Chromosomal Karyotype Differences of the Dark-Purple Tea Cultivar ‘Ziyan’ and Its Natural Hybrid Progenies

Zhen Jin1,†, Bo Sun1,†, Jiacheng Huang1, Liqiang Tan1, and Qian Tang1, *
1College of Horticulture, Sichuan Agricultural University, Chengdu 611130, China
† Zhen Jin and Bo Sun contributed equally to this work.
*)Corresponding author: tangqi2008@126.com

Abstract. ‘Ziyan’ is a new variety of dark purple tender shoots tea tree which is rich in anthocyanins. ZY-1 and ZY-3 were two natural hybridized offspring of ‘Ziyan’, which were cultivated by single plant selection. ‘Ziyan’ is as the female parent, whereas the male parent has yet to be determined. In this study, we try to investigate cytological parameters of the three kinds of tea plants, and analyze the chromosomal karyotype differences among them. The results showed that the max arm ratio of ‘Ziyan’ was determined 2.50, and relative length was between 3.56% and 5.39%. The karyotype asymmetry index was 59.920%, and the karyotype formula was 2n=2x=30=22m (2SAT)+8sm. The max arm ratio of ZY-1 was 2.50, and relative length was between 4.80% and 8.69%. The karyotype asymmetry index was 60.307%, and the karyotype formula was 2n=2x=30=20m+10sm. The max arm ratio of ZY-3 was 2.77, and relative length was between 3.95% and 8.70%. The karyotype asymmetry index was 58.679%, which was lower than ‘Ziyan’ and ZY-3, and the karyotype formula was 2n=2x=30=24m+6sm. Although ‘Ziyan’ was significantly different from its two offspring, the karyotype characteristics of three varieties of tea plants were all type 2B. ‘Ziyan’ had a pair of satellites, but neither ZY-1 nor ZY-3 had a satellite. According to the karyotype asymmetry index and average arm ratio, the hybrid progeny ZY-3 was the most primitive, followed by the female parent ‘Ziyan’, while the hybrid progeny ZY-1 was the most evolutive.

1. Introduction

Natural hybrid is a successful cross between two or more different populations based on one or more heritable traits under natural conditions. In the process of biological evolution, natural crossing has an important influence on species formation[1]. Tea plants are a kind of self-affinity plants, which exist widely as hybrid type in nature. Natural cross breeding is an indispensable part of material source in tea plant breeding[2]. ZY-1 and ZY-3 are natural hybrid offspring of ‘Ziyan’, and both them are rich in anthocyanins. However, according to the observation, the tree type of ZY-1 (small tree type) was different from that of Ziyan (shrubby type), and the yield of ZY-1 was maybe higher than that of ‘Ziyan’. The germination period of ZY-3 in spring was earlier than that of ‘Ziyan’, and the number of blooming and fruiting was significantly lower than that of its female ‘Ziyan’. The differences in plant phenotype are mainly influenced by genetic material and environment, in which genetic material is the main factor.
Karyotype analysis is a basic method to study chromosomes, it is a basic work in cytogenetics research. In this experiment, the karyotype analysis was carried out on the tea variety ‘Ziyan’, ZY-1 and ZY-3 to reveal their chromosome composition differences and diversity, and provide the basis for natural hybrid of ‘Ziyan’.

2. Materials and methods

2.1 Plant materials
The representative ‘Ziyan’, ZY-1 and ZY-3 from Pujiang County, Sichuan Province was used as the experimental material. Among them, ZY-1 and ZY-3 are natural hybrid progenies of ‘Ziyan’.

2.2 Chromosome preparation
The seedlings of ‘Ziyan’, ZY-1 and ZY-3 were cultured in plastic flowerpots in greenhouse to the root length of 1-1.5 cm and cut root tips of about 1 cm. Pretreated in 0.002 mol·L⁻¹ 8-hydroxyquinoline at 4 °C for 24 h, and fixed in Carnoy’s solution at 4 °C for 24 h, subsequently, the root tips were macerated in 1 mol·L⁻¹ hydrochloric acid at 60 °C for 16 min, stained with Carbol Fuchsin, and observed under microscope[3].

2.3 Karyotype analysis
Chromosome counts were performed on 30 well-spread metaphase chromosomes from five different root tips. Karyotype analysis referred to the standard of Li et al.[4]. Following parameters were calculated: chromosome relative length, arm ratio, type of chromosomes, index of chromosomes relative length and centromere index. Karyotypic formula referred to the standard of Levan et al.[5], and the asymmetry coefficient of karyotypes was calculated by the method of Arano[6], the karyotypes were calculated according to Stebbins’s standard[7].

3. Results

3.1 Chromosomal numbers of ‘Ziyan’, ZY-1 and ZY-3
All the chromosomal numbers of ‘Ziyan’, ZY-1 and ZY-3 were 2n=30. Metaphase chromosomes, karyotype and the chromosomal idiogram of ‘Ziyan’, ZY-1 and ZY-3 root tips were shown in Fig. 1, and detailed karyotype parameters of chromosome were listed in Table 1.
Figure 1. Metaphase chromosomes, karyotype and the chromosome idiogram of ‘Ziyan’, ZY-1 and ZY-3 root tips

Note: The number 1-15 represent chromosome No.

3.2 Karyotype analysis
The results showed that the chromosome relative length of ‘Ziyan’ ranged from 3.56% to 5.39%, ZY-1 ranged from 4.80% to 8.69% and ZY-3 ranged from 3.95% to 8.70%, and chromosome length ratio of ‘Ziyan’, ZY-1 and ZY-3 were 1.513, 1.813 and 2.203, respectively. The relative length constitution of ‘Ziyan’ was 14M2+16M1, ZY-1 was 2L+12M2+14M1+2S, and ZY-3 was 4L+12M2+12M1+2S. The centromeric index of ‘Ziyan’ ranged between 28.60% and 49.02%, and arm ratio ranked from 1.04 to 2.50. The centromeric index of ZY-1 ranged between 28.57% and 48.17%, and arm ratio ranked from 1.08 to 2.50. The centromeric index of ZY-3 ranged between 26.51% and 50.08%, and arm ratio ranked from 1.00 to 2.77. The chromosome types of the three tea plants were all metacentric chromosomes (m) or submetacentric chromosomes (sm), and the number of m-type chromosomes was much more than that of sm chromosomes. Moreover, ‘Ziyan’ had a pair of satellites on the tenth pair of chromosomes, while ZY-1 and ZY-3 were not. The karyotype formula of ‘Ziyan’ was 2n=2x=30=22m (2SAT)+8sm, ZY-1 was...
2n=2x=30=20m+10sm and ZY-3 was 2n=2x=30=24m+6sm. Karyotype asymmetry index of ‘Ziyan’ was 59.920%, ZY-1 was 60.307% and ZY-3 was 58.679%. The karyotype characteristics of three kinds of tea plants were all type 2B, according to Stebbins’s classification criteria. Scattered map of karyotype asymmetry of ‘Ziyan’, ZY-1 and ZY-3 were shown in Fig.2.

Table 1. Karyotype parameters of chromosome of ‘Ziyan’, ZY-1 and ZY-3

| No. | Chromosome No. | Short arm length / % | Long arm length / % | Total length / % | Index of relative length | Type of relative length | Arm ratio | Centromere index / % | Centromere type |
|-----|----------------|----------------------|---------------------|------------------|--------------------------|-------------------------|-----------|---------------------|-----------------|
| 1   | 2.11           | 3.28                 | 5.39                | 1.19             | M2                       | 1.56                    | 39.10     | m                   |                 |
| 2   | 2.44           | 2.83                 | 5.27                | 1.17             | M2                       | 1.16                    | 46.34     | m                   |                 |
| 3   | 2.05           | 3.16                 | 5.21                | 1.15             | M2                       | 1.55                    | 39.29     | m                   |                 |
| 4   | 1.54           | 3.64                 | 5.18                | 1.15             | M2                       | 2.37                    | 29.71     | sm                  |                 |
| 5   | 2.20           | 2.66                 | 4.87                | 1.08             | M2                       | 1.21                    | 45.30     | m                   |                 |
| 6   | 1.79           | 2.97                 | 4.76                | 1.05             | M2                       | 1.66                    | 37.64     | m                   |                 |
| 7   | 2.31           | 2.42                 | 4.73                | 1.05             | M2                       | 1.05                    | 48.74     | m                   |                 |
| 8   | 1.56           | 2.92                 | 4.47                | 0.99             | M1                       | 1.87                    | 34.79     | sm                  |                 |
| 9   | 1.85           | 2.50                 | 4.35                | 0.96             | M1                       | 1.35                    | 42.53     | m                   |                 |
| 10* | 1.19           | 2.98                 | 4.17                | 0.92             | M1                       | 2.50                    | 28.60     | m                   |                 |
| 11  | 1.81           | 2.06                 | 3.87                | 0.86             | M1                       | 1.13                    | 46.86     | m                   |                 |
| 12  | 1.57           | 2.58                 | 4.16                | 0.92             | M1                       | 1.64                    | 37.87     | m                   |                 |
| 13  | 1.70           | 2.32                 | 4.02                | 0.89             | M1                       | 1.36                    | 42.33     | m                   |                 |
| 14  | 1.27           | 2.44                 | 3.70                | 0.82             | M1                       | 1.92                    | 34.20     | sm                  |                 |
| 15  | 1.75           | 1.82                 | 3.56                | 0.79             | M1                       | 1.04                    | 49.02     | m                   |                 |
| 1   | 3.60           | 5.09                 | 8.69                | 1.30             | L                        | 1.41                    | 41.42     | m                   |                 |
| 2   | 3.09           | 4.78                 | 7.87                | 1.18             | M2                       | 1.54                    | 39.32     | m                   |                 |
| 3   | 2.65           | 5.11                 | 7.76                | 1.16             | M2                       | 1.93                    | 34.17     | sm                  |                 |
| 4   | 2.95           | 4.53                 | 7.48                | 1.12             | M2                       | 1.54                    | 39.44     | m                   |                 |
| 5   | 2.42           | 4.72                 | 7.15                | 1.07             | M2                       | 1.95                    | 33.91     | sm                  |                 |
| 6   | 3.16           | 3.98                 | 7.14                | 1.07             | M2                       | 1.26                    | 44.20     | m                   |                 |
| 7   | 3.11           | 3.80                 | 6.90                | 1.04             | M2                       | 1.22                    | 44.99     | m                   |                 |
| 8   | 1.87           | 4.68                 | 6.55                | 0.98             | M1                       | 2.50                    | 28.57     | sm                  |                 |
| 9   | 2.41           | 4.00                 | 6.40                | 0.96             | M1                       | 1.66                    | 37.56     | m                   |                 |
| 10  | 2.87           | 3.47                 | 6.34                | 0.95             | M1                       | 1.21                    | 45.24     | m                   |                 |
| 11  | 2.80           | 3.31                 | 6.12                | 0.92             | M1                       | 1.18                    | 45.86     | m                   |                 |
| 12  | 2.23           | 3.81                 | 6.04                | 0.91             | M1                       | 1.71                    | 36.90     | sm                  |                 |
| 13  | 1.92           | 3.70                 | 5.62                | 0.84             | M1                       | 1.93                    | 34.13     | sm                  |                 |
| 14  | 2.30           | 2.83                 | 5.14                | 0.77             | M1                       | 1.23                    | 44.82     | m                   |                 |
| 15  | 2.31           | 2.49                 | 4.80                | 0.72             | S                        | 1.08                    | 48.17     | m                   |                 |
| 1   | 2.97           | 5.73                 | 8.70                | 1.31             | L                        | 1.93                    | 34.18     | sm                  |                 |
| 2   | 4.34           | 4.33                 | 8.67                | 1.30             | L                        | 1.00                    | 50.08     | m                   |                 |
| 3   | 3.54           | 4.43                 | 7.96                | 1.19             | M2                       | 1.25                    | 44.41     | m                   |                 |
| 4   | 3.11           | 4.71                 | 7.82                | 1.17             | M2                       | 1.52                    | 39.72     | m                   |                 |
| 5   | 3.58           | 3.62                 | 7.20                | 1.08             | M2                       | 1.01                    | 49.73     | m                   |                 |
| 6   | 3.20           | 3.75                 | 6.94                | 1.04             | M2                       | 1.17                    | 46.03     | m                   |                 |
| ZY-3| 7              | 1.81                  | 5.01                | 6.82              | M2                      | 2.77                    | 26.51     | sm                  |                 |
| 8   | 2.11           | 4.63                 | 6.74                | 1.01             | M2                       | 2.19                    | 31.37     | sm                  |                 |
|      | 9   | 10  | 11  | 12  | 13  | 14  | 15  |
|------|-----|-----|-----|-----|-----|-----|-----|
| Chrs | 3.15| 3.70| 2.40| 2.80| 2.45| 2.06| 1.10|
| Asym | 3.39| 3.73| 3.53| 3.05| 2.83| 3.11| 2.85|
| Mera | 6.55| 6.42| 5.93| 5.86| 5.28| 5.17| 3.95|
| Ratio | 0.98| 0.96| 0.89| 0.88| 0.79| 0.77| 0.59|
| Type | M1  | M1  | M1  | M1  | M1  | M1  | S   |
| Arms | 1.08| 1.38| 1.47| 1.09| 1.15| 1.51| 2.59|
| Value | 48.15| 41.96| 40.54| 47.89| 46.43| 39.88| 27.85|
| Note | m   | m   | m   | m   | m   | m   | m   |

Note: * means the chromosomes with satellites, and the length of satellites is not included in the chromosome length.

Figure 2. Scattered map of karyotype asymmetry of ‘Ziyan’, ZY-1 and ZY-3

4. Summary
Natural hybrid is one of the ways to form variation, which provides a material basis for the formation of genetic diversity. ‘Ziyan’ is different from its offspring in phenotype and other shapes. Among the results of karyotype analysis of the three tea lines, there was genetic diversity between ‘Ziyan’ and its offspring. Although their chromosome types were metacentric chromosomes (m) and submetacentric chromosomes (sm), only ‘Ziyan’ has a pair of satellites on submetacentric chromosomes. The basic evolutionary trend of plant karyotype is from symmetry to asymmetry. Thus, primitive plants have symmetrical karyotypes. And the more asymmetric the plant karyotype is, the higher its degree of evolution[8]. In our study, the karyotype asymmetry index of the chromosomes of ‘Ziyan’ (59.920%) and ZY-1 (60.307%) were close to that of Camellia sinensis cv. Gulin-Niupicha (60.93%)[9], but ZY-3 (58.679%) was much lower than that of them. Therefore, the degree of evolution of the ‘Ziyan’ and ZY-1 should be relatively high, while ZY-3 was relatively primitive. The results showed that the phenotypic and cytological differences of different offspring of the same female parent were obvious, which may be caused by the variation of natural crossing or the difference between the male parent of ZY-1 and ZY-3. In addition, there were differences in arm ratio range, chromosome length ratio, karyotype type and so on. This study provides a reference for the genetic evolution of tea plants.

Acknowledgments
This work was supported by Tea Breeding Key Technologies R & D Program of Sichuan Province during the 13th Five-Year Plan Period (2016nyz0037).
References

[1] Y. G. Wang. Natural hybrid and specie formation. Biodiversity, 25 (2017) 565-576.
[2] Y. J. Hou, Q. He, G. L. Liang, et al. ISSR analysis of tea hybrid progenies. Journal of Southwest University (Natural Science Edition), 28 (2006) 267-270.
[3] X. R. Wang, H. R. Tang, J. Duan, et al. J. Sys. Evol. 46 (2008) 505–515.
[4] M. X. Li and R. Y. Chen, J. Wuhan Botanic. Res. 3 (1985) 297–302.
[5] A. Levan, K. Fradga and A. A. Sandberg, Hereditas 52 (1964) 201–220.
[6] H. Arano, Japanese J. Bot. 19 (1965) 31–67.
[7] G. L. Stebbins, Chromosomal evolution in higher plants (Edward Arnold Ltd. Press, London, 1971), pp. 87–123.
[8] C. P. Shi. Karyotype analysis of Strawberry. Anhui Agricultural Science. 13 (2011) 7621-7622.
[9] B. Sun, Y. X. Tian, J. P. Xu, et al. Optimization of chromosome preparation conditions and karyotype analysis of Camellia sinensis cv. Gulin-niupicha. Molecular Plant Breeding. 16, 2577-2582 (2018).