CYTOGENETIC STUDIES ON RAPESEED

II. The Analysis of Salient Feature on the Chromosomal Morphology of Mitotic Prophase in Rapeseed.

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Abstract

In this paper the salient feature on the chromosomal morphology of mitotic prophase in B.napus, B.chinensis and B.juncea was studied. The main results were as follow:

1. Mitotic prophase is a continuous process, but it is divided into 4 stages- extreme early prophase, early prophase, middle prophase and late prophase.
2. The frequency distribution of various mitotic prophase in rapeseed was observed. The results indicate that extreme early prophase was about 70%, early prophase about 24.5%, middle prophase about 5% and late prophase about 0.5%.
3. The karyotype of B.napus, B.chinensis and B.juncea was analyzed in late prophase. The results indicate that B.napus 2n=38=18M+4SM+2ST+14T. B.chinensis 2n=20=10M+10T. B.juncea 2n=36=30M+2ST+4T.
4. It was observed that No. 10 of B.chinensis often was attached to the nucleolus. The 2 chromosomes become Xtype in No.19 pairs of chromosomes of B.napus which may be seen from early prophase to late prophase.

Introduction

The studies on morphology of chromosomes in respect to variable analysis of chromosomal ploidy, number and structure, recognition of single chromosomal shape and size and study of taxonomy and evaluation in rapeseed are all very important. China is one of origin countries of rapeseed where there are many varieties and rich resource. There were only a few studies on morphology of chromosomes of

rapeseed. There was not any paper dealing with variable law on the chromosomal morphology of mitotic prophase in rapeseed. Thus, we have studied salient feature on the chromosomal morphology of mitotic prophase in B.napus, B.chinensis and B.juncea.

Materials and methods

Material offering

There are cultivars Xian nong you 2, Gan you 5 of B.napus, Guei hua zi of B.Chinensis and He xian la cai zi of B.juncea from Hunan Agricultural College Changsha, China.

Basic method

1. Material culture:
   Roots from germinating seeds growing at 25°C.
2. Pretreatment:
   Treating the attached root with 0.002M 8-hydroxyquinoline for 1 hour.
3. Pro-hypotonic treatment: Excising the good root tips and immersing them in 0.075M solution for 30 minutes at 25°C.
4. Wall degradation: Treating with 2.5% enzyme mixture for 30-40 minutes.
5. Post-hypotonic treatment: Rinsing the material 2-3 times in 25°C redistilled water and then immersing it in the distilled water for 5-10 minutes.
6. Fixation: Pour out the distilled water. Add 4-5 ml of fresh prepared methanol solution: acetic acid /3:1/ to the material,
7. Preparation of samples.
8. Smear: Put on 3-4 root tipe on the slide which was kept in the freezed distilled water. Add drop fixation solution to them. Tear the material with a pair of forceps and remove the supernatant.
9. Flame-drying: Add 2 drops fixation solution on the slide, then bake it on the alcohol burner.
10. Staining: Stain the dried slides in a 1:10 Giemsa solution for 20 minutes, then rinse them in distilled water, and have them air-dried and mounted in damar balsam.

11. Karyotype analysis: The karyotype analysis and the Giemsa stained region of the mitotic late prophase chromosomes in rapeseed were studied.
The forms of the chromosomes and the special characters of Giemsa stained region of all homologus chromosomes at late prophase were indentified. By the calculation of the arm ratio, according to Levan /1964/ classification method, four principal chromosome types are distinguished based on the position of the centromere. Metacentric - the arm ratio is 1.0-1.7; Submetacentric - the arm ratio is 1.7-3.0; Subtelocentric - the arm ratio is 3.0-7.0; Telocentric - the arm ratio is 7.0.

Results and analysis

The variable law on the chromosomal morphology of mitotic prophase in rapeseed.
Mitotic prophase is a continuous process, but it is divided into 4 stages - extreme early prophase, early prophase, middle prophase and late prophase. At the onset of mitosis, the chromonemata exhibits a chromomeric pattern. The chromomeres appear at extreme early prophase as a series of darker staining granules with different shape. The darker staining granules are joined by chromonomatal fibrils. The thread like chromosomes appear to be associated with darker staining granule./Fig.1 right/ During early prophase the long, thread like chromosomes, each composed of two chromatids, may be seen at the end of chromosome where coiling is lax /Fig.2 left/. In the middle prophase the chromatids are seen to be twisted around each other in relational coils or become chaismata /Fig.3/. At the end of prophase, when the chromosomes attain their maximum contraction and as they become clearer, the frequency distribution of mitotic various prophase on rapeseed was observed.The results indicate that very early prophase occurs in about 70% of cells, early prophase in about 24.5%, middle prophase in about 5% and late prophase in about 0.5%. Because of the four phases of mitotic prophase extreme

early prophase is the longest, with early prophase, middle prophase and late prophase being progressively shorter /Table 1/.

In general, there is only one darker staining nucleolus during prophase.

Table 1. The frequency distribution of various propnase of mitosis on rapeseed.

Item \ phase	extreme early prophase	early prophase	middle	late	total
No. of cells observed	700	245	50	5	1000

Morphological characteristic of chromosomes at late prophase in B.napus /Fig.7/.

The chromosome number of B.napus is 38 /2n=38/. According to the position of the centromere chromosomes can be classified into 4 classes. There are 9 pairs metacentric, 2 pairs submetacentric, 1 pair subtelocentric and 7 pairs telocentric. About 86% telocentric are short or shortest chromosomes /Table 2/.

The karyotypic formula is 2N=38=18M+4sM+2ST+14T.

Morphological characteristic of chromosomes at late prophase in B.chinensis /Fig.1/.

The chromosome number of B.chinensis is 20 /2n=20/. According to the position of the centromere chromosomes can be classified into 2 classes. There are 5 pairs of metacentrics and 5 pairs of telocentrics. We demonstrated in B.chinensis that chromosomes stained with Giemsa showed dark segment and light segment, along the entire length of a late prophase chromosomes next to centromere were dark segment.

Relative length of dark segment and light segment varies between different chromosomes and is expressed by different ratio: dark segment: light segment=1:1 on the chromosomes 3, 4, 8 and 9; dark segment: light segment = 2:1 on the chromosomes 5 and 6 but in chromosomes number 10 the

Table 2. The relative length, arm ratio and type of chromosome in B.napus.

No. of chromosomes	Relative length /‰/ /$\bar{X} \pm$ S.E./	Arm ratio /l/s/ /$\bar{X} \pm$ S.E./	Chromosomal type
K_1	8.86 ± 0.34	2.50 ± 0.10	SM
K_2	6.96 ± 0.23	1.25 ± 0.05	M
K_3	6.30 ± 0.30	1.25 ± 0.04	M
K_4	6.30 ± 0.30	1.25 ± 0.08	M
K_5	6.56 ± 0.26	4.50 ± 0.12	ST
K_6	6.56 ± 0.26	-	T
K_7	6.06 ± 0.24	1.00 ± 0.04	M
K_8	6.06 ± 0.21	2.00 ± 0.06	SM
K_9	6.05 ± 0.23	1.25 ± 0.09	M
K_{10}	6.05 ± 0.27	1.20 ± 0.04	M
K_{11}	6.06 ± 0.25	1.60 ± 0.06	M
K_{12}	6.05 ± 0.28	1.60 ± 0.02	M
K_{13}	6.06 ± 0.22	-	T
K_{14}	6.06 ± 0.18	-	T
K_{15}	4.33 ± 0.27	1.20 ± 0.05	M
K_{16}	4.32 ± 0.19	-	T
K_{17}	4.32 ± 0.18	-	T
K_{18}	3.80 ± 0.16	-	T
K_{19}	3.16 ± 0.13	-	T

ratio dark segment: light segment = 1:2; In addition, in chromosomes number 1 the ratio dark segment: light segment = 1:1.2; While in chromosomes number two the ratio dark segment: light segment = 1:3.5 /Table 3, 4/.

Morphological characteristic of chromosomes at late prophase in B.juncea.

There is a diploid chromosome number of 36 in B.juncea, the karyotype of which consists of 15 pairs of median, 1 pair of submedian and 2 pairs of terminal centric chromosomes. The karyotypic formula is 2n=36=30M+2SM+4T /Table 5/.

Behaviour of chromosomes at late prophase.

We observed that one pair of shortest chromosomes is asso-

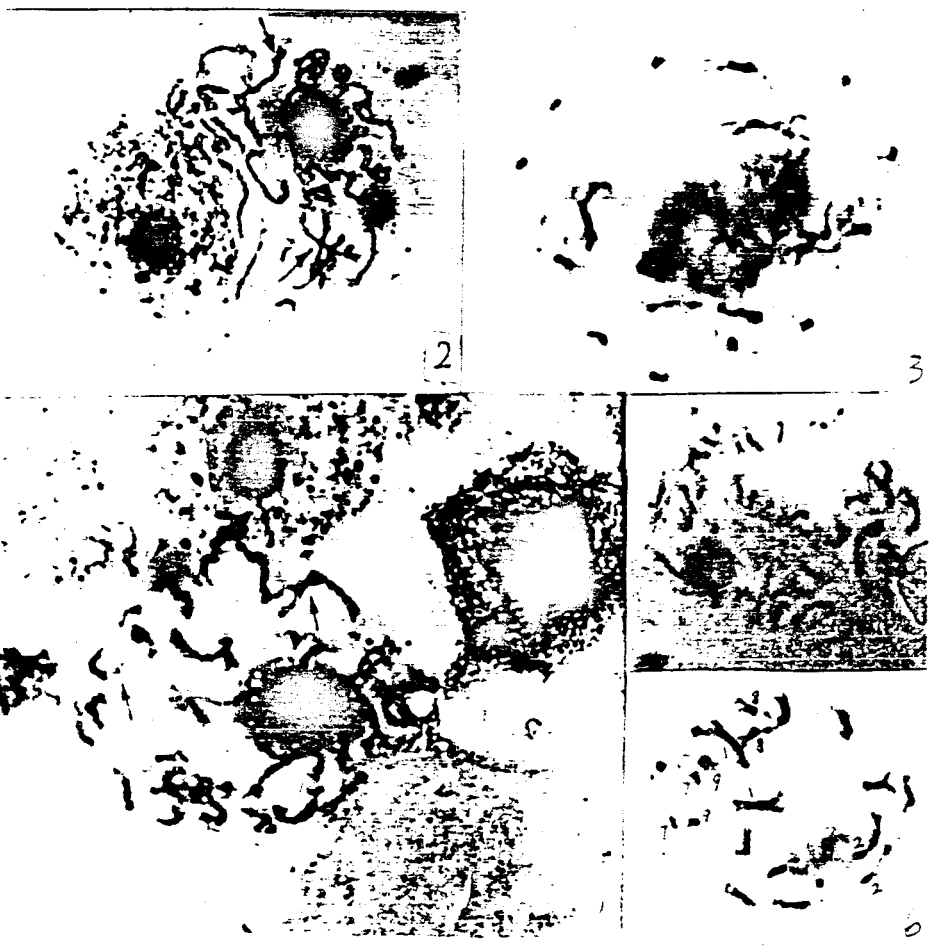
ciated with nucleolus during middle prophase and late prophase in B.chinensis. During late prophase homologous pairs of chromosomes often are brought together, for instance about 40%-60% in B.chinensis /Fig.6/. We also observed that 2 chromosomes become X type in No. 19 pairs of chromosomes in B.napus which may be seen from early prophase to late prophase /Fig. 2, 7/.

Table 3. The relative length, arm ratio and type of the chromosome in B.chinensis.

No. of chromosome	Relative length /%/ /$\bar{X} \pm$ S.E./	Arm ratio /L/S/ /$\bar{X} \pm$ S.E./	Chromosomal type
K_1	15 ± 0.20	1.25 ± 0.05	M
K_2	15 ± 0.19	-	T
K_3	15 ± 0.12	1.25 ± 0.06	M
K_4	10 ± 0.21	1.25 ± 0.07	M
K_5	10 ± 0.21	1.25 ± 0.07	M
K_6	10 ± 0.10	1.26 ± 0.08	M
K_7	10 ± 0,09	-	T
K_8	6.6 ± 0.11	-	T
K_9	4.5 ± 0.12	-	T
K_{10}	3.8 ± 0.11	-	T

Table 4. Proportion of relative length of dark stained and light stained chromosome segments in B.chinensis.

No. of chromosomes	Effects with Giemsa staining of the chromosomes		dark segments : light segments
	dark segments /%/	light segments /%/	
K_1	44	56	1:1.2
K_2	22	78	1:3.5
K_3	50	50	1:1
K_4	50	50	1:1
K_5	67	33	2:1
K_6	67	33	2:1
K_7	33	67	1:2
K_8	50	50	1:1
K_9	50	50	1:1
K_{10}	33	67	1:2



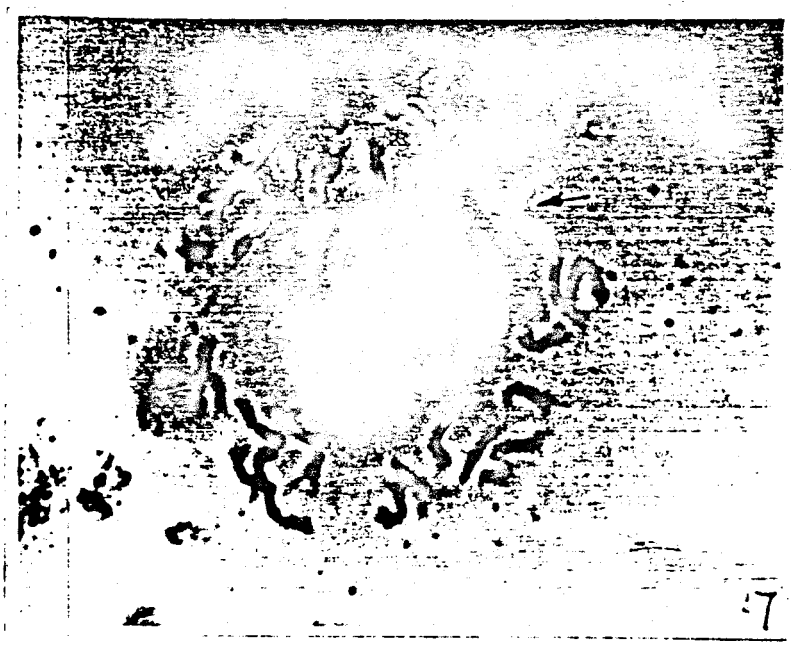


Table 5. The relative length, arm ratio and type of the chromosome in B.juncea.

No. of chromosome	Relative length /‰/ /$\bar{X} \pm$ S.E./	Arm ratio / L/S / /$\bar{X} \pm$ S.E./	Chromosomal type
K_1	9.2 ± 0.25	1.25 ± 0.09	M
K_2	8.4 ± 0.18	-	T
K_3	8.2 ± 0.23	1.50 ± 0.08	M
K_4	7.2 ± 0.19	1.40 ± 0.04	M
K_5	7.0 ± 0.12	1.50 ± 0.05	M
K_6	7.0 ± 0.18	3.0 ± 0.06	SM
K_7	6.0 ± 0.17	1.00 ± 0.07	M
K_8	5.8 ± 0.16	1.00 ± 0.08	M
K_9	5.6 ± 0.15	1.00 ± 0.04	M
K_{10}	5.0 ± 0.13	1.50 ± 0.06	M
K_{11}	5.2 ± 0.16	1.40 ± 0.06	M
K_{12}	4.0 ± 0.16	1.0 ± 0.09	M
K_{13}	4.0 ± 0.10	1.40 ± 0.07	M
K_{14}	4.0 ± 0.10	-	T
K_{15}	3.8 ± 0.11	1.10 ± 0.04	M
K_{16}	3.2 ± 0.09	1.00 ± 0.03	M
K_{17}	3.2 ± 0.08	1.40 ± 0.04	M
K_{18}	3.0 ± 0.05	1.50 ± 0.03	M

1. Late prophase /left/ and karyotype /right/ of mitotic cell in B.chinensis /X2812/.
2. Early prophase /right/ and interphase /left/ of mitotic cell in B.napus /X3000/. The end of chromosome where relational coil is lax /short arrows/.
3. Middle prophase of mitotic cell in B.chinensis /X2812/.
4. Very early prophase /right/, middle prophase /left/ and interphase /upper left/ of mitotic cell in B.napus /X3460/. The chromatids are seen to be twisted around each other in relational coil /short arrows/.
5. Late prophase of mitotic cell in B.juncea /X2500/.
6. Homologous chromosomes are side by side at near position in B.chinensis /X2812/. Numbers indicate homologous chromosomes.

7. Late prophase and karyotype of B.napus /X3600/. There are 2 chromosomes which become X type /short arrow/.

References

Li Xun, 1978. Cytological observation on the interspecific hybrid F, between Brassica napus L. and Brassica chinensis L. 5/1/: 41-44.

Levan A.K., Fredya and A.A.Sandbery, 1964. Hereditas Band 52/2/: 201-220.