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西藏自治区日喀则市白草核型分析

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摘要：白草(*Pennisetum centrasiaticum*)广泛分布于世界各地，被牛羊所采食。本试验采用常规根尖压片法对采自西藏日喀则市的白草进行染色体数目统计及核型分析。结果表明：白草染色体总长度为 45.87 μm，平均臂比为 1.23，核型不对称指数为 54.83；所有染色体的臂比在 1.08 至 1.70 范围内，其中近中部着丝粒染色体(sm)为 1 对，其余 17 对均为中部着丝粒染色体(m)，所有染色体未观测到随体出现；白草染色体基数为 9，核型为 $2n = 4x = 36 = 34\ m + 2\ sm$，属于四倍体植物，核型类型为 1A，是进化较原始的物种。

关键词：白草；染色体；核型；西藏

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Karyotype Analysis of *Pennisetum centrasiaticum* in Shigatse, Tibet

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Abstract: *Pennisetum centrasiaticum* is widely distributed all over the world, and it is kind of forage grazed by cattle and sheep. In this study, a conventional squash technique was used to analyze the karyotype of *P. centrasiaticum* collected from shigatse. Results showed that the sum of chromosome lengths was 45.87 μm, the average arm ratio of chromosome was 1.23, and the index of karyotypic asymmetry was 54.83. All chromosomes with the arm ratio ranged from 1.08 to 1.70, one pair of chromosomes was sm, and the remaining chromosomes were m. There was no satellite on the chromosome of *P. centrasiaticum*. The basic chromosome number was 9, and *P. centrasiaticum* was a tetraploid plant with karyotype formula $2n=4x=36=34\ m+2\ sm$, and belonged to Stebbins' 1A type. It meant *P. centrasiaticum* was a primitive species in evolution.

Key words: *Pennisetum centrasiaticum* Tzvel., Chromosome, Karyotype, Tibet

Changes in chromosome structure and quantity play an important role in reproductive isolation between populations[1]. Karyotype is the phenotypic characteristics of chromosomes[2], and karyotype analysis is a basic method in cytogenetics research by observing the chromosome morphological characteristics of species following certain standards, pairing, numbering, and grouping chromosomes artificially[3]. Therefore, investigating karyotype of chromosomes is essential for studying plant evolution[2-6].

Pennisetum centrasiaticum is a perennial herb growing mostly at hillside and dry place. It is an

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excellent forage with good nutritional value, and widely distributed in Heilongjiang, Jilin, Liaoning, Inner Mongolia, Hebei, Shanxi, Shaanxi, Gansu, Qinghai, northwest Sichuan, north Yunnan and Tibet of China[7]. Mehra and Sharma[8] reported the chromosomes number was $2n=18$ in *P. centrasiaticum* collected from Kashmir in the Northwest Himalayas. Mehra and Remanandan[9] found that *P. centrasiaticum* had two intraspecific races, diploid ($2n=18$) and pentaploid ($2n=45$) in Kumaon. Li[10] investigated karyotype and C-banding of *P. centrasiaticum* collected from the stallion station of Changling County, Jilin Province, and found that the chromosome number was $2n=2x=18=16$ m+2 st. However, karyotype information on *P. centrasiaticum* is still scarce.

Therefore, we conducted the karyotype analysis of *P. centrasiaticum* from Tibet using conventional squash technique. This would provide a theoretical basis for diversity protection and utilization of *P. centrasiaticum* germplasm resources.

1 Materials and methods

1.1 Plant materials

The seeds of *P. centrasiaticum* were collected from Aima Township (89°15′1″ E, 29°26′17″ N; masl: 4 010 m), Nanmulin County, Shigatse City, Tibet, China in October 2019. The matured seeds were placed on a petri dish with moist filter paper and germinated at 25℃. When the radicle grew to 10～15 mm, the root tips were cut off for the cytological study.

1.2 Chromosome preparation

The preparation of chromosomes at metaphase of mitotic was followed the methods of Lu[11] as below. The root tip of *P. centrasiaticum* was put into a 1.5 mL tube, the tube was kept open and moist, then treated the tube in a N_2O gas chamber under eight standard atmospheres for 2 h. Five hundred microlitter Carnoy's fluid (anhydrous ethanol: glacial acetic acid=3∶1, volume ratio) was added to each tube at 4℃ for more than 30 minutes. The fixed root tip was put on a clean glass slide with a drop of 45% acetic acid and a cover glass on the top. The cover glass was taped gently with tweezers to disperse the root tip cells evenly, then heated on the alcohol lamp for a few seconds, press it down vertically with thumb. The morphological characteristics and chromosome number in scattered chromosomes with clear morphology were observed under a microscope. The glass slide was frozen in the −80℃ freezer for 1.5 h, and then removed the cover glass, stained the slide with DAPI (REF H-1200), and taken pictures using an Olympus fluorescence microscope under the ultraviolet channel.

1.3 Karyotype analysis

Five pictures of metaphase plates with good dispersion were selected, then the cutting, the homologous chromosome pairing, numbering and the karyotypes were completed by Adobe Photoshop CS5 software. Image J software was used to measure the length of each chromosome, and Microsoft Excel 2016 was used to draw the ideogram.

Karyotype analysis were conducted according to the standard protocol reported by Li and Chen[12], the position of the centromere was determined by the method of Levan[13], index of the karyotypic asymmetry (As. K%) was calculated by Arano[14] as follows.

$$\text{As. K\%} = \frac{\text{The sum of length of the long arms in chromosome set}}{\text{The sum of chromosome length in its set}} \times 100$$

The karyotype classification was conducted according to the standard set by Stebbins[15].

2 Results and analysis

2.1 Chromosome number of *P. centrasiaticum*

Thirty cells at metaphase of mitosis were counted, except for individual cells with 15, 32, 35 chromosomes, 90% of cells possessed 36 chromosomes. According to the observation results, the chromosomes number of *P. centrasiaticum* in Shigatse, Tibet was $2n=36$. The chromosome phase of *P. centrasiaticum* at metaphase of mitotic was shown in figure 1.

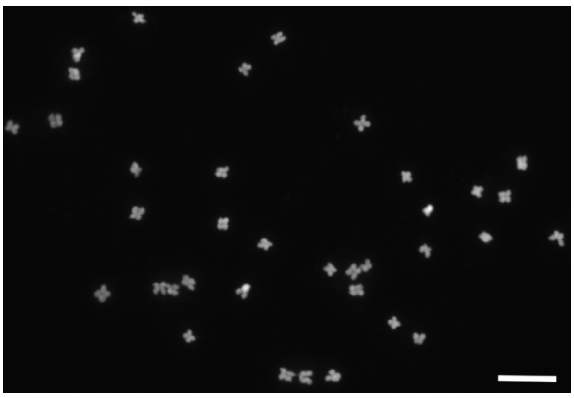

Fig. 1　Chromosome phase of *P. centrasiaticum* at metaphase of mitotic

Note: Scale bar=10 μm

2.2　Karyotype of *P. centrasiaticum*

All 36 chromosomes were sorted in pairs and numbered according to the length of the chromosome from high to low as shown in figure 2. The idiogram for chromosome of *P. centrasiaticum* was shown in figure 3.

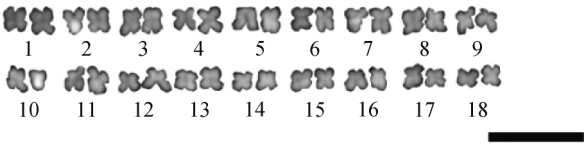

Fig. 2　Karyogram of *P. centrasiaticum*

Note: Scale bar=10 μm

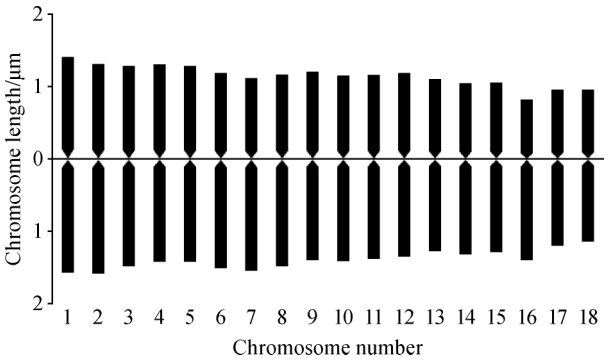

Fig. 3　Ideogram for chromosome of *P. centrasiaticum*

The karyotype statistical results were shown in Table 1. The sum of chromosome lengths was 45.87 μm, the chromosome length ranged from 2.10 μm to 2.98 μm, the relative length ranged from 4.57% to 6.49%, the long arm length ranged from 1.14 μm to 1.58 μm, the short arm length ranged from 0.82 μm to 1.41 μm, and there was no satellite on the chromosome. All chromosomes with the arm ratio ranged from 1.08 to 1.70, one pair of chromosomes was sm, and the remaining chromosomes were m. The average arm ratio of the chromosome was 1.23, and the index of karyotypic asymmetry was 54.83. The ratio of the longest chromosome to the shortest chromosome was 1.42, and there was no chromosome with arm ratio greater than 2, the karyotype of *P. centrasiaticum* in Tibet was asymmetry belonged to Stebbins' 1A type.

Table 1　Chromosome parameters of *P. centrasiaticum*

Chromosome number	Long arm/μm	Short arm/μm	Total length/μm	Relative length/%	Arm ratio	Type
1	1.57±0.07	1.41±0.01	2.98±0.08	6.49±0.15	1.11±0.04	m
2	1.58±0.01	1.31±0.02	2.89±0.03	6.31±0.01	1.21±0.01	m
3	1.48±0.03	1.29±0.01	2.77±0.05	6.04±0.08	1.15±0.02	m
4	1.42±0.07	1.31±0.02	2.73±0.09	5.94±0.16	1.08±0.03	m
5	1.42±0.01	1.29±0.02	2.71±0.03	5.90±0.09	1.10±0.00	m
6	1.51±0.01	1.19±0.01	2.70±0.02	5.88±0.07	1.27±0.00	m
7	1.54±0.02	1.12±0.01	2.66±0.02	5.80±0.03	1.39±0.01	m
8	1.48±0.01	1.16±0.01	2.65±0.02	5.77±0.08	1.27±0.00	m
9	1.40±0.03	1.21±0.02	2.60±0.05	5.68±0.08	1.16±0.00	m
10	1.41±0.01	1.15±0.01	2.56±0.02	5.58±0.01	1.22±0.00	m
11	1.38±0.05	1.16±0.02	2.54±0.07	5.54±0.17	1.19±0.02	m
12	1.35±0.02	1.19±0.02	2.53±0.04	5.52±0.06	1.13±0.00	m
13	1.28±0.03	1.10±0.01	2.38±0.04	5.18±0.07	1.16±0.01	m
14	1.32±0.02	1.05±0.03	2.36±0.05	5.15±0.12	1.26±0.02	m
15	1.29±0.02	1.05±0.01	2.34±0.03	5.10±0.04	1.22±0.01	m
16	1.40±0.01	0.82±0.01	2.22±0.02	4.84±0.02	1.70±0.00	sm
17	1.20±0.01	0.96±0.01	2.16±0.01	4.70±0.05	1.25±0.01	m
18	1.14±0.00	0.96±0.00	2.10±0.00	4.57±0.01	1.19±0.00	m

Note: m means the arm ratio ranged from 1.01 to 1.70, the centromeric point is located in the middle of the chromosome; sm means the arm ratio ranged from 1.71 to 3.00, the centromere is located near the middle of the chromosome

3 Discussion

3.1 Chromosome number and ploidy of *P. centrasiaticum*

The experiment identified for the first time that the chromosome number of *P. centrasiaticum* in Tibet was 36, which was the same as previously reported in six *Pennisetum* species[16-17]. However, Mehra and Sharma[8] found that *P. centrasiaticum* collected from Kashmir hills possessed 18 chromosomes. Mehra and Remanandan[9] also found that *P. centrasiaticum* existed in two intraspecific races, diploid ($2n=18$) and pentaploid ($2n=45$) in Kumaon. The karyotype of the *P. centrasiaticum* in Changling County, Jilin Province is $2n=18$[10]. The differences in chromosome number of *P. centrasiaticum* from different collection locations might be related to the environmental adaptation.

Swaminathan and Nath[18] reported that the basic chromosome numbers of *Pennisetum* were 7 and 9, and some *Pennisetum* with the basic chromosome numbers 5 and 8 were also found in Africa. Li and Wu[16] pointed out that the basic chromosome numbers of *Pennisetum* were divided into two categories: one was 7, such as *P. americanum*, $2n=14$, and *P. purpureum*, $2n=28, 56, 21$; the other was 9, such as *P. alopecuroides*, $2n=18$. Zhang[17] observed the chromosome of four strains of *P. longissimum* var. *intermedium* and found that they were tetraploid with the basic chromosome numbers of 9. Zhang and Kang[19] reported that the chromosome number of *P. langissimum* var. *intermedium* was $2n=6x=54$, and the basic chromosome number was 9. Followed this rule, *P. centrasiaticum* in Shigatse, Tibet should be classified as a tetraploid with the basic chromosome number of 9, and the karyotype formula was $2n=4x=36=34$ m+2 sm.

3.2 Polyploidization of *P. centrasiaticum* and its adaptation to high-altitude areas

P. centrasiaticum collected from Kashmir hills, Kumaon and Jilin Province of China is diploid and possesses a chromosome number of 18[8-10]. However, pentaploid ($2n=5x=45$) is also found in Kumaon[9], and tetraploid ($2n=4x=36$) in our research in Tibet of China. A large number of studies showed that the overall doubling of the genome was the main driving force for plant evolution. In angiosperms, the overall doubling of the genome occurrs once or multiple times in about 70% of species in their evolutionary history[20]. With high altitude and low oxygen content in air, vegetation is difficult to grow in Tibet. The results of this study showed that *P. centrasiaticum* belonged to tetraploid, which might contribute to its adaptation to the hard environment of high-altitude areas.

4 Conclusions

P. centrasiaticum collected from Shigatse, Tibet in this study was identified as a tetraploid plant with the basic chromosome number of 9, and the karyotype formula was $2n=4x=36=34$ m+2 sm, belonged to Stebbins' 1A type, which meant *P. centrasiaticum* was a primitive specie in evolution.

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