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Full Length Research Paper

Karyotype studies on *Tagetes erecta* L. and *Tagetes patula* L.

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Karyotypes of nine *Tagetes erecta* L. accessions and three *Tagetes patula* L. accessions were studied. The chromosome numbers of *T. erecta* and *T. patula* were 2n=2x=24 and 2n=4x=48, respectively. The karyotype formulae of *T. erecta* L. 'Scarletade' and 'Perfection Yellow' are 2n=2x=24=4sm+20m; '9901AB' and 'Harvest', 2n=2x=24=2sm+22m; 'Taishan', 2n=2x=24=14sm+10m; 'Marvel' and 'Perfection Orange', 2n=2x=24=24m. The karyotype formulae of *T. patula* L.: 'GoldenGate' and 'Janie' are 2n=4x=48=4sm+44m; 'Little Hero', 2n=4x=48=48m.

Key words: *Tagetes erecta* L.*, Tagetes patula* L.*,* chromosome, karyotype.

INTRODUCTION

Tagetes erecta L. and *Tagetes patula* L. belonged to composites family. They originated from Central America, mainly distributed in western Mexico and southeastern Arizona (Robert, 1962). The genus *Tagetes* (Asteraceae) contains 56 species, of which only few species were currently cultivated as horticultural crops. Some companies, such as, Thompson and Morgan, Pan-American Seed and SluisGroot etc. cultivate new cultivars every year. Examples are, 'Marvel' line, 'Taishan' line of *T. erecta* L. and 'Bonanza' line, 'Boy' line of *T. patula* L. which have been widely used in the world. Most of the cultivars were produced in the traditional hybridization breeding way (Wang, 2003, 2009; Tian et al., 2007). Besides, some works also have been done on the breeding of transgenic marigold (Gregorio et al., 1992; Charles et al., 2001). Nowadays, the species widely used throughout the world were *T. erecta* L.*, T. patula* L. and *T. tenuifolia* (Soule, 1996). In China, *T. erecta* L. and *T. patula* L. were introduced and widely cultivated as important garden plants. In addition, the inflorescence of pigment *T. erecta* L. flowers were also ideal materials for extracting lutein. Therefore, it was very important to study *Tagetes* plant with their great economic value.

The plant taxonomy was mainly based upon morphological, cytological, and molecular biological analysis, etc. As an important means of cytological analysis, chromosome karyotype analysis has been widely used in biological genetic variation, systematic evolution or relationship identification (Zheng et al., 2005; He et al., 2005; He and Zhang, 2009). Up till now, there have been massive reports about chromosome karyotype analysis in Asteraceae plants (Kong, 2000; Yang, 2001; Xie and Zheng, 2003; Chen, 2008; Zhang et al., 2009). For instance, Li et al. (2007) studied the karyotype of fourteen cultivars of cut chrysanthemum, and Zhang et al., (2009) conducted a cytological study on the genus *Syncalathium* (Asteraceae-Lactuceae). But the karyotypes of *Tagetes* plants were rarely studied. In our paper, we widely collected *Tagetes* species and cultivar materials which were popular in China for a systematic study on their chromosome numbers and karyotypes, while related researches have not been reported. The objective of this study was to provide cytological information for systematic classification, breeding and germplasm resources study.

MATERIALS AND METHODS

This research studied on twelve accessions of genus *Tagetes* which were popular in the domestic market, including seven ornamental *T. erecta* cultivars, two pigment *T. erecta* cultivars (*T. erecta* L. 'Scarletade' and '9901AB'), three *T. patula* cultivars (Table1).

All karyotype observations were made from root tips. Seeds were germinated on wet filter paper in Petri dishes at 25°C. Fresh root

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Table 1. The source of materials investigated.

Cultivars	Source
T. erecta L. 'Scarletade'	Inner Mongolia Bureau of Parks
T. erecta L.'9901AB'	Inner Mongolia Bureau of Parks
T. erecta L. 'Harvest'	Beijing Institute of Landscape and Garden
T. erecta L. 'Taishan'	Beijing Institute of Landscape and Garden
T. erecta L. 'Marval'	Beijing Institute of Landscape and Garden
T. erecta L. 'Perfection Yellow'	Beijing Institute of Landscape and Garden
T. erecta L. 'Perfection 'Orange'	Beijing Institute of Landscape and Garden
T. erecta L. 'Inca Orange'	Beijing Institute of Landscape and Garden
T. erecta L. 'Inca Yellow'	Beijing Institute of Landscape and Garden
T. patula L. 'GoldenGate'	Beijing Institute of Landscape and Garden
T. patula L. 'Little Hero'	Beijing Institute of Landscape and Garden
T .patula L. 'Janie'	Beijing Institute of Landscape and Garden

Table 2. Parameters of chromosomes of *T.erecta* and *T.patula.*

Cultivar	Karyotype formula	A.A.R	Lt/St	Type	Length type	As.K(%)
T. erecta L.'Scarletade'	2n=2x=4sm+20m	1.5	2.36	1B	2n=4L+8M2+6M1+6S	60.39
T .erecta L.'9901AB'	2n=2x=2sm+22m	1.51	2.2	1B	2n=4L+8M2+8M1+4S	60.38
T. erecta L. 'Harvest'	2n=2x=6sm+18m	1.66	2.34	1B	2n=4L+6M2+10M1+4S	62.7
T. erecta L.'Taishan'	2n=2x=14sm+10m	1.74	2.5	2B	2n=6L+4M2+10M1+4S	64.02
T. erecta L. 'Marval'	2n=2x=24m	1.48	2.37	1B	2n=4L+8M2+8M1+4S	59.92
T. patula L. 'GoldenGate'	2n=4x=4sm+44m	1.56	2.62	1B	2n=12L+8M2+16M1+12S	61.34
T .patula L.'Little Hero'	2n=4x=4sm+44m	1.45	2.8	1B	2n=12L+8M2+16M1+12S	59.67
T. erecta L. 'Inca Orange'	2n=2x=2sm+22m	1.32	2.50	1B	2n=4L+8M2+8M1+4S	57.15
T .erecta L. 'Inca Yellow'	2n=2x=24m	1.30	2.84	1B	2n=4L+4M2+12M1+2S	56.95
T. patula L. 'Janie'	2n=4x=48m	1.46	2.64	1B	2n=12L+12M2+12M1+12S	59.7
T. erecta L. 'Perfection 'Yellow'	2n=2x=4sm+20m	1.31	3.35	1B	2n=6L+6M2+6M1+6S	56.85
T .erecta L. 'Perfection 'Orange'	2n=2x=24m	1.17	2.43	1B	2n=6L+8M2+6M1+4S	54.06

A.A.R= Average arm ratio; Lt= Longest arm; St-Shortest arm; As.k(%)= Index of the karyotypic asymmetry.

tips were cut approximately 1 cm long before pretreated in 0.002 mol/L 8-hydroxyquinoline solution for 4 h; then, fixed with Carnoy I (glacial acetic acid : 70% ethanol = 1:3) for 20 h. After hydrolysis in 1 mol/L HCl at 60°C for 8 -10 min, the root tips were rinsed in distilled water twice for approximately 20 min. Prior to observation, stained with phenol fuchsin solution for 30 min, and squashed for chromosome observation. Observations were made of somatic mitotic metaphase. At least thirty cells of each cultivar have been observed to ensure their chromosome number. Five cells's chromosome parameters of each cultivar were surveyed and calculated according to Li et al. (1985); karyotype asymmetry (KA) was classified according to Arano (1963) and karyotype classification was according to Stebbins (1971).

RESULTS

Chromosome number of 2n=2x=24 was found among the *T. erecta* L. cultivars; *T. patula* L. cultivars have a chromosome number of 2n=4x=48. Satellite has not been found in the tested plants. Their detailed parameters and karyotype formulae are listed in Table 2. The chromosomes, karyograms and idiograms are shown in Figures 1, 2 and 3, respectively. Brief descriptions of the cytological features of each cultivar were as follows:

T. erecta L. 'Scarletade'

The karyotype formula of *T. erecta* L. '*Scarletade'* was 2n=2x=4sm+20m. The ratio of the longest to the shortest chromosome was 2.36, the KA was of type 1B, average arm ratio was 1.5 and the length type was 2n=4L+8M2+6M1+6S.

T. erecta L. '9901AB'

The karyotype formula of the *T. erecta* L. '9901AB' was 2n=2x=2sm+22m. The ratio of the longest to the shortest chromosome was 2.2, the length type was 2n=4L+8M2+8M1+4S, average arm ratio was 1.51 and the KA was of type 1B.

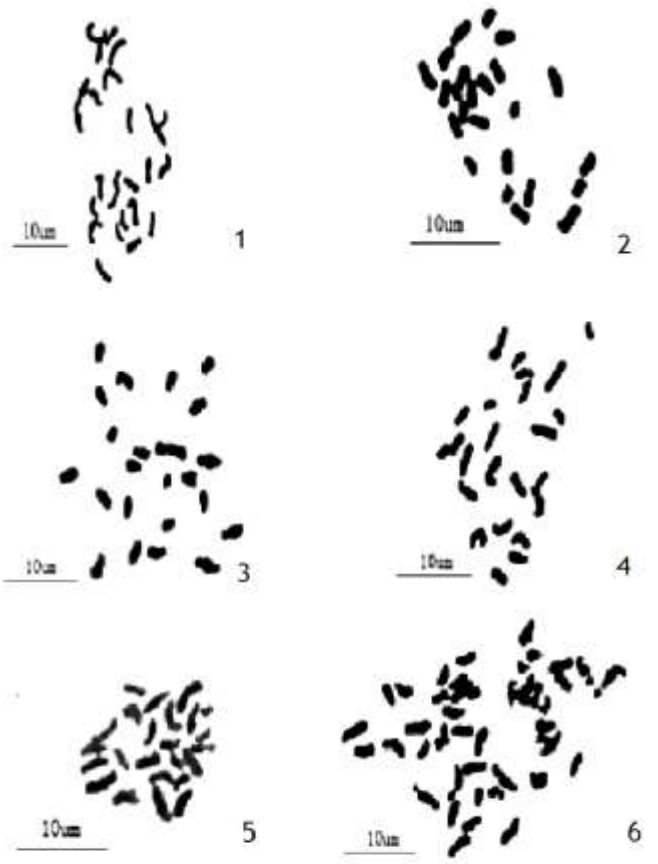

Figure 1. Chromosomes of *Tagetes erecta* L. and *Tagetes patula* L. 1-5, 9-12. Chromosomes of *T. erecta* L. 1. 'Scarletade'. 2. '9901AB'. 3. 'Harvest'. 4. 'Taishan'. 5. 'Marvel'. 9. 'Perfection 'Yellow'. 10. 'Perfection Orange'. 11. 'Inca Orange'. 12. 'Inca Yellow'. 6-8. Chromosomes of *T.patula* L. 6. 'GoldenGate'. 7. 'Janie'. 8. 'Little Hero'.

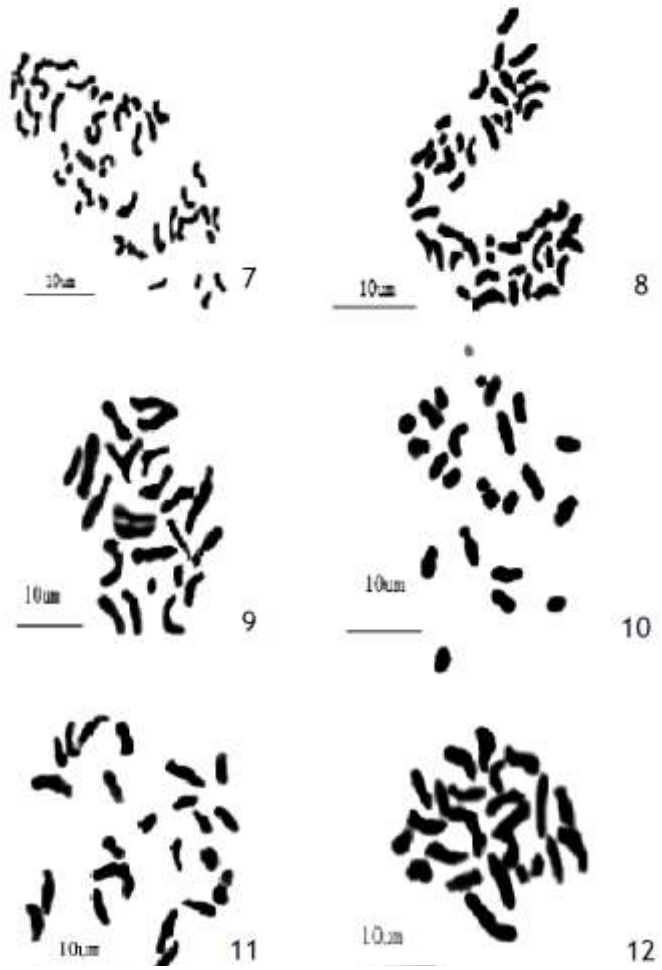

Figure 1. Contd.

T. erecta L. 'Harvest'

The karyotype formula of *T. erecta* L. 'Harvest' was 2n=2x=6sm+18m. The ratio of the longest to the shortest chromosome was 2.34, the length type was 2n=4L+6M2+10M1+4S, average arm ratio was 1.66 and the KA was of type 1B.

T. erecta L. 'Taishan'

The karyotype formula of *T. erecta* L. 'Taishan' was 2n=2x=14sm+10m; the ratio of the longest to the shortest chromosome was 2.5, the KA was of type 2B, average arm ratio was 1.74 and length type was2n=6L+4M2+10M1+4S.

T. erecta L. 'Marval'

The karyotype formula of *T. erecta* L. 'Marval' was 2n=2x=24m. The ratio of the longest to the shortest chromosome was 2.37, the KA was of type 1B, average arm ratio was 1.48 and length type was 2n=4L+8M2+8M1+4S.

T. patula L. 'GoldenGate'

The karyotype formula of *T. patula* L. 'GoldenGate' was 2n=4x=4sm+44m. The ratio of the longest to the shortest chromosome was 2.62, 2n=12L+8M2+16M1+12S, average arm ratio was 1.56 and the KA was of type 1B.

T. patula L. 'Little Hero'

The karyotype formula of *T. patula* L. 'Little Hero' was 2n=4x=4sm+44m. The ratio of the longest to the shortest chromosome was 2.8, length type was 2n=12L+8M2+16M1+12S, average arm ratio was 1.45 and the KA was of type 1B.

Figure 2. Karyograms of *Tagetes erecta* L. *and Tagetes patula* L. 1-5, 9-12. Karyograms of *T.erecta* L. 1. 'Scarletade'. 2. '9901AB'. 3. 'Harvest'. 4. 'Taishan'. 5. 'Marvel'. 9. 'Perfection 'Yellow'. 10. 'Perfection Orange'. 11. 'Inca Orange'. 12. 'Inca Yellow'. 6-8. Karyograms of *T.patula* L. 6. 'GoldenGate'. 7. 'Janie'. 8. 'Little Hero'.

***T. patula* L. 'Janie'**

The karyotype formula of *T. patula* L. 'Janie' was 2n=4x=48m. The ratio of the longest to the shortest chromosome was 2.64, length type was 2n=12L+12M2+12M1+12S, average arm ratio was 1.46

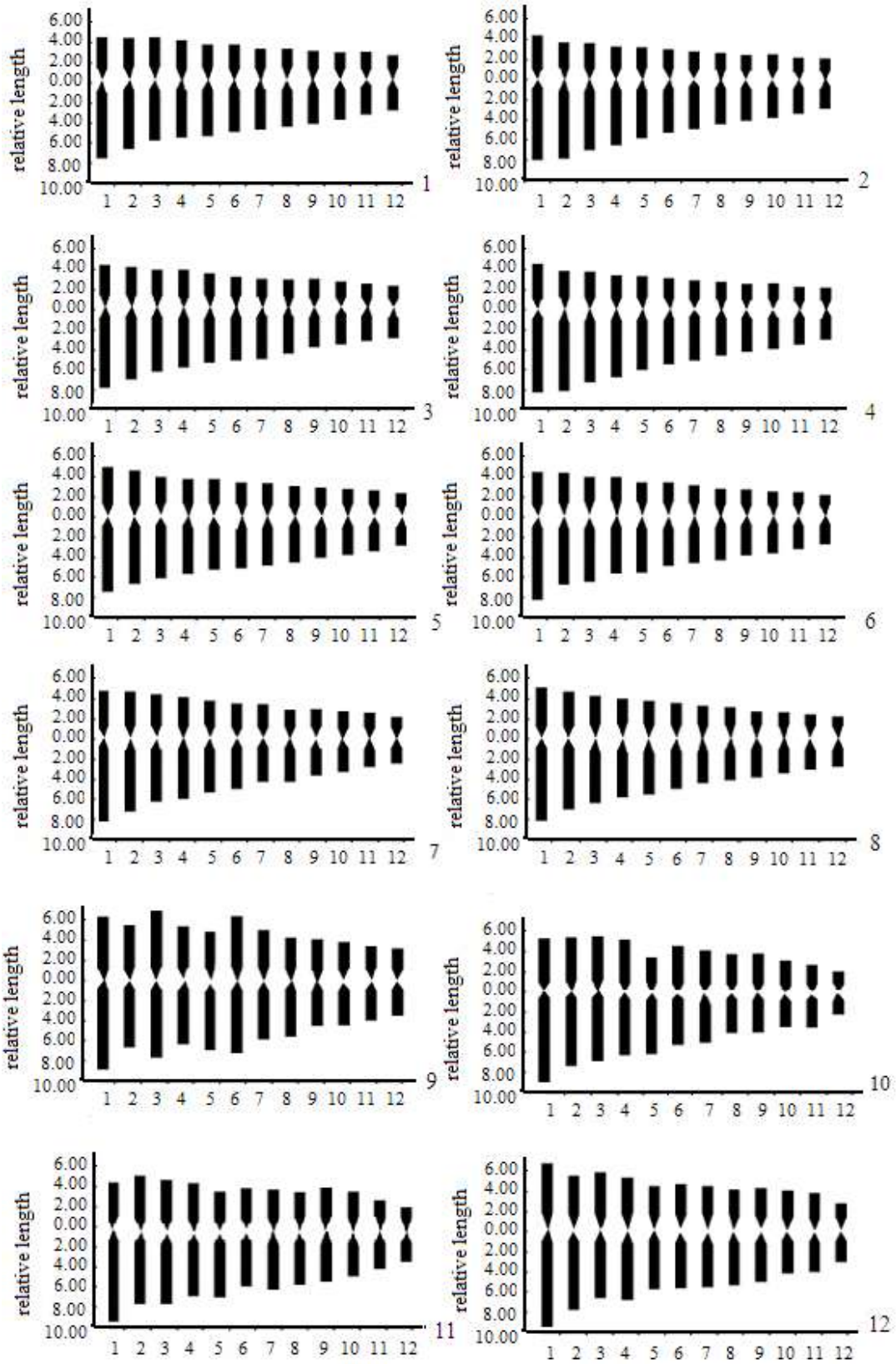

Figure 3. Idiograms of *Tagetes erecta* L. and *Tagetes patula* L. 1-5, 9-12. Idiograms of *T.erecta* L. 1. 'Scarletade'. 2. '9901AB'. 3. 'Harvest'. 4. 'Taishan'. 5. 'Marvel'. 9. 'Perfection 'Yellow'. 10. 'Perfection Orange'. 11. 'Inca Orange'. 12. 'Inca Yellow'. 6-8. Idiograms of *T.patula* L. 6. 'GoldenGate'. 7. 'Janie'. 8. 'Little Hero'.

and the KA was of type 1B.

T. erecta L. 'Perfection Yellow'

The karyotype formula of *T. erecta* L. 'Perfection 'Yellow' 'was 2n=2x=4sm+20m. The ratio of the longest to the shortest chromosome was 3.35, length type was 2n=6L+6M2+6M1+6S, average arm ratio was 1.31 and the KA was of type 1B.

T. erecta L. 'Perfection Orange'

The karyotype formula of *T. erecta* L. 'Perfection 'Orange' ' was 2n=2x=24m. The ratio of the longest to the shortest chromosome was 2.43, length type was 2n=6L+8M2+6M1+4S, average arm ratio was 1.17 and the KA was of type 1B.

T. erecta L. 'Inca Orange'

The karyotype formula of *T. erecta* L. 'Inca Orange' was 2n=2x=2sm+22m. The ratio of the longest to the shortest chromosome was 2.50, length type was 2n=4L+8M2+8M1+4S, average arm ratio was 1.32 and the KA was of type 1B.

T. erecta L. 'Inca Yellow'

The karyotype formula of *T. erecta* L. 'Inca Yellow' was 2n=2x=24m. The ratio of the longest to the shortest chromosome was 2.84, length type was 2n=4L+4M2+12M1+2S, average arm ratio was 1.30 and the KA was of type 1B.

DISCUSSION

The main carrier of genetic substances was chromosome. The size, number and even morphology characters of chromosome were relatively stable in plants, alternation of generations are not easily affected by environmental conditions. Therefore, the karyotype and chromosome number could provide cytological information for the plant classification, phylogeny and relationship identification.

Our results indicate that no satellite existed in the tested *Tagetes* plants, all with submetacente (sm) or metacenters (m). In the last several years, some efforts have been offered to karyotype analysis on a few *Tagetes* plants. Li et al. (2005) studied on the chromosome number of *T. erecta* 'ACHY021' 'PBLY026' and *T. patula* 'PBHO029', the result was consistent with ours. Qi et al. (2008) only studied on karyotype type of *T. erecta* L. 'Little Hero'; their result 2B was different from ours 1B. Wang and Li (1987) have studied the chromosome number and karyotype formula about ten composites. In their paper, the karyotype formula of genus *Tagetes* was 2n=24=6sm+16st(2SAT)+2t, which was different from ours. However, compared to theses reports, our research was more systematic. From a lot of work for a long time, we can ensure that *T. erecta* L. and *T. patula* L. had the same basic chromosome number twelve, and the chromosome numbers were different, *T. erecta* L. was diploid 2n=2x=24, *T. patula* L. was tetraploid 2n=4x=48. The difference between our result and others probably came from the experimental error, while the true causes still needed more researches to illustrate.

Karyotype differences of nine *T. erecta* L.cultivars and three *T. patula* L. cultivars were mainly displayed in such aspects as average arm ratio, karyotype formula and index of the karyotypic asymmetry etc. For the *T. erecta* L cuiltivars, As.K% ranged from 54.06% to 64.02%, average arm ratio was from 1.17 to 1.74 and their primary karyotype types were 1B except for 'Taishan' was 2B. Metacentric chromosomes existed in the every tested cultivar, while submetacentric chromosomes did not. Among 12 pairs of chromosomes in 'Taishan', 7 pairs were submetacenter (sm). But, no pairs of submetacenter chromosomes was found in both *T. erecta* L 'Marvel 'and *T. erecta* L 'Inca'. Likewise, within the *T. patula* L. cultivars, As.K% ranged from 59.67 to 61.34%, average arm ratio was from 1.45 to 1.56, with all karyotype types belong to 1B. Chromosome constitution was same as *T. erecta* L. and no submetacenter existed in 'Jenie'. In recent years, the karyotypes studies have been not only on different species but also on different cultivars (Gao and Zhuang, 2009; Zhan et al., 2009, 2010; Wang et al., 2010). In these reports, the karyotype differences between cultivars were also included. This difference maybe as a result from during long-term breeding process, the chromosomal hybridization occurred between different populations or individuals with different karyotypes.

According to Levitzky, (1931) and Stebbins (1971), the basic trend of karyotype evolution was from symmetrical to asymmetrical for the angiosperm. Meanwhile, according to Arano (1963), when the As.k% was less than 60%, karyotype symmetries were high. It could be deduced that genus *Tagetes* asymmetries were relatively low. Some cultivars have the same karyotype formula, so they may have a near genetic relationship. This result will provide basic cytological information for the breeding work on marigold. But the correct genetic relationship between these cultivars also needs to be researched combined with some other methods. Previous studies by Wang and Li (1987), Li et al. (2005) and Qi et al. (2008) got the same results in basic chromosome number of genus *Tagetes* plants steadily as twelve. The basic chromosome number was single and maybe support that the evolutionary process was relatively simple than those whose basic chromosome number were not single (He

and Zhang, 2009). However, more related molecular biotechnology researches such as gene sequencing and molecular markers were need to be carried out on more *Tagetes* plants.

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