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Full Length Research Paper

Karyotype analysis of three *Solanum* plants using combined PI-DAPI staining and double fluorescence *in situ* hybridization with 45S and 5S rDNA probes

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In this study, mitotic metaphase chromosomes of *Solanum surattense* Burm., *Solanum lyratum* Thunb. and *Solanum photeinocarpum* Nakam. were well prepared using an advanced chromosome preparation method. The chromosomes were distinguished by combined PI-DAPI (CPD) staining and double fluorescence *in situ* hybridization (FISH) with 45S and 5S rDNA probes and their molecular cytogenetic karyotypes were established. Although, the karyotype of *S. surattense* Burm. and *S. photeinocarpum* Nakam was first established, the karyotype formulas of the three *Solanum* plants (*S. surattense* Burm., *S. lyratum* Thunb. and *S. photeinocarpum* Nakam) can be described as follows: 2n=24=18m +4sm+2st (2SAT), 2n=24=18m +4sm+2st (2SAT) and 2n=24=18m +6sm (2SAT). Moreover, the karyotype asymmetry of the three species belongs to 2A type. After CPD staining, the centromeres of all chromosomes in the three species were shown as red CPD bands, indicating the presence of GC-rich DNA sequences in the chromosomes. Subsequential double FISH shows that *S. surattense* Burm., *S. lyratum* Thunb. and *S. photeinocarpum* Nakam, all have a pair of 45S rDNA sites located on chromosomes, and all of these 45S rDNA sites correspond to the respective prominent CPD banded regions. *S. surattense* Burm., *S. lyratum* Thunb. and *S. photeinocarpum* Nakam correspond to the CPD banded regions at the ends of chromosomes 8, 10 and 12, respectively. The three *Solanum* plants all have a pair of 5S rDNA sites. In *S. surattense* Burm., the 5S rDNA sites are located on the long arm of the 6th chromosome pair, while the 5S rDNA sites of *S. lyratum* Thunb. and *S. photeinocarpum* Nakam are all located on the short arms of the 4th chromosome pair. Our study shows that the CPD bands and rDNA FISH signals provide effective chromosome markers allowing us to establish accurate molecular cytogenetic karyotypes of the three species tested.

Key words: *Solanum surattense* Burm. *Solanum lyratum* Thunb., *Solanum photeinocarpum* Nakam, karyotype, CPD staining, fluorescence *in situ* hybridization (FISH).

INTRODUCTION

Solanum is the largest species of solanaceae plants. There are more than 2000 kinds of plants all over the world, distributed in tropical and subtropical regions, some of which are produced in temperate zone, and most are produced in tropical South America. There are 39 categories and 14 varieties of *Solanum* distributed throughout China. Twenty of them have been applied to traditional and folk medicine (Xie and Li, 2006). Many of the *Solanum* plants have the effect of clearing heat, detoxification, activating blood and absorbing clots, and are even effective on furunculosis, overstaffing, traumatic injury, winter cough, acute glomerulonephritis, rheumatoid

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Abbreviations: CPD, Combined PI-DAPI; **FISH,** fluorescence *in situ* hybridization; **FITC,** tetramethyl rhodamine isothiocyanate; **TRITC,** tetramethyl rhodamine isothiocyanate.

arthritis, leukorrhea, edema and gonorrhea. However, the three species of Solanum are traditional medicinal plants. Their chemical composition varies with significant efficacy and diversification of anti-cancer, anti-tumor, anti-allergic, antibacterial, anti-inflammatory, and enhanced immune function and so on. There is a wide application in the clinical practice.

Solanum surattense Burm. belongs to the family of Solanaceae, with perennial herbs and subshrub. It is often seen in wasteland, around villages, roadside, hemi dankness and fertile soil. Spine is observed in the whole plant branches and trunk, and bristle is observed in the young part. Its fruit contain a variety of alkaloids such as solamargine, solasonine, etc. *S. surattense* Burm. is an important medicinal plant, in that its root, fruit or whole herbs can all be used. It has many medicinal efficacies such as promoting blood, anesthesia, sedation, antitussive and antiasthmatic, and is used as the main treatment in traumatic injury, lumbocrural pain, stomachalgia, toothache, chilblain, asthma, and so on. It is widespread in tropical regions, and is found to be distributed widely in Henan, Liaoning and south of the Yangtze River in China (Lin et al., 2006). However, karyotype analysis of *S. surattense* Burm. is yet to be reported in the literature.

There are few reports related to *S. surattense* Burm, but they only refer to its botanical characteristics and the characterizations of pharmacognosy research; as such, its karyotype analysis is yet to be reported in the literature.

Solanum lyratum Thunb. is a species of *Solanum*, belonging to the perennial herbaceous climber. It grows in the hillside or on-street, and is mainly reproduced by seed and also by cutting propagation and ramet reproduction. The major producing provinces are Jiangsu, Shandong, Fujian, Jiangxi, Guangdong and Sichuan in China. *S. lyratum* Thunb. is a traditional medicinal plant. The whole plant can be used as medicines, for cold in property, sweet taste, low toxicity, with the effect to anti-tumor, heat-clearing and detoxification, absorb clots and so on. It is used to treat damp-heat, jaundice, anemopyretic headache, leukorrhagia and rheumatoid arthritis (Sun et al., 2006).

S. lyratum Thunb. is a diploid species. Its chromosome number is 2n=24, and its karyotype formula is 2n=24=20m+4sm. However, it belongs to Stebbins's 1A type (Ge and Li., 1990; Yang 2001), and the composition of its chromosome relative length is $2n = 24 = 10M_2 + 14 M_1$.

Solanum photeinocarpum Nakam. is a species of *Solanum*. It is found to be distributed in some places in China (for example, south Yunnan, Jiangxi, Hunan, Guangxi, Guangdong and Taiwan), and it grows in nearby brooks and jungles, damp place or the waste land of the forest edge for a long period of time. The whole plant can be used as medicines, cold in property and bitter taste, though their main functions are to clear heat, detoxify toxin, clear liver, eliminate dampness and normalize the gallbladder, for the treatment of jaundice, blood urination, hot and painful strangury, dysentery, red eyes, sore throat, etc. In Dianxin Minority Area, people like eating the whole plant as a potherb. Although, diseases and insect pests rarely attack the plant, the pesticide contamination is evitable (Liu et al., 2006).

Solanum nigrum. L has two species and one variety, namely *S. nigrum* L., *S. nigrum* L. var pauciflorun Liou and *S. nigrum* L.var. suaveolens G. L. Guo. It has been reported that the chromosome number of the three types of *S. nigrum* L. are 2x=24 for *S. nigrum* L. var. suaveolens G. L. Guo, 4x=48 for *S. nigrum* L. var pauciflorun Liou, and 6x=72 for *S. nigrum* L. (Yang, 1996; Xu, 2004), which shows the existence of polyploidy of this plant. According to the report given so far, the research mainly focuses on the chromosome behavior of hybridized *Solanum nigrum* L. and polyploid *S. nigrum* L. in meiosis., elucidate formation, evolution and systematic classification of multiple species of *S. nigrum* L.

Judging from the karyotype analysis in the report, the classification foundation of *solanum* chromosome is not sufficient, not to mention the banding analysis of the chromosome. In this research, by using modified CPD dyeing procedure (She et al., 2006), we dyed the chromosome of *S. surattense* Burm., *S. lyratum Thunb.* and *S. photeinocarpum* Nakam. in mitosis, and then did dual color fluorescence *in situ* hybridization to biotin labeled 5S rDNA and Dig-Labeled 45S rDNA in order to identify the abundant GC chromosome region in their genome, and provide more identification marks for the chromosome of the three species. We could establish their accurate molecule cell genetic karyotype on this basis, and provide some evidence for interspecific systematic relationship and evolution trend. In the present study, the variability of 5S and 45S rDNA site was investigated in three species of *Solanum*.

MATERIALS AND METHODS

Material was collected in the field for cytological analysis (Table 1). Young root tips were pre-treated with saturated α-bromonaphthalene at 28℃ for 1 h, fixed overnight at room temperature in Carnoy 3:1 (ethanol : acetic acid). Leaf buds of *Solanum* were collected in the field and fixed directly in Carnoy. Slides were stained briefly with 4`,6-diamidino-2-phenylindol (DAPI)/glycerol stored at -20℃. To locate the 45S rDNA sites, probes SK18S and SK25S, containing 18S and 25S rDNA of *Arabidopsis thaliana* L. were used (Unfried et al., 1989; Unfried and Gruendler, 1990). The 5S rDNA was obtained from total genomic DNA of cabbage by PCR using primers 5-'GGATGCGATCATACCAGCAC-3' and 5'-GGGAATGCAACACGAGGACT-3'. The *in situ* hybridization procedure followed that of Moscone et al. (1996). Probe 45S was labelled with digoxigenin-11-dUTP and detected with FITC (tetramethyl rhodamine isothiocyanate), while the 5S rDNA probe was labelled with biotin-11-dUTP and detected with TRITC (tetramethyl rhodamine isothiocyanate). Chromosomes were counterstained with DAPI. Cells were photographed with a BX60 OLYMPUS epifluorescence microscope, and their images were captured with a CoolSNAP-CCD video camera using Meta Imaging Series software.

In this study, Adobe Photoshop software was used to take photos of the chromosomes, and karyotype analysis was studied by Li and Chen (1985) methods. Relative length was expressed as a

Table 1. Source of materials.

Taxon	Locality
Solanum surattense Burm.	Huaihua, Hunan
Solanum lyratum Thunb.	Huaihua, Hunan
Solanum photeinocarpum Nakam.	Huaihua, Hunan

Table 2. Relative length coefficient and chromosome length classification.

Relative Length coefficient	Chromosome Length classification
I, R, L<0.76	short chromosome (S)
0.76≤I, R, L≤1.00	medium-short chromosome (M1)
1.01≤I, R, L≤1.25	middle long chromosome (M2)
I, R, L≥1.26	long chromosome (L)

Table 3. Arm ratio and centromere location classification.

Arm ratio	Centromere location	Abbreviation
1.00	Median sternotomy centromere	M
1.01~1.70	median region	m
1.71~3.00	submedian region	sm
3.01~7.00	subterminal region	st
7.01以上	terminal region	t
∞	terminal point	T

Table 4. Arm ratio and karyotype classification.

Longest/ shortest	Chromosome percent of arm ratio >2			
	0.0	0.01~0.5	0.51~0.99	1.0
<2:1	1A	2A	3A	4A
(2:1)~ (4:1)	1B	2B	3B	4B
>4: 1	1C	2C	3C	4C

percentage of the calculation method by Levan et al. (1964) formula, which shows that the relative length = (chromosome length / total length of genome) × 100 (Table 2). The relative length of the chromosome coefficient = chromosome length / whole group of the average chromosomes length. However, the corresponding chromosome length classification is listed in Tables 1 and 2. According to chromosome arm ratio, arm ratio = long arm / short arm was used to determine the types of centromere position (Table 3).

According to the method proposed by Stebbins (1971) as the standard for karyotype classification, the length ratio of the chromosomes and arm ratio was determined, and the karyotypic degree of symmetry and asymmetry was distinguished (Table 4).

RESULTS

Karyotype

Solanum surattense Burm. karyotype

The number of metaphase chromosome in somatic cells is 2n=24 in *S. surattense* Burm. The karyotype parameters are listed in Table 5. From the table, we see that *S. surattense* Burm.`s karyotype formula is 2n=24=18m+4sm+2st (2SAT), the chromosomes of 1st, 2nd, 3rd, 5th, 7th, 9th, 10th, 11th, 12th are in the median region chromosome, the chromosomes of 4th and 6th are in the submedian region chromosome, the 8th chromosome is in the subterminal region, and the satellite is located in short arm terminal. According to the length of chromosomes, the 7th, 8th, 9th, 10th, 11th and 12th chromosomes are medium-short chromosomes (M1), while the others are medium-long chromosomes (M2). The arm ratio of the longest and shortest chromosome is 1.54. The chromosomes with arm ratio <2 : 1 account for 0.25. The karyotype belongs to "2A" type. The absolute length of *S. surattense* Burm. is in the range of 2.30 to 3.55 μm. The chromosome morphology and karyotype is shown in Figure 1, while the idiogram is

Table 5. *Solanum surattense* Burm. karyotype parameters.

Serial number	relative length			Index of relative length	Centromere Index	Arm ratio	Type
	Long Arm	Short Arm	Total Length				
1	6.00	4.58	10.58	1.27	43.29	1.31	m
2	5.20	4.41	9.61	1.15	45.89	1.18	m
3	4.87	4.10	8.97	1.08	45.71	1.19	m
4	5.96	2.84	8.80	1.06	32.27	2.10	sm
5	4.89	3.60	8.49	1.02	42.40	1.36	m
6	5.77	2.57	8.34	1.01	30.82	2.25	sm
7	4.49	3.46	7.95	0.95	43.52	1.30	m
8*	6.04	1.91	7.95	0.95	24.03	3.16	st*
9	4.44	3.33	7.77	0.93	42.86	1.33	m
10	4.24	3.20	7.44	0.89	43.01	1.33	m
11	4.26	2.98	7.24	0.87	41.16	1.43	m
12	3.86	3.00	6.86	0.82	43.73	1.29	m

Marker "*" represents satellite chromosome, the length of satellites is not included.

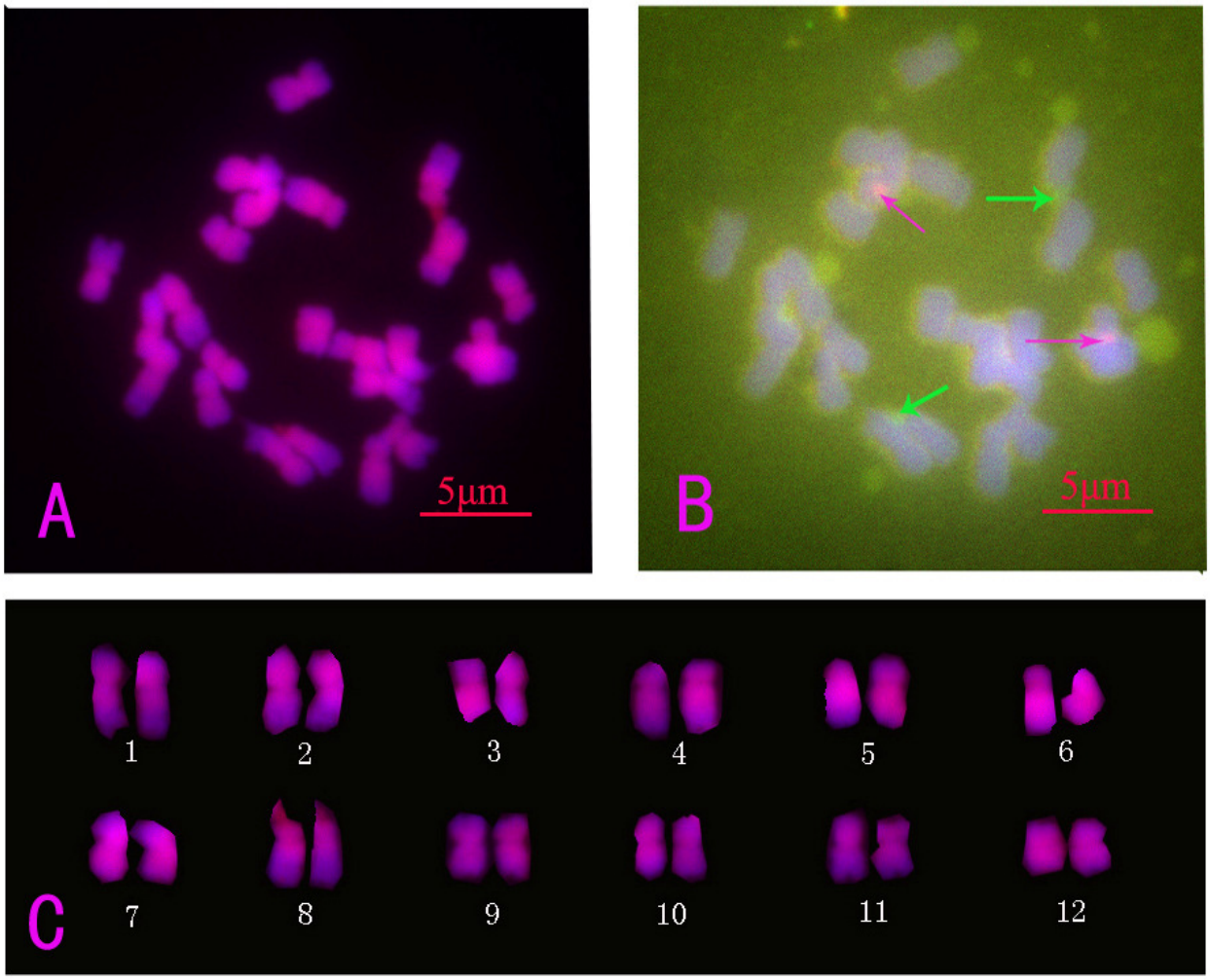

Figure 1. *Solanum surattense* Burm. CPD staining, double FISH and chromosome karyotype. A: *S. surattense* Burm CPD Staining B: *S. surattense* Burm double FISH C: *S. surattense* Burm chromosome karyotype. Red arrows indicate 5S rDNA, green arrows indicate 45S rDNA.

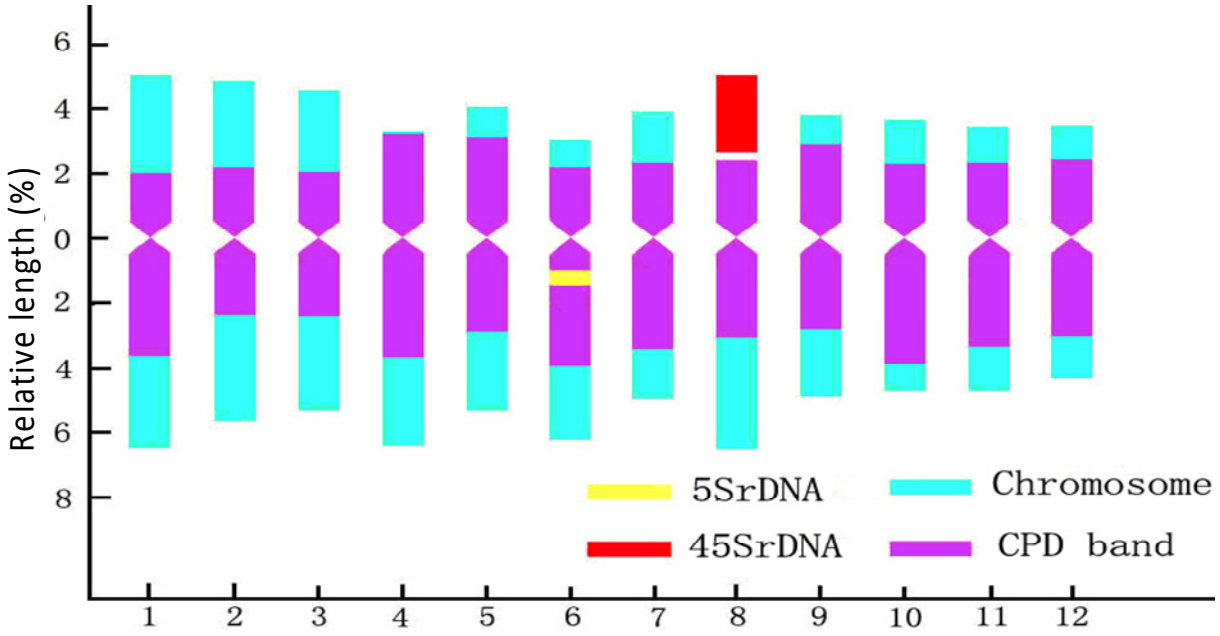

Figure 2. *Solanum surattense* Burm. ideogram.

Table 6. *Solanum lyratum* Thunb. karyotype parameters.

Serial number	Relative length			Index of relative length	Centromere index	Arm ratio	Type
	Long arm	Short arm	Total Length				
1	5.37	4.27	9.64	1.16	44.29	1.26	m
2	5.10	4.14	9.24	1.11	44.81	1.23	m
3	4.91	3.89	8.80	1.06	44.20	1.26	m
4	6.11	2.69	8.80	1.06	30.57	2.27	sm
5	4.83	3.80	8.63	1.04	44.03	1.27	m
6	4.82	3.68	8.50	1.02	43.29	1.31	m
7	4.66	3.71	8.37	1.00	44.32	1.26	m
8	4.84	3.37	8.21	0.99	41.05	1.44	m
9	5.58	2.62	8.20	0.98	31.95	2.13	sm
10	4.61	3.29	7.90	0.95	41.65	1.40	m
11	4.36	3.01	7.37	0.88	40.84	1.45	m
12*	4.95	1.39	6.34	0.76	21.92	3.56	st*

Marker "*" represents satellite chromosome, the length of satellites is not included.

shown in Figure 2.

Solanum lyratum Thunb. karyotype

The number of metaphase chromosome in somatic cells is 2n=24 in *S. lyratum* Thunb. The karyotype parameters are listed in Table 6. The results show that *S. lyratum* Burm`s karyotype formula is 2n=20=18m+4sm+2st (2SAT), the chromosomes of the 1st, 2nd, 3rd, 5th, 6th, 7th, 8th and 11th are median region chromosomes, the chromosomes of the 4th and 9th are submedian region chromosomes, the 12th chromosome is in the subterminal region, and the satellite is located in the short arm terminal. According to the length of chromosomes, the 7th, 8th, 9th, 10th, 11th and 12th chromosomes are medium-short chromosomes (M1), while the others are medium-long chromosomes (M2). The arm ratio of the longest and shortest chromosome is 1.52, although the chromosomes with arm ratio <2 : 1 account for 0.25. The karyotype belongs to "2A" type. The absolute length of *S. lyratum* Burm is in the range of 2.23 to 3.39 μm. The chromosome morphology and karyotype is shown in Figure 3, while the idiogram is shown in Figure 4.

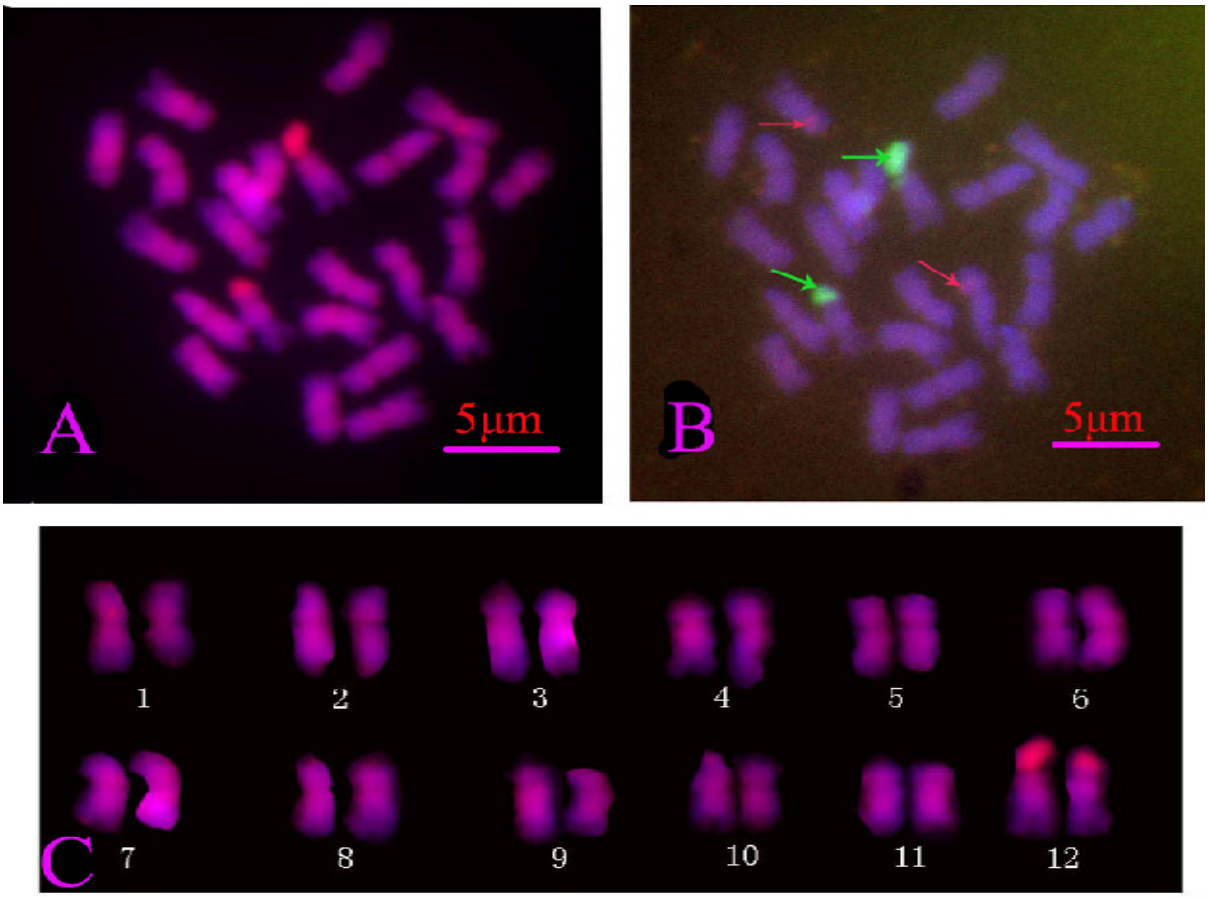

Figure 3. *Solanum lyratum* Thunb. CPD staining, double FISH and chromosome karyotype. A: *S. lyratum* Thunb. CPD Staining B: *S. lyratum* Thunb. double FISH C: *S. lyratum* Thunb. chromosome karyotype. Red arrows indicate 5S rDNA, green arrows indicate 45S rDNA.

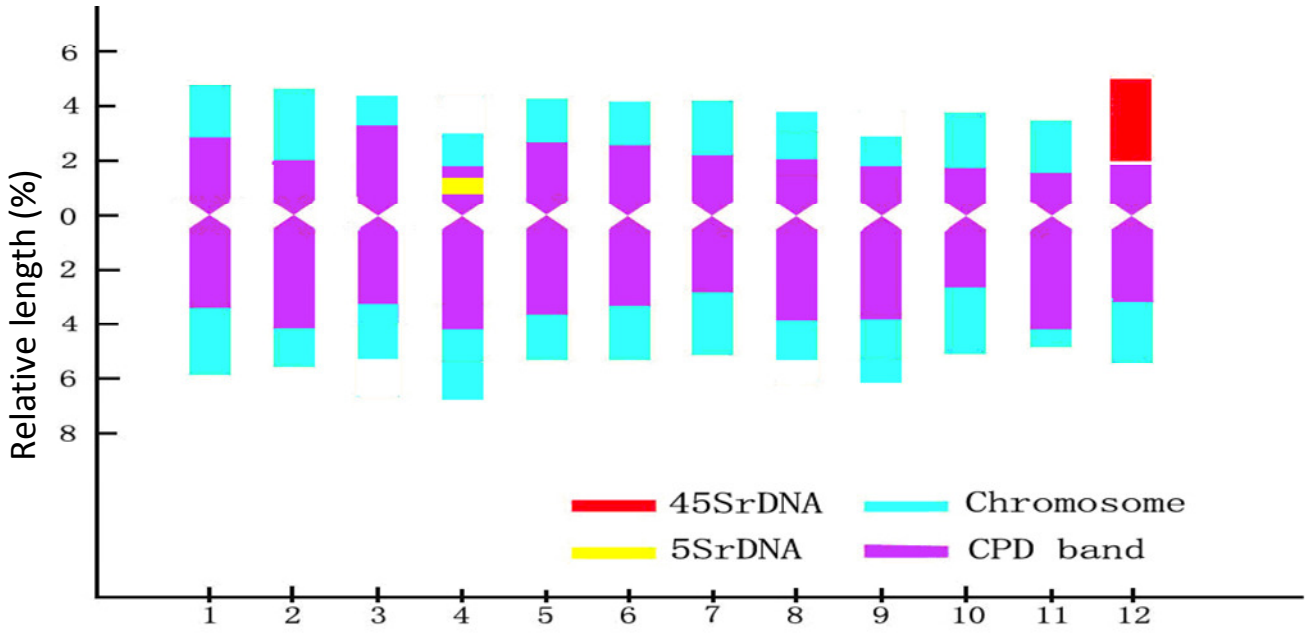

Figure 4. *Solanum lyratum* Thunb. idiogram.

Table 7. *Solanum photeinocarpum* Nakam. karyotype parameters.

Serial number	Relative length			Index of relative length	Centromere index	Arm ratio	Type
	Long arm	Short arm	Total length				
1	5.69	4.25	9.94	1.19	42.76	1.34	m
2	5.31	4.00	9.31	1.12	42.96	1.33	m
3	5.07	3.92	8.99	1.08	43.60	1.29	m
4	4.96	3.84	8.80	1.06	43.64	1.29	m
5	5.73	2.84	8.57	1.03	33.14	2.02	sm
6	4.88	3.59	8.47	1.02	42.38	1.36	m
7	4.68	3.58	8.26	0.99	43.34	1.31	m
8	4.56	3.53	8.09	0.97	43.63	1.29	m
9	4.52	3.26	7.78	0.93	41.90	1.39	m
10	5.19	2.31	7.50	0.90	30.80	2.25	sm
11	4.17	3.28	7.45	0.89	44.03	1.27	m
12*	4.61	2.23	6.84	0.82	32.60	2.07	sm*

Marker "*" represents satellite chromosome, the length of satellites is not included.

Solanum photeinocarpum Nakam. chromosome karyotype

The number of metaphase chromosome in somatic cells is 2n=24 in *S. photeinocarpum* Burm. The karyotype parameters are listed in Table 7. The results show that *S. photeinocarpum* Burm.`s karyotype formula is 2n=24=18m+6sm+2st (2SAT), the 1st, 2nd, 3rd, 4th, 6th, 7th, 8th, 9th and 11th chromosomes are median region chromosomes, the 5th, 11th and 12th chromosomes are submedian region chromosomes, and the 12th chromosome is in the subterminal region. According to the length of chromosomes, the 7th, 8th, 9th, 10th, 11th and 12th chromosomes are medium-short chromosomes (M1), while the others are medium-long chromosomes (M2). The arm ratio of the longest and shortest chromosome is 1.45, whereas the chromosomes with an arm ratio of <2 : 1 account for 0.25. The karyotype belongs to "2A" type. The absolute length of *S. photeinocarpum* Burm. varies from 2.38 to 3.46 µm. The chromosome morphology and karyotype is shown in Figure 5, while the idiogram is shown in Figure 6.

CPD staining and FISH detection

After CPD staining, all chromosomes display CPD banding inf *S. surattense* Burm., *S. lyratum* Thunb. and *S. photeinocarpum* Nakam. This shows that the GC content is rich in them (Figures 1A, 3A and 5A).

45S and 5S rDNA double FISH display of *S. surattense* Burm. possesses a pair of 45S rDNA sites (green signal), located in the eighth chromosome, corresponding to the satellite of the chromosome short arm (Figure 1B). *S. lyratum* Thunb. and *Solanum photeinocarpum* Nakam. possess a pair of 45SrDNA sites respectively, and are all located in the twelfth chromosome short arm satellite (Figures 3B and 6B). However, there is a strong hybridization signal of 45S rDNA in the three species.

S. surattense Burm., *S. lyratum* Thunb. and *S. photeinocarpum* Nakam. all possess a pair of 5S rDNA site (red signal). The 5S rDNA site of *S. surattense* Burm. is located in the sixth chromosome long arm (Figure 3.1:B), but in *S. lyratum* Thunb. and *S. photeinocarpum* Nakam., it is located in the fourth chromosome short arm (Figures 3B and 5B). By comparing the hybridization signal of 45S rDNA site and 5S rDNA site, 45S rDNA site displays strong hybridization signal in the three species. The position of CPD banding, 45S and 5S rDNA FISH are tabulated in Table 8.

DISCUSSION

In general, both the number and position of 45S rDNA sites, detected by FISH, coincided with the CPD bands and secondary constrictions observed in the karyotypes. However, some 45S rDNA regions that were not detected as secondary constrictions could be identified as CPD bands. On the other hand, the number of 5S rDNA sites did not correlate with any other cytological parameter. In *S. lyratum* Thunb. and *S. photeinocarpum* Nakam., variation in the number and position of rDNA sites was very limited and did not constitute an important cytological marker to characterize species. *S. surattense* Burm. can be easily identified by the difference in the position of 45S and 5S rDNA sites.

It was always observed on chromosome VI or IV in several species that visualization of the 5S rDNA was limited by its small size. For instance, in *S. surattense* Burm, the 5S rDNA sites were minuscule points inside the chromosome mass (Figure 2). The small chromosome size and the tendency of fluorescence to expand beyond the region marked by the probe did not allow us

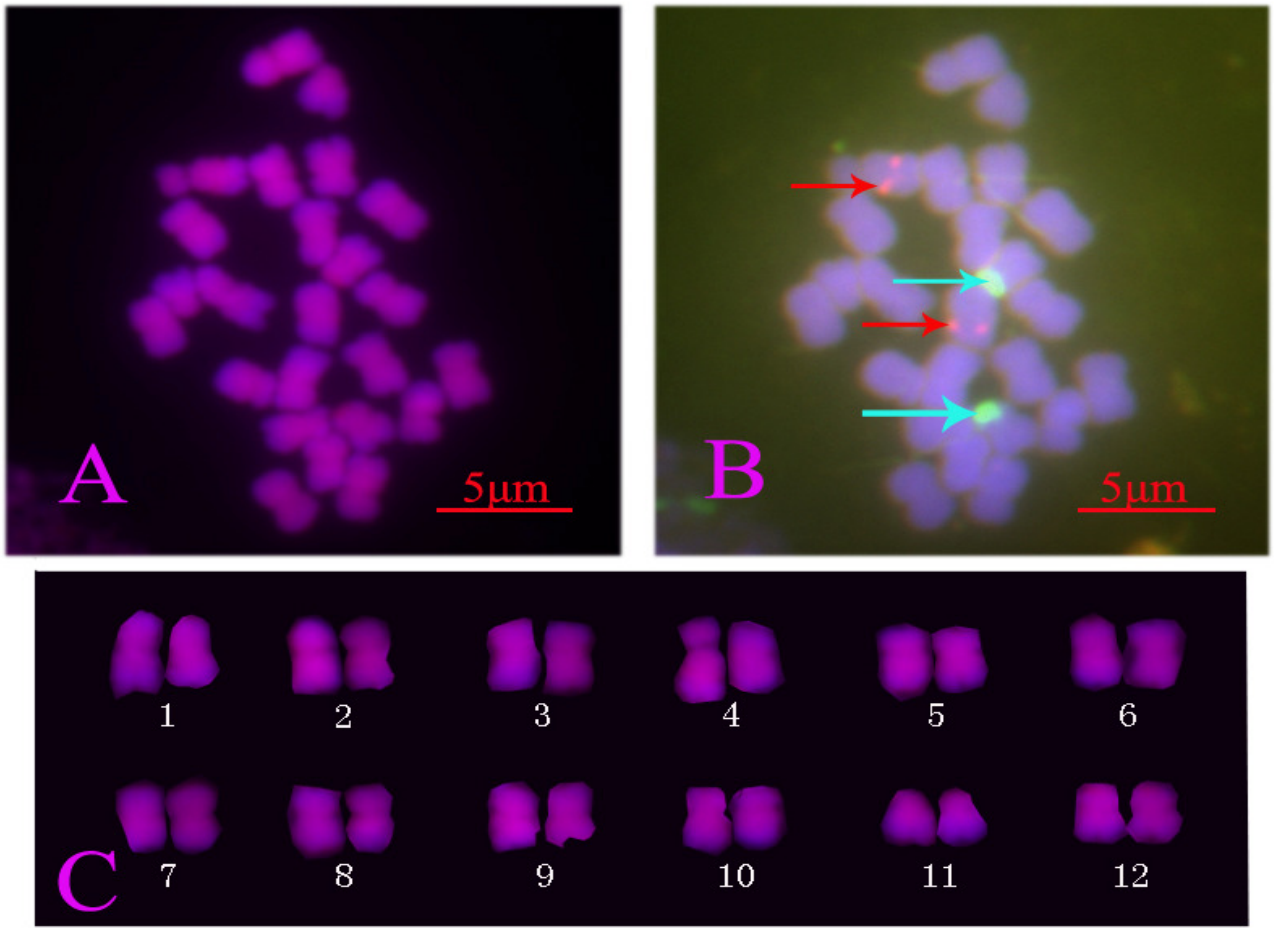

Figure 5. *Solanum photeinocarpum* Nakam. CPD staining, double FISH and chromosome karyotype Fluorescent *in situ* hybidization with 5S (red) and 45S (green) in three *Solanum* species. A: *S. photeinocarpum* Nakam CPD Staining B: *S. photeinocarpum* Nakam. double FISH C: *S. photeinocarpum* Nakam. chromosome karyotype. Red arrows indicate 5S rDNA, green arrows indicate 45S rDNA.

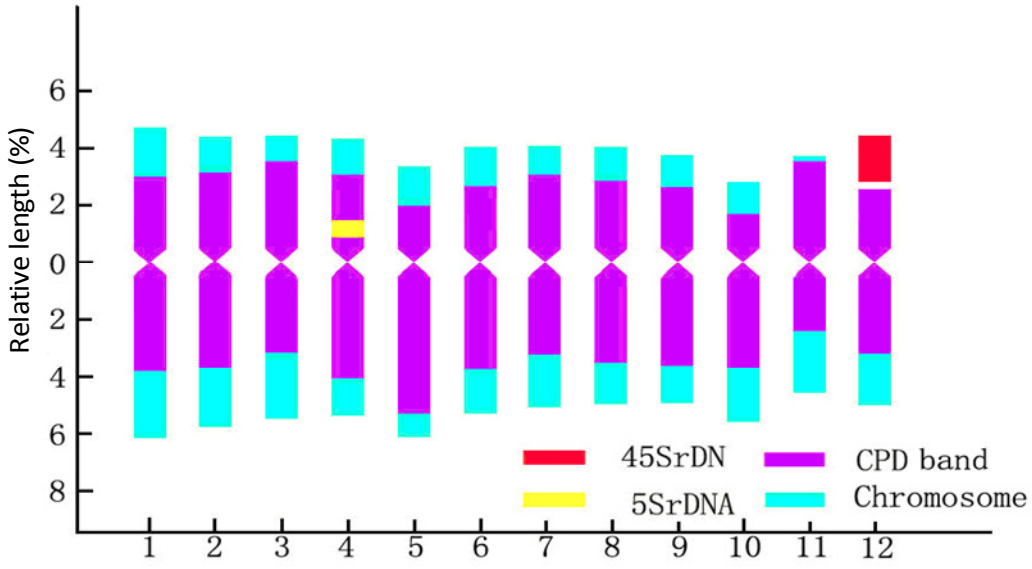

Figure 6. *Solanum photeinocarpum* Nakam. ideogram.

Table 8. CPD banding, 45S and 5S rDNA FISH signal.

Variable	*Solanum surattense* Burm. (chromosome position)	*Solanum lyratum* Thunb. (chromosome position)	*Solanum photeinocarpum* Nakam. (chromosome position)
CPD banding	All chromosome	All chromosome	All chromosome
45S rDNA FISH signal	8 (Satellite)	12 (Satellite)	12 (Satellite)
5S rDNA FISHsignal	6 (Long arm)	4 (Short arm)	4 (Short arm)

Table 9. Basic situation statistics of three *Solanum* plants karyotype.

Species	Karyotype formula	Chromosome relative composition	Longest / shortest chromosome	Chromosome percent of arm rato>2	Karyotype asymmetric type
Solanum surattense Burm.	2n=2x=24=18m+4sm+2st (2SAT)	6M2+6M1	1.54	0.25	2A
Solanum lyratum Thunb.	2n=2x=24=18m+4sm+2st (2SAT)	6M2+6M1	1.52	0.25	2A
Solanum photeinocarpum Nakam.	2n=2x=24=18m+6sm (2SAT)	6M2+6M1	1.45	0.25	2A

to distinguish clearly between terminal and subterminal rDNA site. In general, the 45S rDNA were larger, more numerous and more variable than 5S rDNA signals. In some group of diploid species, the 45S rDNA sites varied from one pair to two pairs. In *Solanum*, this variation was small.

This number of metaphase chromosome for the three *Solanum* species was same as that of the previous literature reported on chromosome number, where the plants of *Solanum* chromosome number x=12 were stable. This karyotype of *S. surattense* Burm. and *S. lyratum* Thunb. was 2n=24=18m+4sm+2st, but *S. photeinocarpum* Nakam. was 2n=24=18m+6sm, of which *S. surattense* Burm. and *S. photeinocarpum* Nakam. were reported for the first time, and *S. lyratum* Thunb.'s karyotype had some differences with the study result of Ge and Li (1990) (2n=24=20m+4sm). As in this study, flame-drying method was used for chromosome preparation, whereas in the study of Ge and Li (1990), conventional production methods were used. Maybe some of the differences observed in the studies' methods resulted in karyotype differences, and is also possible that the result was caused by the regional difference of test materials. The test materials of Ge and Li (1990) came from the medicine garden of Shangdong traditional chinese medicine college, and were taken from Huaihua College Campus.

The plant taxonomist and chemist, Stebbins, pointed out that a large number of symmetrical karyotypes were often found in ancient and primordial plants in the system of evolution, and a large number of asymmetric karyotypes were often seen in the late and specialized plants. Karyotype evolution goes from symmetry to asymmetry, which is supported by much research on botanic karyotype and thus accepted. Based on the foregoing analysis (Tables 8 and 9), it is concluded that all the chromosomes of the three species belong to meta centric chromosome and submetacentric chromosome. According to the report of Chen and Qin, (2004) and Stebbins (1971), cytogenetics is primitive. Compared with *S. surattense* Burm., *S. lyratum* Thunb. is at a relatively late evolutionary stage, and *S. photeinocarpum* Nakam. is even at a more relatively late evolutionary stage, but all of them preserve most of the original properties.

Conclusion

Three species of *Solanum* were investigated for the first time. In the three species of the group with x = 12, and with 2n = 24, 45S rDNA and 5S rDNA sites were observed to be terminally located (Figures 2, 4 and 6). The positions of 5S and 45S rDNA sites are summarized in Table 8. The karyotype formula, chromosome relative length of the longest /shortest chromosome ratio, the chromosomes with arm ratio <2 : 1, and the nuclear asymmetric types were all listed in Table 9. They exhibited the same number of chromosomes and chromosome morphology. The number of 45S and 5S rDNA sites was almost constant in these species, but the positions of 5S and 45S rDNA sites varied. The *S. lyratum Thunb.* displayed an asymmetrical karyotype, with 7 to 12 chromosomes as medium-short chromosomes (M1), and the others as medium-long chromosomes (M2) (Table 6). The 45S rDNA site was located on chromosome, while the 5S rDNA site was located on the short arm of pair. Although *S. photeinocarpum* Nakam. showed a karyotype very similar to that of *S. lyratum Thunb.*, it displayed 45S rDNA in chromosome pair, and 5S rDNA

in pair (Table 8), whereas in *S. surattense* Burm., these sites were located in pairs (45S) and (5S), and 5S rDNA was located on the long arm. However, these sites of 45S rDNA were similar in the three species.

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