

KARYOTYPES IN 12 POPULATIONS OF *ALLIUM CAERULEUM* FROM TIANSHAN MOUNTAINS, XINJIANG, CHINA

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Abstract

Xinjiang is an important natural habitat for the wild genus *Allium* in Central Asia. The karyotypes of 12 populations of *Allium caeruleum* from Tianshan Mountains, Xinjiang, China were investigated in this paper. Results showed that all of the tested materials were diploid ($2n=2x=16$) which had M, m, sm and st types and the percentage of the total chromosome number was 1.04%, 68.75%, 25.00% and 5.21%, respectively. The satellite existed in six populations. The compositions of chromosome relative length were L, M2, M1 and S types. The percentage karyotype asymmetry index was 58.58% to 62.70%. Karyotypes was 2A or 2B. The higher evolutionary trend of karyotypes was in Zeketai population. The four groups were clustered at 5.5 genetic distance. The genetic diversity of chromosome compositions and unstable satellites were in 12 populations. The changes of chromosomes structure results in the genetic variability for adaptation of new habitats and it is one of the evolutionary strategy.

Key words: Tianshan Mountains, Xinjiang, *Allium caeruleum*, Karyotype analysis.

Introduction

The genus *Allium* is an important edible, medicinal, forage and ornamental germplasm resources with high economic value (Kole, 2011). There are about 850 species of the genus *Allium* all around the world (Wheeler *et al.*, 2013). Xinjiang is an important distribution area for distribution of the wild genus *Allium* in Central Asia (Zhu, 2008; Lin & Tan, 2017). There are some wild relatives which are *A. caeruleum*, *A. oreoprasum*, *A. galanthum* used by local people. Due to its special geographical environmental conditions, the study of genetic diversity and genetic relationship of wild *Allium* relatives has attracted much more attention (Lin & Tan, 2015; Xi *et al.*, 2015; Lin & Tan, 2017). Perennial wild *A. caeruleum* is called blue globe onion, blue ornamental onion, blue-of-the-heavens, blue-flowered garlic or azure-flowered garlic and it native to Central Asia, Xinjiang of China, Kazakhstan, Russia, Tajikistan, Uzbekistan, and Kyrgyzstan (Xu & Kamelin, 2000). It is distributed in seven ecological zones of Xinjiang, along the north of the Tianshan Mountains and south of Altai Mountains. It is an important feeding plant for cattle and sheep in early spring in special circumstances in Xinjiang. The kazak herdsmen called it as the cattle and sheep good grass. There were genetic diversity characteristics of phenotypic traits among different populations. The traits of roots, bulbs and floral form characteristics closely related to habitat characteristics (Yang *et al.*, 2014; Ma *et al.*, 2016).

The structural features of chromosomes are relatively constant. Its relative length, the position of centromeres and other indicators have genus and species specificity reference. Therefore, the karyotype analysis, morphological taxonomic characteristics and DNA molecular markers are become the basic contents of plant taxonomic identification and distant hybridization identification successfully. While, it also provide important cytological evidence to reveal the genetic relationship between the wild relatives and the genetic evolution process (Fritsch *et al.*, 2010; Hosseini & Go, 2010; Wei *et al.*, 2011; Genc *et al.*, 2013; Friesen & Fragman-Sapir, 2014; Aruga *et al.*, 2015). The study found

that there were $2n=16$, $2n=24$, and $2n=32$ chromosome ploidy differentiation in the Baluuke Mountains in Xinjiang (Yang *et al.*, 2014). It reflected *A. caeruleum* contained the rich genetic information under the special geographical environment in Xinjiang, and it had the cytological basis of genetic diversity characteristics. However, Tianshan Mountains in Xinjiang is an important distribution area, whether are there other genetic differentiation in different populations of *A. caeruleum*? How is the karyotype evolutionary and the evolution trend? The study was based on karyotype of 12 different populations of *A. caeruleum* distributed in Tianshan Mountains in Xinjiang to reveal the differences in chromosome karyotypic characteristics and explore its evolutionary trends and to provide a certain cytological basis for new varieties innovation.

Materials and Methods

Materials: The mature and plump seed used for karyotype analysis were collected from original habitats and stored in a cool and dry place. We followed Xu & Kamelin (2000) for identification of the species. A list of material collection site information used in this study is given in Table 1.

Methods: Seeds material germinated at 15°C under dark condition. When the radicle broke through the seed coat and root length was about 1cm, it was put in saturated p-Dichlorobenzene solution to treat for 8 h and used the distilled water to wash. Carnoy's fluid (absolute ethanol: glacial acetic acid = 3:1, volume ratio) fixed it 20~24h. 1mol L^{-1} HCl acid dissociated at 60°C for 8 min and 45% glacial acetic acid softened for 5 min. After washing with distilled water and drying excess water with filter paper, it was stained with improved phenol magenta for 15 min (Yang *et al.*, 2014). Each population selected 30 complete metaphase cells for chromosome observation and counting. Five chromosomes were mounted with neutral gel as they well-distributed at medium metaphase cells and made of permanent slides.

Table 1. Origins of the materials.

No's	Origin	Habitat	Population and abbreviation
1.	Batebake, Xinyuan	Dry slope	Batebake population, BT
2.	Kurdish, Gongliu	Wet land	Kurdish population, KE
3.	Zeketai, Xinyuan	Dry slope	Zeketai population, ZK
4.	Xijier, Mulei	Rock crack	Xijier population, XJ
5.	Xijier Reservoir, Mulei	Shrub slope	Reservoir population, RE
6.	Yinggepu, Mulei	Shrub slope	Yinggepu population, YG
7.	Tianchi, Fukang	Shrub slope	Tianchi population, TC
8.	Gelin, Urumqi	Gravel land	Gelin population, GL
9.	Haxionggou, Miqian	Plain	Haxionggou population, HX
10.	Lingyuan, Urumqi	Gravel land	Lingyuan population, LY
11.	Hongqi reservoir, Urumqi	Gravel land	Hongqi population, HQ
12.	Yamalike, Urumqi	Dry slope	Yashan population, YS

Statistical analysis

Under Nikon Eclipse 80i Microscope Photograph with Nis-Elements F 3.0, the length of the chromosome (compounding the length of satellites) was measured, karyotype parameters were calculated, and karyotype formulas and chromosome composition were analyzed and compared with Motic Images Advanced 3.2 (Stebbins, 1971; Genc *et al.*, 2013; Yang *et al.*, 2014). Based on the arm ratios to determine chromosome type, the arm ratio of the metacentric chromosome (M) is 1, the metacentric chromosome (m) is 1.01~1.70, the submetacentric chromosome (sm) is 1.71~3.00, and the subtelocentric chromosome (st) is 3.01~7.00. Based on AR, Lc/Sc and As K to extraction principal components then cluster analysis. Based on As K and Lc/Sc the geographical correlation and karyotype evolutionary trend were analyzed (Zhang *et al.*, 2011; Liu *et al.*, 2013). The data analysis was processed with Excel 2010 and Photoshop 8.0. The cluster analysis was with SPSS 17.0.

Results

Karyotype: *A. caeruleum* from 12 different populations had the same number of chromosomes $2n=2x=16$. They were all diploids and had 16 chromosomes. No other ploidy cells were found. There were differences in composition of chromosome types among different populations. There were total four types, M, m, sm and st in 12 populations. Statistical analysis of the percentages of different types of chromosomes in the total number of chromosomes was known, that M chromosomes was 1.04% of total chromosomes, m chromosomes was 68.75%, sm chromosomes was 25.00% and st chromosomes was 5.21%. The ZK population and the LY population were composed of the M, m and sm chromosome types. The XJ population and the TC population were composed of the m and sm chromosome types. The rest, 67% of the test population, were composed of the m, sm, and st chromosome types. Besides, there were six populations, 50% of the test population, existed satellite chromosome (SAT), The long arm of the m chromosome of the BT population, the HQ population and the YS population, the short arm of the m chromosome of the KE population were each followed one SAT. In the TC population, there were one and two SATs appeared on the long arm of chromosome m and the

long arm of chromosome sm respectively. Five SATs were found in the HX population. Two were located on the short arm of the m chromosome and three were located on the long arm and short arm of the sm chromosome (Figs. 1, 2 and 3).

The average length of the long arm of the chromosome in the 12 populations was 143.56 μ m. The average length of the short arm was 91.74 μ m. The ratio between them was 1.56:1. The total long arm length varied from 242.84 μ m (HX population) to 89.19 μ m (BT population). The ratio between maximum and minimum was 2.72:1. The total short arm length varied from 144.38 μ m (HX population) to 63.06 μ m (BT population). The ratio between maximum and minimum was 2.28:1.

The average relative length of the chromosomes in the 12 populations was between 3.81% and 9.26%. Chromosome relative length varied between 2.47% (HQ population) to 10.44% (LY population). Differences existed in the composition of chromosome lengths among different populations. Based on analysis of the relative length of chromosomes. *A. caeruleum* from 12 populations were composed of four types: long chromosomes (L), medium length chromosomes (M2), middle short chromosomes (M1), and short chromosomes (S). Statistical analysis of the percentages of different length types of chromosomes in the total number of chromosomes was known, that L chromosomes was 11.98% of total chromosomes, M2 was 39.58%, M1 was 35.94% and S was 12.50%. Among them, the BT population contained only two types of chromosomes was M2 and M1. The rest contained L, M1, M2 and S (Table 2).

The average of relative length difference (DRL) on *A. caeruleum* from 12 populations was 5.45. The change ranged from 4.2 (KE population) to 6.68 (BT population). The average of centromere index (TF) was 39.27% with a range of 36.00% (GL population) to 41.42% (BT population). The average arm ratio (AR) for the 12 populations was 1.53 (KE population) to 1.91 (GL population). Ratio of chromosomes with AR > 2 ranged from 12.5% (BT population; KE population) to 37.5% (LY population). Chromosome length ratio was ranged from 1.57 (BT population) to 2.83 (ZK population). Karyotype asymmetry coefficients (AS K) was ranged from 58.58% (BT population) to 64.00% (GL population). The karyotype type were type 2A and type 2B, the BT population was type 2A, and the rest of 11 populations were type 2B (Table 2).

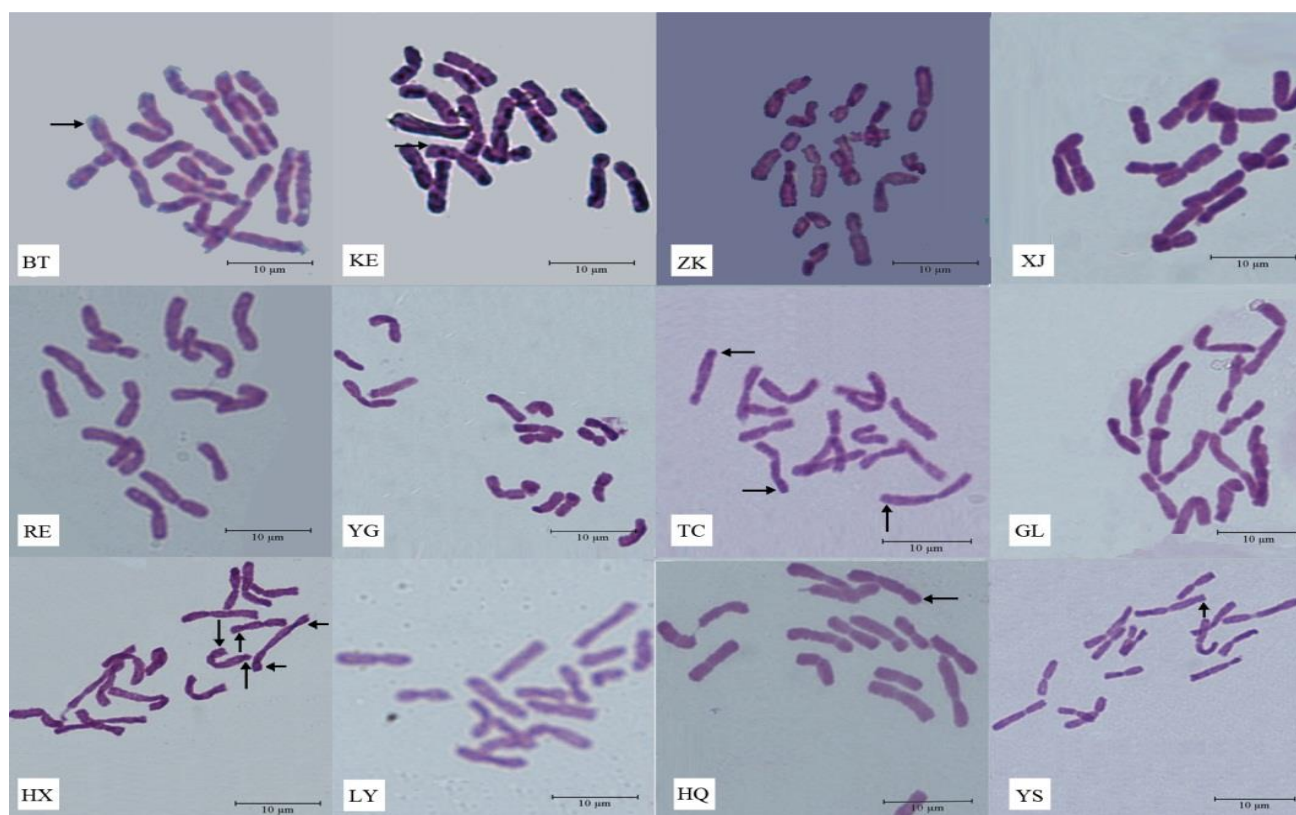


Fig. 1. The chromosome of *A. caeruleum* in 12 populations from Tianshan Mountains, Xinjiang, China.
 Note: BT: Batebake population; KE: Kurdish population; ZK: Zeketai population; XJ: Xijier population; RE: Reservoir population; YG: Yinggepu population; TC: Tianchi population; GL: Gelin population; HX: Haxiongou population; LY: Lingyuan population; HQ: Hongqi population; YS: Yashan population; The arrow indicates SAT.



Fig. 2. The karyotypes of *A. caeruleum* in different populations from Tianshan Mountains, Xinjiang, China
 Note: BT: Batebake population; KE: Kurdish population; ZK: Zeketai population; XJ: Xijier population; RE: Reservoir population; YG: Yinggepu population; TC: Tianchi population; GL: Gelin population; HX: Haxiongou population; LY: Lingyuan population; HQ: Hongqi population; YS: Yashan population.

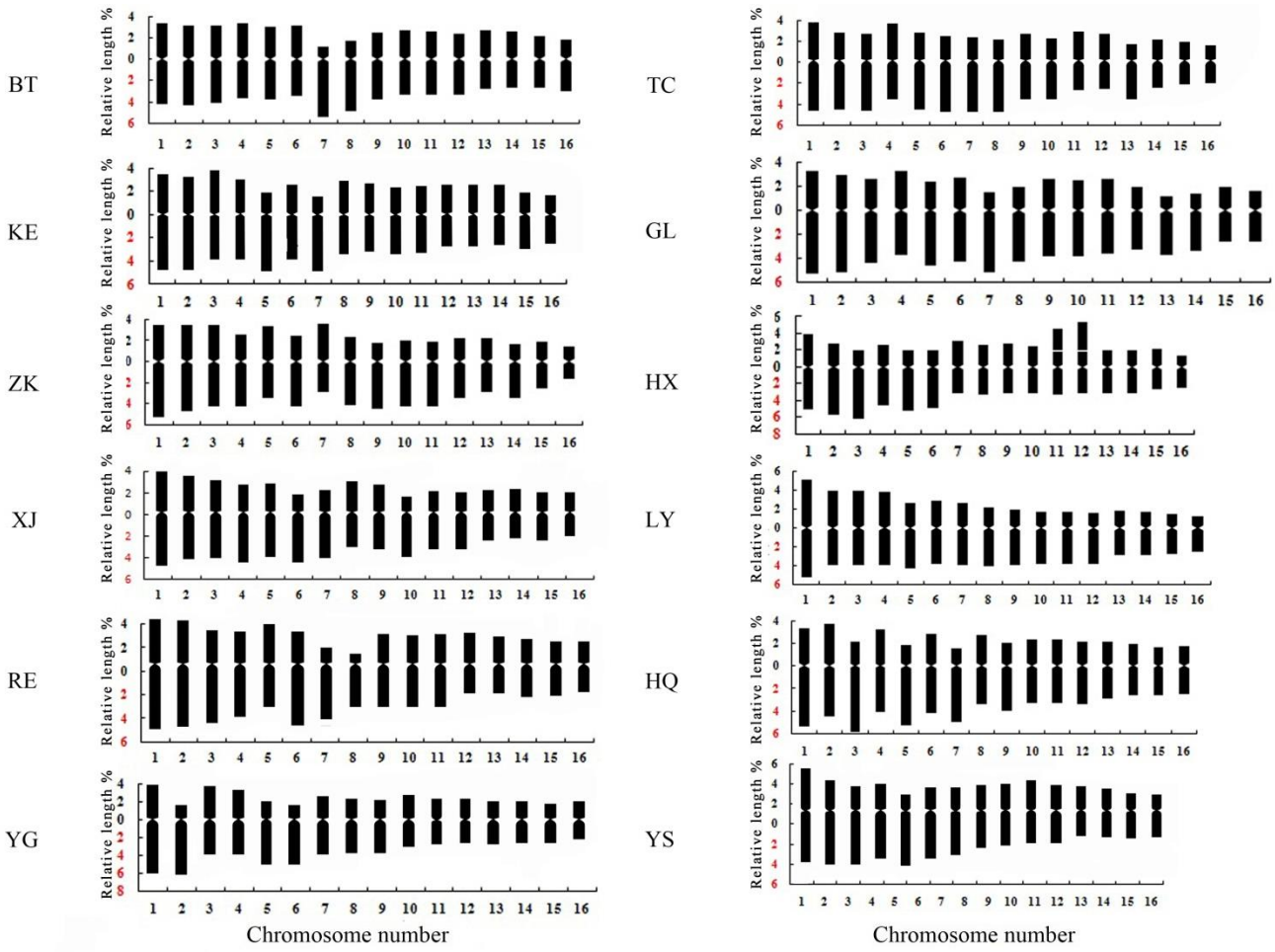


Fig. 3. The idiograms of *A. caeruleum* in different populations from Tianshan Mountains, Xinjiang, China
 Note: BT: Batebake population; KE: Kurdish population; ZK: Zeketai population; XJ: Xijier population; RE: Reservoir population; YG: Yinggepu population; TC: Tianchi population; GL: Gelin population; HX: Haxiongou population; LY: Lingyuan population; HQ: Hongqi population; YS: Yashan population.

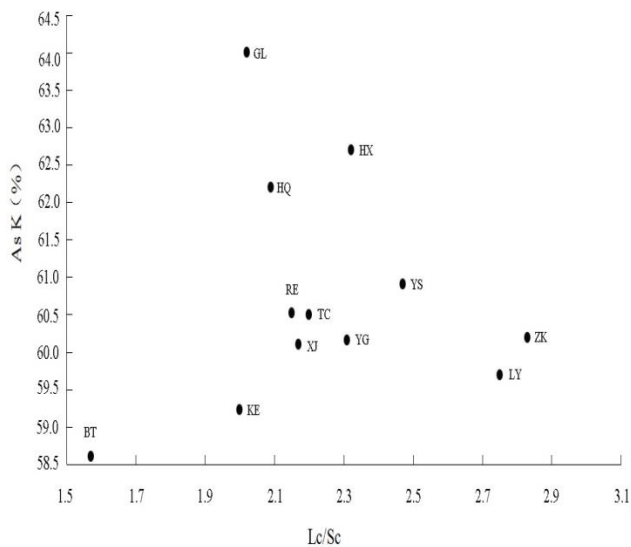


Fig. 4. The evolutionary trend of *A. caeruleum* in 12 populations from Tianshan Mountains, Xinjiang, China
 Note: BT: Batebake population; KE: Kurdish population; ZK: Zeketai population; XJ: Xijier population; RE: Reservoir population; YG: Yinggepu population; TC: Tianchi population; GL: Gelin population; HX: Haxiongou population; LY: Lingyuan population; HQ: Hongqi population; YS: Yashan population.

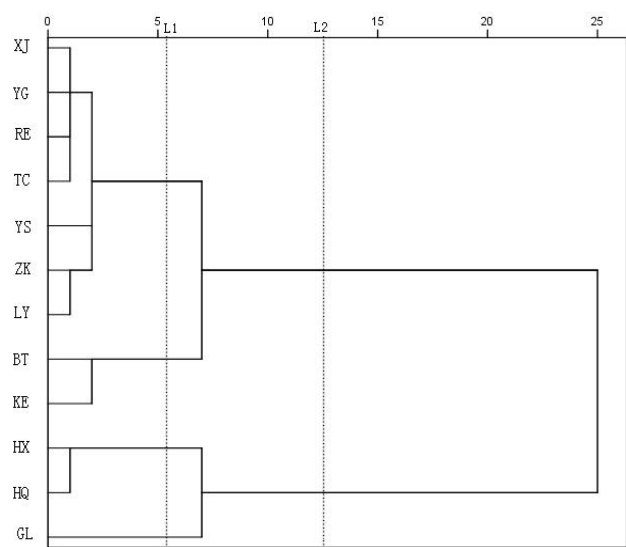


Fig. 5. The cluster of difference *A. caeruleum* based on karyotype from Tianshan Mountains, Xinjiang, China
 Note: BT: Batebake population; KE: Kurdish population; ZK: Zeketai population; XJ: Xijier population; RE: Reservoir population; YG: Yinggepu population; TC: Tianchi population; GL: Gelin population; HX: Haxiongou population; LY: Lingyuan population; HQ: Hongqi population; YS: Yashan population.

Table 2. The karyotype parameters of *A. caeruleum* in 12 populations from Tianshan Mountains, Xinjiang, China.

Code	LA (μm)	SA (μm)	Range of relative length (%)	DRL	Index of relative length	Lc/Sc	As K (%)	TF (%)	AR	PCA>2 (%)	Types	Karyotype formula and chromosome composition of relative length
BT	89.19	63.06	3.45-10.13	6.68	0.76-1.21	1.57	58.58	41.42	1.58	12.50	2A	2x=16=14m (1SAT)+1sm+1st=9M2+7M1
KE	110.72	76.24	4.20-8.40	4.20	0.67-1.34	2.00	59.22	40.78	1.53	12.50	2B	2x=16=14m (1SAT)+1sm+1st=2L+6M2+7M1+1S
ZK	130.20	86.11	3.10-8.78	5.68	0.50-1.41	2.83	60.19	39.81	1.60	25.00	2B	2x=16=1M+10m+5sm=2L+7M2+5M1+2S
XJ	125.44	83.27	4.22-9.13	4.91	0.67-1.46	2.17	60.10	39.90	1.58	12.50	2B	2x=16=11m+5sm=2L+7M2+6M1+1S
RE	124.22	81.03	4.30-9.26	4.96	0.69-1.48	2.15	60.52	39.48	1.76	18.75	2B	2x=16=13m+1sm+2st=2L+5M2+7M1+2S
YG	115.27	76.33	4.29-9.94	5.65	0.69-1.59	2.31	60.16	39.84	1.61	25.00	2B	2x=16=13m+2sm+1st=2L+6M2+6M1+2S
TC	153.07	101.59	3.87-9.00	5.13	0.59-1.36	2.20	60.50	39.50	1.57	18.75	2B	2x=16=8m (1SAT)+8sm (2SAT)=1L+8M2+4M1+3S
GL	182.61	102.70	4.18-8.45	4.27	0.67-1.35	2.02	64.00	36.00	1.91	31.25	2B	2x=16=10m+4sm+2st=2L+8M2+4M1+2S
HX	242.84	144.38	3.86-8.98	5.12	0.62-1.44	2.32	62.70	37.30	1.75	25.00	2B	2x=16=9m (2SAT)+6sm (3SAT)+1st=3L+4M2+8M1+1S
LY	113.53	76.56	3.79-10.44	6.65	0.61-1.67	2.75	59.70	40.30	1.67	37.50	2B	2x=16=1M+8m+7sm=1L+7M2+5M1+3S
HQ	161.28	97.88	2.47-8.74	6.27	0.67-1.40	2.09	62.20	37.80	1.73	18.75	2B	2x=16=12m+3sm (1SAT)+1st=3L+4M2+6M1+3S
YS	174.30	111.72	4.01-9.90	5.89	0.64-1.58	2.47	60.90	39.10	1.62	18.75	2B	2x=16=10m (1SAT)+5sm+1st=3L+5M2+4M1+4S

Note: LA: Total length of the long arm of the chromosome; SA: Total length of the short arm of the chromosome; AR: Arm ratio; Lc/Sc: the longest chromosome relative length/the shortest chromosome relative length; DRL: The difference between minimum and maximum, relative length of chromosomes; PCA: The proportion of arm ratio greater than two; As K: Percentage karyotype asymmetry index; TF: Total form percentage

Correlation analysis and karyotype evolutionary trend analysis: The correlation were analyzed between the DRL, TF (%), As K and Lc/Sc and geographic distance respectively, the results showed that there was no correlation between each index and geographical distance. After the As K and Lc/Sc indicators were analyzed comprehensively, it were known that, the karyotype evolutionary trend of the *A. caeruleum* and the order of the karyotype evolution from low to high was BT, KE, GL, HQ, RE, XJ, TC, YG, HX, YS, LY and ZK. The closer to the right, the higher the degree of karyotype evolution, the evolutionary trend map was displayed, The ZK population was the highest and the BT population was the lowest in the 12 populations distributed in Tianshan Mountains, Xinjiang, China (Fig. 4).

Cluster analysis: Based on the average of AR, Lc/Sc, and As K of 12 populations, the primary data of principal components were used to cluster. The results indicated that the genetic distance was 12.5 (L2), the 12 populations were divided into two groups. The first group contained the GL population, the HQ population, and the HX population. The second group contained the rest nine populations. When the genetic distance was 5.5 (L1), the 12 populations were divided into four groups. The first group only contained the GL population. The second group contained the HQ population and the HX population. The third group contained the KE population and the BT population. The fourth group contained the ZK population, the YS population, the TC population, the RE population, the YG population and the XJ population (Fig. 5).

Discussion

This study clarified the *A. caeruleum* from 12 populations of Tianshan Mountains that the chromosome base number of were x=8, 2n=2x=16, and both were diploid. Chromosomes were the medium-sized size, and were basically the same size as other *Allium* species (Hosseini & Go, 2010; Genc *et al.*, 2013; Yang *et al.*, 2014; Friesen & Fragman-Sapir, 2014; Aruga *et al.*, 2015). The collection area of this test material was concentrated in the east to the west of Tianshan Mountains. No triploid and tetraploid or other ploidy differentiation was found among 12 populations. Changes in chromosome numbers were related to species evolution. And it had obvious geographic features. Such as, there were diploid and tetraploid ploidy differentiation in *A. nutans* distributed in the Haba River on Altai Mountains. *A. caeruleum* distributed on Balruk Mountain was also found a ploidy differentiation. *A. caeruleum* distributed on Tacheng Yumin were tetraploid. *A. caeruleum* distributed on Tacheng Axer were triploid. The karyotypes of the three populations of *A. caeruleum* were all type 2A (Yang *et al.*, 2014).

Compared with the present study, this reflects to a certain extent that the Balruk Mountains, which located between the Tianshan Mountains and the Altai Mountains, were the centers of diversity of chromosome ploidy differentiation of *A. caeruleum*. Secondly, there were 2n=2x and 2n=3x chromosome ploidy differentiations in *A. fetisowii* that distributed in the same

population (Yang *et al.*, 2014). Polyploidy is one of the most important ways for wild *Allium* plants to survive (Duchoslav *et al.*, 2010; Wei *et al.*, 2011). Based on the development of chromosome ploidy from diploid to polyploidy (Sharma & Gohil, 2008; Friesen & Fragman-Sapir, 2014; Aruga *et al.*, 2015). We thought that the BT population distributed in Tianshan Mountains was the ancestral population of this species. Secondly, as the important distribution of wild *Allium* which appeared the phenomenon of polyploidy needs further in-depth cytogeographic analysis. The geographical origin and evolution of *A. caeruleum* that distributed in Balruk Mountains and Tianshan Mountains would be discussed.

There were m, sm, and st three chromosomes types of 12 populations that distribution in Tianshan Mountains. The eight populations also had asymmetrical st chromosomes, accounting for 66.67% of the investigated populations. The chromosomes of *Allium* plants are mostly symmetrical (m and sm). Rarely asymmetric is st and t type (He & Xu, 2001; Friesen & Fragman-Sapir, 2014; Aruga *et al.*, 2015). This asymmetric type of chromosome had a higher frequency in 12 populations. The seven populations with st chromosomes all had 2B karyotype evolutionary characteristics.

If the *Allium* species appeared the morphological changes and structural hybridity of the SAT that showed some groups were in active variation. The difference of SAT chromosome is the expression of survival adaptation (He & Xu, 2001; Friesen & Fragman-Sapir, 2014 and Aruga *et al.*, 2015). *Allium* species may appear on m chromosome, sm chromosome and st chromosome. The polymorphism existed in inter-species and sub-species (He & Xu, 2001; Sharma & Gohl, 2008; Zhang *et al.*, 2009; Friesen & Fragman-Sapir, 2014; Aruga *et al.*, 2015). The *A. caeruleum* distributed in Balruk Mountains was found the SAT appeared on the short arm of tetraploid the m chromosome, but no SAT was found in diploid and triploid.

In this study, SATs appeared on the m and sm chromosomes of 12 populations distributed in Tianshan Mountains. At the same time, the frequency of SAT was also different. It showed the polymorphism and instability of SAT among different populations. It is explained that the chromosomal structure mutation is occurring. Chromosome structural variation is a source of karyotypic changes. With more genetic variation and heterozygosity, it has enhanced the ability to adapt to new environment and has important evolutionary significance. The combination of polyploidization and asexual reproduction is an important evolutionary strategy for *A. caeruleum* (Ye *et al.*, 2017). It is a manifestation of the plant evolution and is similar to species studied in *Allium* section *Rhizonum* in China (Zhang *et al.*, 2009; Friesen & Fragman-Sapir, 2014; Aruga *et al.*, 2015). There were no B chromosomes found in the 12 populations distributed in Tianshan Mountains, and in the same way as the Balruk Mountains population. Then, 12 populations distributed in Tianshan Mountains that the chromosomal length consists mainly of M2 and M1 chromosomes, was similar to that diploid distributed in Balruk Mountains (Yang *et al.*, 2014).

The evolutionary trend of karyotype is developed from symmetry to asymmetry. The evolutionary trend of the karyotype is 1A→2A→2B→2C. The karyotype of *Allium* is symmetrical, most of which are symmetrical 2A type. There are also types 1A, 2B, 2C, and 3A (He & Xu, 2001; Sharma & Gohil, 2008; Zhang *et al.*, 2009; Hosseini & Go, 2010; Wei *et al.*, 2011; Genc *et al.*, 2013). No 1A, 2C, and 3A types were founded in the 12 populations. Of these, only the BT population had 2A type. The rest of were 2B about 91.6% of the test populations. From the perspective of cytology, combining the Karyotypes of *A. caeruleum* in Balruk Mountains, this species is in an active stage of differentiation.

Cluster analysis showed that the LY population, the ZK population, the RE population and the XJ population were clustered in one group, and the same results of cluster analysis based on morphological traits, that called mini ornamental group (Ma *et al.*, 2016). It is characterized by relatively short plants, short scapes and dense flowers, which are important ornamental germplasm resources. The cluster analysis with its closer karyotype features further explained their more closely related phylogenetic relationships.

Conclusion

The composition of chromosome of *A. caeruleum* in 12 different populations of Tianshan Mountains, Xinjiang, has diversity and instability of satellites. Chromosome structure variation has more genetic variation and heterozygous advantages, and it can enhance the ability to adapt to the new environment and it is an important evolutionary strategy.

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