proved to be outstanding material for cytogenetic research for more than a century. Despite their phenotypic differences, most *Lilium* spp have the same chromosome number (2n=2x=24) and almost identical chromosome portraits (Stewart, 1947). *Lilium longiflorum* and *Lilium rubellum* ranged in nuclear DNA content material by less than 2% (Noda, 1991), (Van Tuyl & Boon, 1997) were also found that have similar karyotypes. In *Lilium* spp., most chromosomes (4-6, 7-9, and 10-12) are morphologically too similar to be identified without additional diagnostic landmarks.

Karyotype analysis has played an important role in the identification and designation of chromosomes in many plant species. Karyotype analysis was usually based on somatic mitotic metaphase chromosomes. Karyotype research has been

[1]Demonstrator, Botany Department, Lashio University,

mainly primarily based totally at the concept that symmetrical karyotypes are extra primitive than asymmetrical ones; longer chromosomes than shorter ones; median centromeres with chromosome arms of equal length are more primitive than chromosomes with arms of unequal length; low basic numbers give rise to higher ones. These features are based on the comparison between karyotypes of known relative antiquity, as determined through classical taxonomy (Sharma, 1990, Kamel, 2006).

The cytogenetic investigation is one of the best-documented experimental proofs for the elucidation of the mode of speciation on different groups of plants (Zohary, 1984) and (Kumar and Kumar, 2007). Cytological techniques determine the chromosomes (Singh, 1993). The study of genetic and cytogenetic variation is essential for hybridization between genotypes (Siahsar *et al*., 2005). Knowledge of karyotype relationships is an important prerequisite for effective plant genetic and breeding studies (Martinez-Gomez *et al*., 2003).

Karyotype provides basic information about the morphology of an individual chromosome, number, chromosomal homology, and ploidy level in plants. The karyological analysis assisted in the identification of the different cultivars in the same species according to the cytotaxonomically.

The present study was conducted to observe its karyomorphology, using chromosomal landmarks such as chromosome length, arm ratio, and secondary constrictions. This study aimed to provide more information on karyotype analysis and was also beneficial for further research concerns with the *Lilium* genus.

Materials and Methods

The sample used in this research was collected from the “Soe” Lily Nursery Farm, Pyin Oo Lwin, Mandalay Region. The present investigation was carried out at the Department of Botany, Lashio University.

The chromosomes were measured with a digital clipper for karyotype analysis and arranged decreasing in order of chromosome lengths, according to Stewart, (1947). The degree of asymmetry or symmetry of karyotypes was classified according to Stebbins, (1971). Measurement of the (a) length of long arms, short arms, and the whole length of the chromosome, (b) arm ratio, centromeric index, and relative length of each chromosome were calculated. Satellites were included in the total length to calculate arm ratios. Karyological data were obtained from the ten most definitive cells. Arm ratios, centromeric index, and relative length of each chromosome were calculated according to the following formulae:

$$(1)\ \text{Arm ratio} = \frac{\text{Length of the long arm}}{\text{Length of the short arm}}$$

$$(2)\ \text{Centromeric index} = \frac{\text{Length of short arm}}{\text{Total length of chromosome}}$$

$$(3)\ \text{Relative length} = \frac{\text{Total length of each chromosome}}{\text{Total complementary length of chromosome}} \text{ x } 100$$

Classification of chromosome types was calculated by using Stebbins, (1971), i.e. (a) Satellite chromosome (SAT), (b) Median chromosome (M) with arm ratio between 1.00 and 1.25, (c) Submedian chromosome (SM) with arm ratio between 1.26 and 1.75, (d) Subterminal chromosome (ST) with arm ration between 1.76 and 2.5 and (e) Terminal chromosome (T) with arm ratio exceeding 2.6.

The mean value of short arm length, long arm length, and satellite were used to prepare the idiograms.

Results

The basic chromosome number of *L. longiflorum* was quite stable with 2n=2x=24. The karyotype, karyotypic formula, and the index of asymmetry were listed in Table 1 and Figure 4C. The karyotypes from ten studied sample cells were illustrated in Figure 1, 2, and 3. On observing the size and morphology of somatic chromosomes were assigned into four groups and did not detect aneuploidy or B-chromosomes. The one pairs of large median centromere chromosomes, one pair of large submedian, three pairs of small subterminal, and the smallest seven pairs of chromosomes with terminal centromeres. The secondary constriction observed in the number 4 subterminal chromosome, was satellite. Based on Levan *et al.,* (1964) method, the karyotype formula was determined to be 1m+1sm+3st+7t. The ideogram was presented in Figure 5.

Analyses of mitotic chromosome characters were recorded in Table 1. The metaphase spreads and karyograms of this species was shown in Fig. 1, 2, 3, and 4. It indicated that the longest chromosome was the median and the shortest one was terminal. The total complementary length of chromosomes was 159.78 µm. The chromosome length and relative length of the chromosome ranged from 9.22 µm and 6.04 µm in chromosome 12 to 20.86 µm and 12.83 µm in chromosome pair 1. The length of the long arm varied from 7.56 µm in chromosome pair 12 to 10.58 µm in chromosome pair 2. The longest and shortest values of the short arm length were for chromosome pairs 1 and 12, with averages of 9.58 µm and 1.65 µm, respectively. Meanwhile, chromosome pair 12, with an average of 4.74 µm, had the highest arm ratio index and the minimum value of this ratio was obtained in chromosome 1 with an average of 1.09 µm.

The following signs have been adopted to elucidate the position of primarily or spindle fiber attachment constructions, m=median, sm=submedian, st=subterminal, and t=terminal. The satellite chromosome was assigned to the subterminal.

The chromosome pair 1 was the median and named m. and the mean length was 20.86 ± 6.16 µm. The arm ratio was 1.09 ± 1.04 µm. The centromeric index was 0.48 ± 0.01 µm. The relative length range was 12.83 ± 1.84 µm. The submedian chromosome was observed in chromosome pair 2 and the mean total length was 17.82 ± 4.34 µm, arm ratio, centromeric index, and relative length were 1.46 ± 0.15, 0.41 ± 0.03 and 11.55 ± 1.61 µm respectively.

The chromosome pair 3, 4, and 5 was subterminal and named st_1, st_2, and st_3 respectively. Those ranged from mean total length of 13.75 ± 2.83 to 15.04 ± 3.15 µm. The ranged from 2.28 ± 0.27 µm in st_1 to 2.34 ± 0.23 µm in st_3. The centromeric index ranged from 0.30 ± 0.02 µm in st_3 to 0.31 ± 0.03 µm in st_1. The relative length ranged from 8.94 ± 1.02 µm in st_3 to 9.71 ± 1.26 µm in st_1. The subterminal chromosome pair st_2 possessed satellite as shown in Table 1, Figure 4 A, B, C, and D.

Table 1. Mitotic chromosome characteristics of *Lilium longiflorum* Thunb. cv. Easter Lily

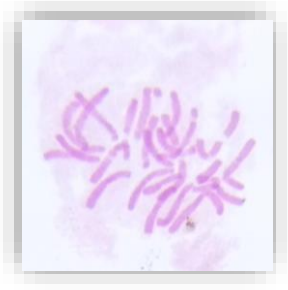
Chro-mosome No.	Chro-mosome Type	Satellite Length (µm)	Short Arm Length (µm)	Long Arm Length (µm)	Total Length (µm)	Arm Ratio LA/SA	Centromeric Index SA/TL	Relative Length TL/TCL x 100
		Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E
1	m	-	9.58 ± 2.70	10.37 ± 2.90	20.86 ±6.16	1.09 ± 0.04	0.48 ± 0.01	12.83±1.84
2	sm	-	7.28 ± 1.75	10.58 ±2.73	17.82 ± 4.34	1.46 ± 0.15	0.41 ± 0.03	11.55±1.61
3	st_1	-	4.55 ± 0.82	10.39 ± 2.49	15.04 ± 3.15	2.28 ± 0.27	0.31 ± 0.03	9.71±1.26
4	st_2^*	1.49±0.38	4.43 ± 0.74	9.95 ± 2.47	14.38 ± 3.19	2.23 ± 0.28	0.31± 0.02	9.32±0.99
5	st_3	-	4.16 ± 0.92	9.65 ± 1.95	13.75 ± 2.83	2.34 ± 0.23	0.30 ± 0.02	8.94±1.02
6	t_1	-	2.39 ± 0.70	10.48 ± 2.59	12.86 ± 3.10	4.51± 0.89	0.19 ± 0.03	8.29±0.88
7	t_2	-	2.24 ± 0.24	9.97± 2.50	12.21 ± 2.67	4.43 ± 0.92	0.19 ± 0.03	7.89±0.67
8	t_3	-	2.23 ± 0.51	9.38 ± 1.92	11.61 ± 2.28	4.29 ± 0.81	0.19 ± 0.03	7.55±0.81
9	t_4	-	2.11 ± 0.43	9.11 ± 2.09	11.19 ± 2.38	4.34 ± 0.79	0.19 ± 0.03	7.25±0.85
10	t_5	-	1.98 ± 0.47	8.77 ± 1.89	10.75 ± 2.26	4.52 ± 0.79	0.19 ± 0.03	6.97±0.69
11	t_6	-	1.91 ± 0.58	8.21 ± 1.66	10.09 ± 2.05	4.49 ± 0.93	0.19 ± 0.03	6.58±0.82
12	t_7	-	1.65 ± 0.37	7.56 ± 1.46	9.22 ± 1.60	4.74 ± 1.09	0.18 ± 0.04	6.04±0.76

m= Median; sm= Submedian; SA= Short Arm; LA= Long Arm; TL= Total Length; TCL= Total Complementary Length;

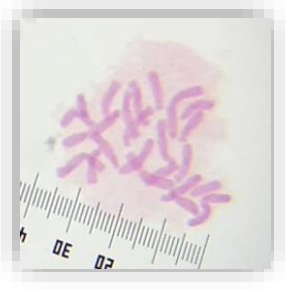
SE=Standard Error; (µm)= micrometer; *= Satellite chromosome length including in short arm; Karyotype formula =1m + 1sm + 3st* +7t

Metaphase of Cell No.1

Metaphase of Cell No.2

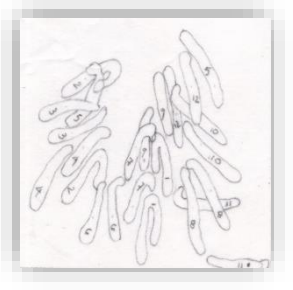


Metaphase of Cell No.3

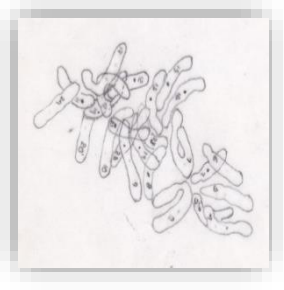


Metaphase of Cell No.4

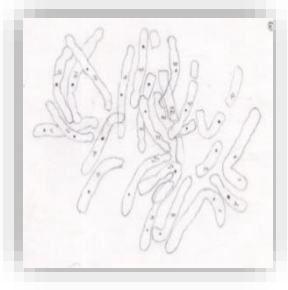
Metaphase of Cell No.5



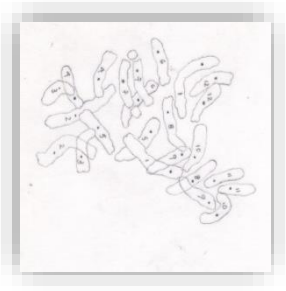
Chromosomes Outline of Cell No.1



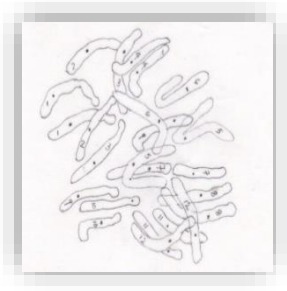
Chromosomes Outline of Cell No.2



Chromosomes Outline of Cell No.3



Chromosomes Outline of Cell No.4



Chromosomes Outline of Cell No.5

Fig.1. Five selected cells (1-5) Metaphase and Chromosome Outlines of *Lilium longiflorum* Thunb. cv. Easter Lily

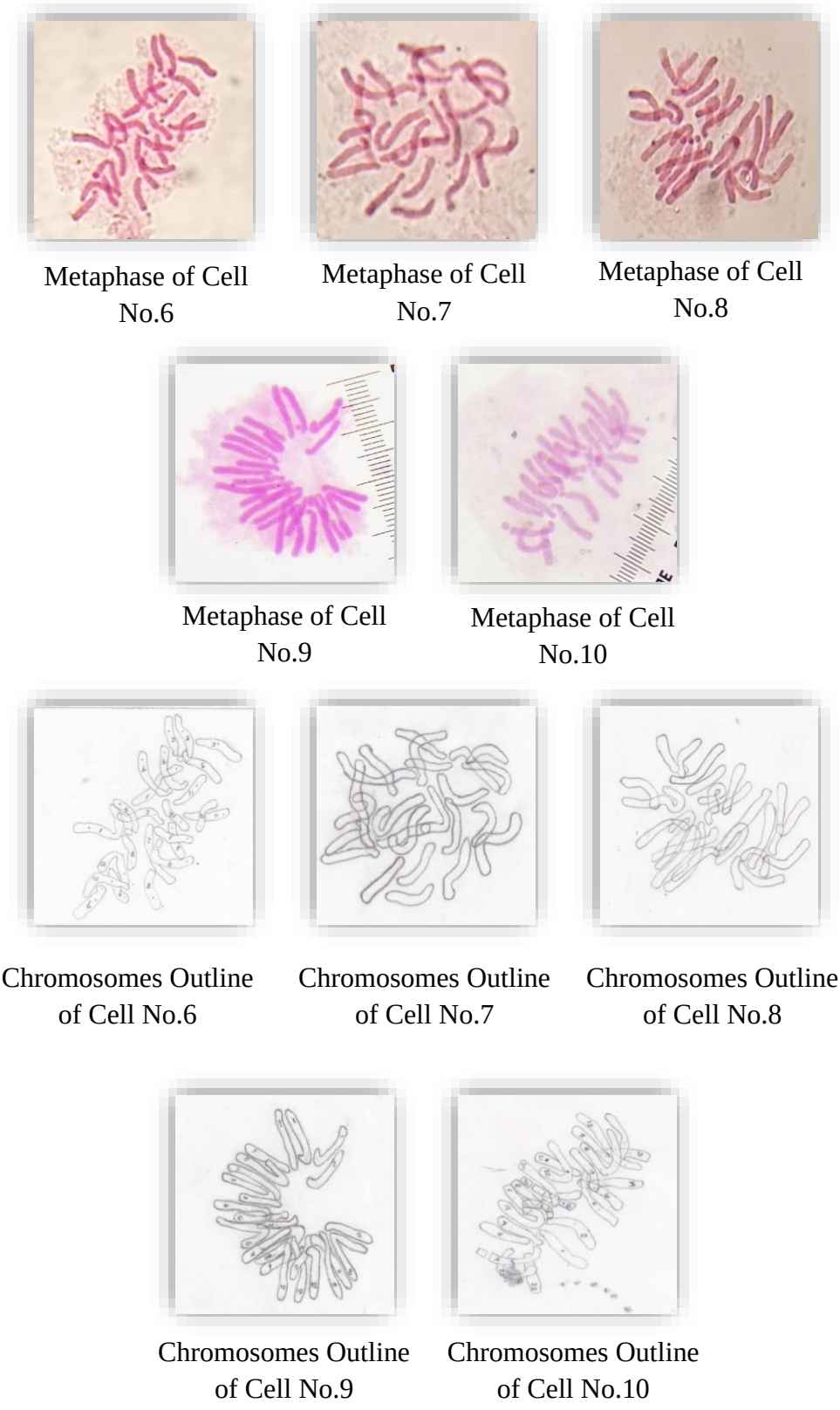


Fig.2. Five selected cells (6-10) Metaphase and Chromosome Outlines of *Lilium longiflorum* Thunb. cv. Easter Lily

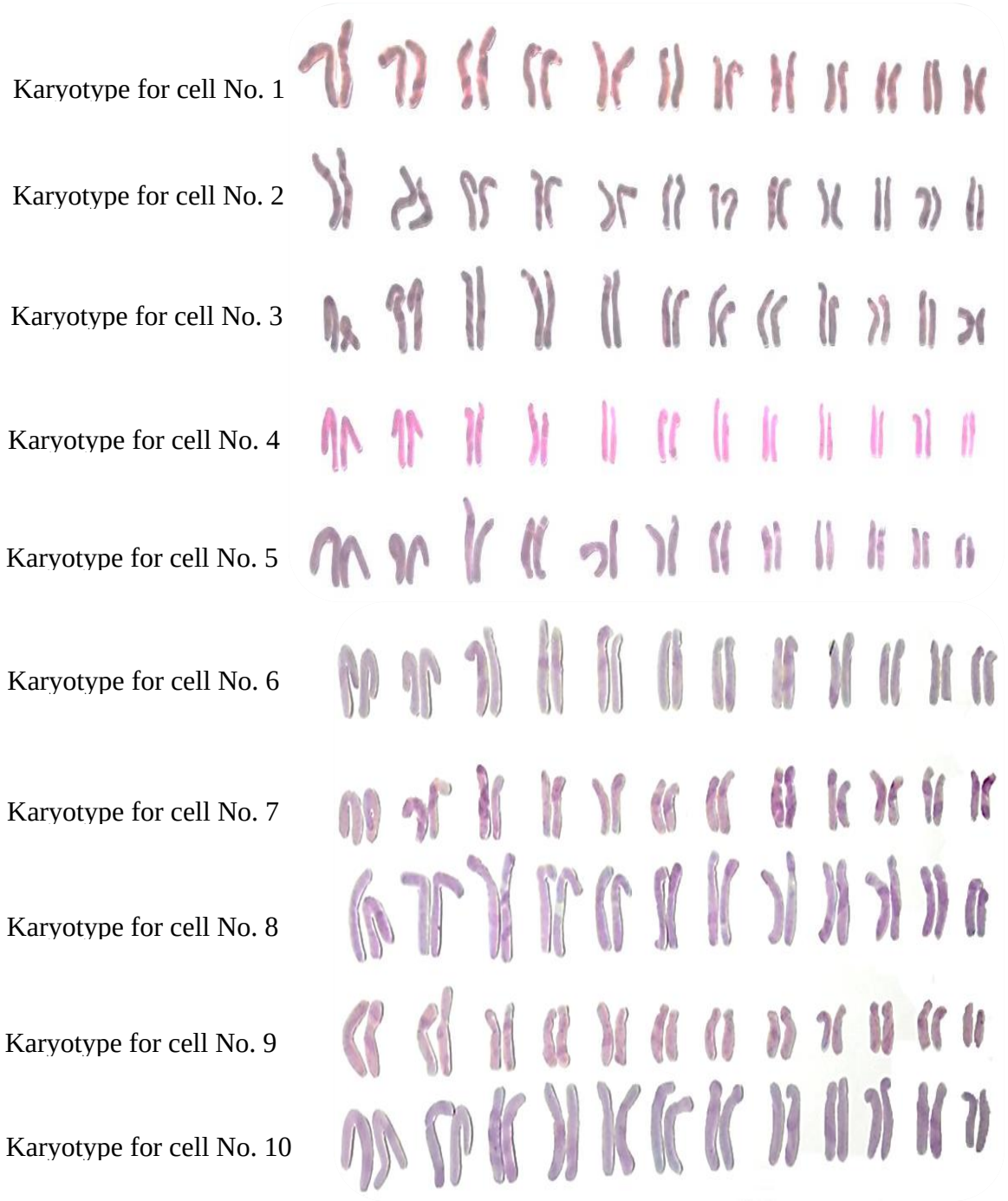
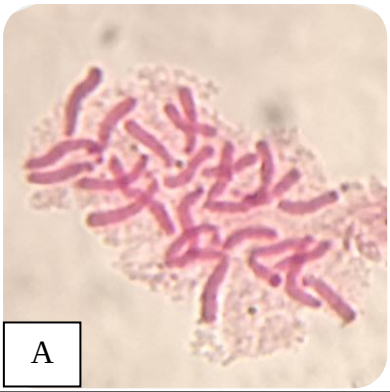
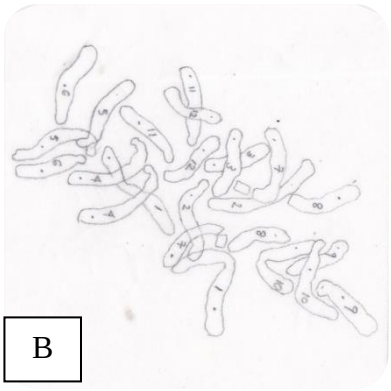


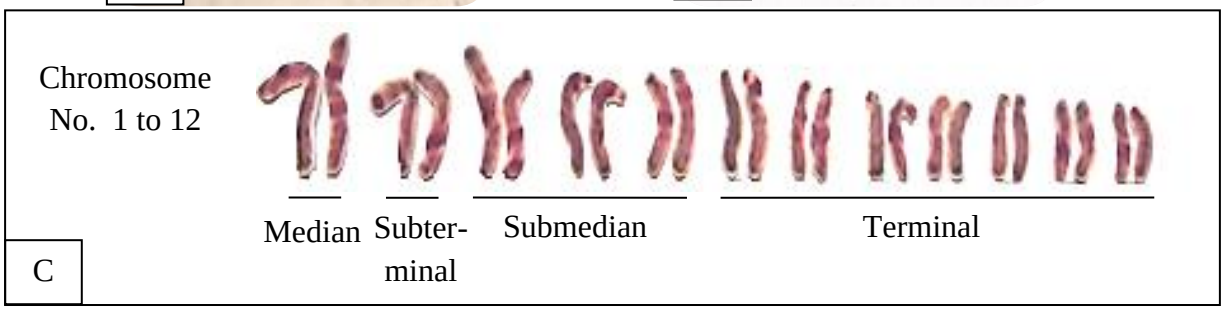
Fig.3 Ten selected cells (1-10) for karyotypes analysis of *Lilium lonaiflorum* Thunb. cv. Easter Lilv



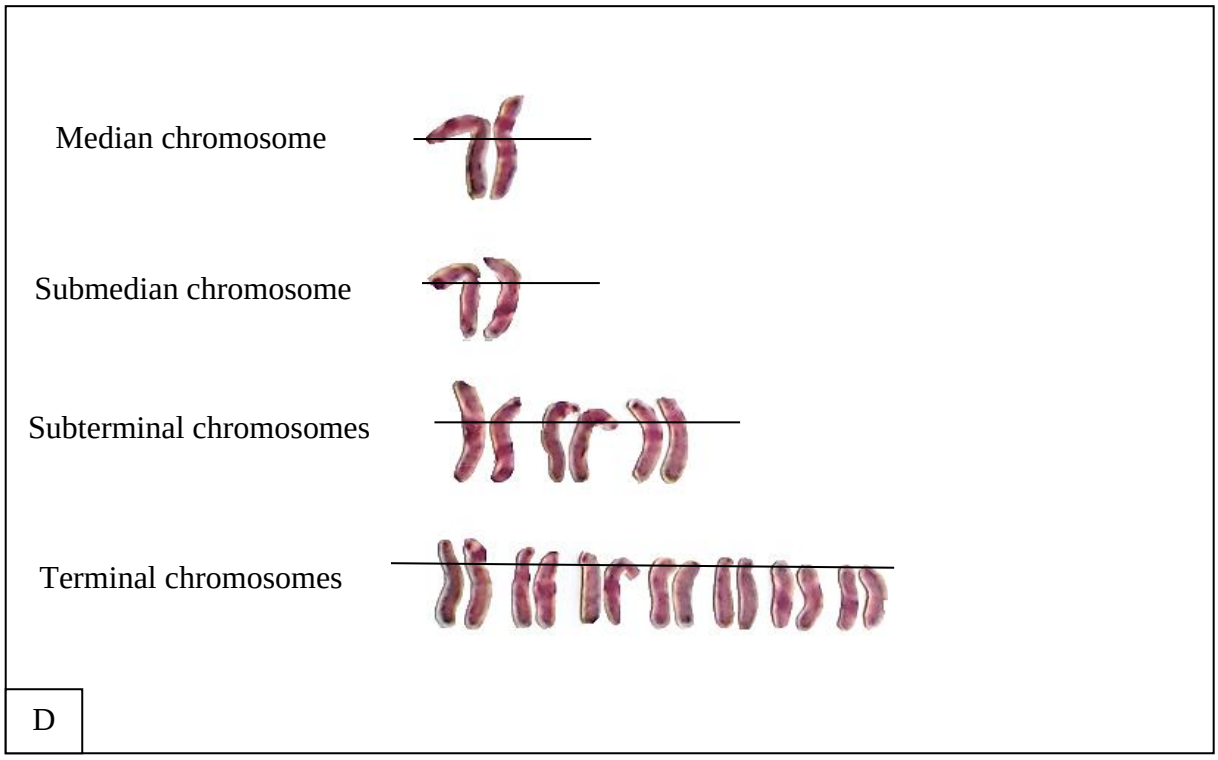
A



B



C



D

Fig. 4 A. Mitotic cell of *Lilium longiflorum* Thunb.cv. Easter Lily

B. Outline mitotic cell of *Lilium longiflorum* Thunb.cv. Easter Lily

C. Karyotype of *Lilium longiflorum* Thunb.cv. Easter Lily

D. Four group of Chromosomes *Lilium longiflorum* Thunb.cv. Easter Lily

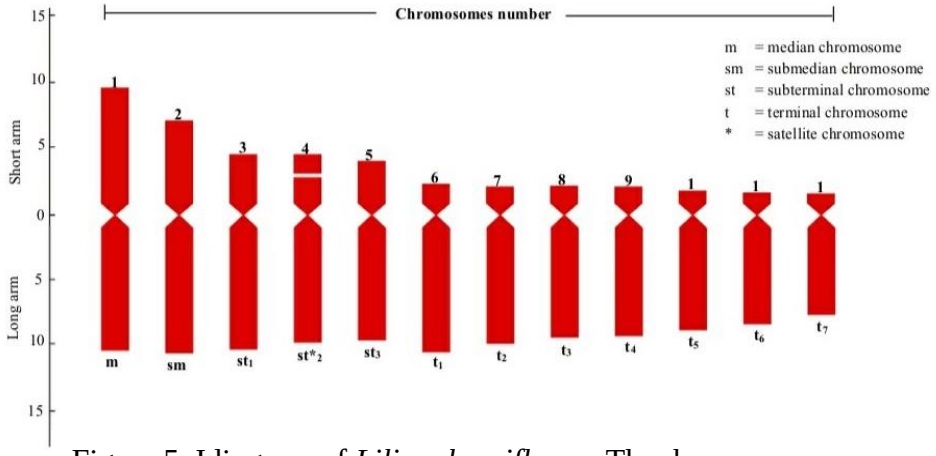


Figure 5. Idiogram of *Lilium longiflorum* Thunb.

Discussion and Conclusion

Its karyotype formula was determined to be 1m+1sm+3st+7t and only one satellite chromosome was observed in the number 4 subterminal chromosomes. Accordingly the indicating data, *L. longiflorum* was karyotypic asymmetry. This observation of the karyotype formula was agreed with the finding of Gao *et al*., (2011). According to Stewart, (1947), all species of *Lilium* have two large pairs of chromosomes with submedian centromeres and ten pairs with subterminal centromeres and 2n=24 chromosomes. Gao *et al*., (2011) also stated that among 12 pairs of chromosomes, the first two pairs are quite stable, and thus, can be recognized as a special trait of this genus. This result was also consistent with previous reports on *L. longiflorum* (Noda, 1991, Lim *et al*., 2001, Inceer *et al*., 2002).

However, Tahamil *et al*., (2014) reported that 1m+4sm+7st for *L. longiflorum* with only one satellite in chromosome 6. Stewart, (1943), Son, (1977), and Gao *et al*., (2009, 2011) recommend that there appears to be only little karyotypic variation between the investigated populations.

The present finding agreed with the following findings of; Noda, (1991) also investigated that the basic chromosome complement (x=12) consists of two large V-shaped chromosomes and ten shorter I-shaped ones: 2V+10I. The metacentric chromosome is slightly larger than the submetacentric, so they can be distinguished from each other during mitosis as well as during meiosis. The I-shaped chromosomes are classified into two groups subterminal and terminal, it was difficult to distinguish between chromosomes. Marasek, (2003) also clarified in all lily genotypes analyzed; only the longest chromosomes, metacentric or submetacentric, were easily recognizable without measurements. Concerning the results, chromosome morphology can be used as a reliable marker to assess the variations among lily species (Tahamil, 2014).

In this observation, the total complementary lengths of chromosomes were 159.78 µm. This result was a variation from the finding of Lim *et al*., (2001) who recorded it as about 286 µm, and Tahamil *et al*., (2014) measured 330.75 µm. About this finding, Song, (1987) mentioned that the conventional staining methods have some limitations in the unequivocal identification of individual chromosomes of similar size and morphology in *Lilium* species. Heimburger, (1962) also reported that

chromosome size may also be affected by growth conditions, and may also depend on the duration of the analysis.

In the present study, the chromosomes were significant for the first two chromosome pairs; median and submedian and they lacked the satellite. This result agreed with the finding of Gao *et al*. (2011) who also reported that *L. longiflorum* has a karyotype that lacks satellites in the first two homologous chromosome pairs. Stewart (1947) recommended that basically, all species of *Lilium* have two large pairs of chromosomes with submedian centromeres and ten pairs with subterminal centromeres and 2n=24 chromosomes. Meanwhile, it has become evident that secondary constructions are more important and complicated in this genus.

The previous work by Stewart (1947) and Gao *et al*. (2011) indicated that the amount and position of secondary constrictions are the most variable features in *Lilium*. This means among 12 pairs of chromosomes, the first two pairs are quite stable, and thus, can be recognized as a special trait of this genus.

It believes that the results will favor the taxonomic studies in *Lilium* of other areas. This study provides baseline information regarding the effective exploitation and use of *L. longiflorum* Thunb. cv. Easter Lily, resources for breeding research to be used as cut flowers and potted plants. And it provides comprehensive morphological and cytogenetic information which can be helpful for its cytotaxonomy.

References

Bowser, D., 1986. Florist reviews. 177: 29-32.

Gao, Y. D., D.Z. Song and J.H. Xing, 2011. Karyotype studies in thirty-two species in *Lilium* (Liliaceae) from China.

Gao, Y. D., S.D. Zhou and X.J. He, 2009. Karyotypes of four genera in Liliaceae (s.str.) from Hengduan Mountains of southwest China. Acta Bot. Yunnan. 31: 399405.

Heimburger, M., 1962. Comparison of chromosome size in species of Anemone and their hybrids. Chromosoma 13: 328-340.

Inceer, H., S. Hayirliogly and O. Beyazogly, 2002. Karyological study on some taxa of the genus *Lilium L*. (Liliceae). Pak. J. Bot. 34: 33-40.

Kamel, E. A. R., 2006. Karyotype Characterization and Polyploid Variations in Some Genotypes of *Triticum aestivum* L. Cytologia 71(2): 169-174, The Japan Mendel Society.

Kumar, G. and P. Kumar Rai, 2007. EMS induced karyomorphological variations in maize (*Zea mays* L.) Inbreds. Tunk. J. Biol. 31:187-195.

Levan, A., K. Fredga, and A. A. Sandberg, 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52: 201-220.

Lim, K. B., J. D. Wennekers, E. Hans, J. Jong, E. Jacobsen, and J. M. Van Tuyl, 2001. Karyotype analysis of *Lilium longiflorum* and *Lilium rubellum* by chromosome banding and florescence in situ hybridization. Genome 44: 911-918.

Marasek, A. and T. Orlikowska, 2003. Morphology of chromosomes of nine lily genotypes and usefulness of morphological markers for hybrid verification. Acta Biol. Crac. Ser. Bot. 45: 159-168.

Martinez-Gomez, P., Y. Vaknin, T.M. Gradziel, and F. Dicenta, 2003. Karyotype analysis in Almond. ISHS ActaHorticulturae 622. http://www.actahort.org/ books/622/.

Noda, S., 1991. Chromosomal variation and evolution in the genus *Lilium*. In Chromosome engineering in plants: genetics, breeding, evolution. Part B. Edited by T. Tsuchiya and P.K. Gupta. Elsevier, Amsterdam. pp. 507-524.

Peruzzi, L., I.J. Leitch, and K.F. Caparelli, 2009. Chromosome diversity and evolution in Liliaceae. Ann. Bot. 103: 459-475.

Sharma, A., 1990. Taxonomy as related to genetic diversity in plants. J. Ind. Bot. Soc. 69: 1-3.

Siahsar, B. A., S. Ghaffari Pour, G. Karimzadeh, M. Sahebi, and H. Akbari Moghadam, 2005. Karyotypic and Morphologic Variations in some Hull-less Barley (*hordeum vulgare L.*) Genotypes. Proceedings of 9th ICABR International Conference on Agricultural Biotechnology; Ten years later. July 6 to July 10, 2005. Ravello (Italy).

Singh, R. J., 1993. Plant Cytogenetics. CRC Press Inc.

Son, J., 1977. Karyotype analysis of *Lilium longiflorum* Thunberg by means of C-banding method. Japan. J. Genetics 52: 217-221.

Song, N. H., 1987. Analysis of C-banded karyotypes and chromosomal relationships of *Lilium* species. Ph. D. Thesis, Kyungpook Natl. Univ. Daegu, Korea.

Stebbins, G. L., 1971. Chromosomal evolution in higher Plants. Edward Arnold (Publisher) Ltd, London, pp. 87-89.

Stewart, R. N., 1943. Occurrence of aneuploids in *Lilium*. Bot. Gaz. 104: 620-626.

Stewart, R. N., 1947. The morphology of somatic in *Lilium*. American Journal of Botany 34: 9-26

Tahamil, S. K, C. Esmaeil, Z. Nasser, A. Z. Rasool, M. Mehdi and J. Daryl, 2014. Karyological Characteristics of *Lilium ledebourii* Boiss and *Lilium longiflorum* Thunb.

Van Tuyl, J.M., and E. Boon, 1997. Variation in DNA-content in the genus *Lilium*. Acta Hortiv. 430: 829-835.

Zohary, D., 1984. Modes of evolution in plants under domestication. In Grant WF. ed. Plant Biosystematics, Academic Press, Canada, 579-596.