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Cytological Analysis of the Effect of Gametocidal Chromosome 2C on ChineseSpring- E. elongatum 7E Disomic Addition

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Abstract

Elytrigia Desv is an important parent plant in wheat breeding because of its good characters. In this study, according to the complete or incomplete gametocidal effect, ChineseSpring- Ae.cylindrical-2C disomic addition were used to cross with ChineseSpring- E. elongatum （E. elongatum, 2n=14EE) 7E disomic addition. The chromosome aberration were induced and the translocation of wheat- E. elongatum 7E were created. By observing the F1 and BF1 meiosis of pollen mother cell, it showed that the number of univalents, rod bivalents , multivalents were more than theoretical value. In meiosis metaphase Ⅰ of CE-7E"×CS-2C", the configuration of chromosome pairing was 3.88Ⅰ+16.18Ⅱ+0.51Ⅲ+0.23Ⅳ。Even in anaphase Ⅰ、Ⅱ, there were a lot of abnormal phenomena, such as lagging chromosomes and fragments. By using C-banding, the materials with translocation were identified. After checked 102 plants of F2, we obtained 7 translocation plants, including 1 inter-wheat-genome translocation. The translocation frequency was 6.86%.

*Keywords:*Gametocidal chromosome; E. elongatum;Crossing; Meiosis; C-banding; Translocation

1. Introduction

Triticum aestivum L. (2n = 6x = 42, AABBDD) is allohexaploid, containing three genome: A, B and D. *Elytrigia Desv*, perennial wild plants of *Triticeae Dumort*, is a kindred genus of wheat. It is widely distributed in nature and has a lot of good properties that *Triticum aestivum* don't have which make it a huge

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distant genetic exchange resource of wheat. Through distant hybridization and other methods, the good properties of *Elytrigia Desv* can be transferred to cultivated wheat varieties. Therefore, it can be used to enhance the resistance and improve the quality of wheat, breed disease-resistant, high-quality, high-yielding varieties (lines). In Liu Shubing *et al*.'s study, they found *Elytrigia elongatum*'s 7 chromosomes had some homologous relation with that in wheat chromosomes, respectively, through RFLP (Restriction Fragment Length Polymorphism) and IEF (Isoelectric Focusing) [1]. Therefore, *Elytrigia elongatum* and *Middle elongatum* were used most successfully in wheat genetic improvement. In this study, according to the gametocidal effect, Chinese Spring-*Ae.cylindrical*-2C disomic addition was used to cross with Chinese Spring-*E. elongatum* 7E disomic addition, the translocation and deletion of *E. elongatum* were induced. Observing the meiosis of F_1、 B_1 F_1 pollen mother cell, the mutant with translocation were identified. Furthermore, the translocation and deletion were identified using C-banding.

2. Materials and Methods

2.1 Materials and Disposal

The materials used in the study are Chinese Spring-*E. elongatum* 7E (2n=14 EE, 2n＝44 AABBDD＋2E) disomic addition, Chinese Spring- *Ae.cylindrical* (2n＝44 AABBDD＋2C) disomic addition and Chinese Spring control which are provided by Professor Li Jilin from Genetics Lab, Life and Environment Science College, Harbin Normal University.

Chinese Spring-*Ae.cylindrical*-2C (♂) disomic addition and Chinese Spring-*E. elongatum* 7E (2n=14EE) (♀) disomic addition were sowed separately and crossed in the fields. After the hybridism F1 was sowed, self cross and back cross was conducted and the F_2、B_1F_2 were sowed line by line.

Cross was conducted as routine method. Crossing was conducted in the field. Chinese Spring control was sowed in the field simultaneously.

2.2 Chromosome Morphology

Conventional squashing method was adopted in chromosome slides making of chromosome in meiosis of F1 pollen mother cell. The anther of F1 at meiosis of anther panicle was chosen, and fixed with acetic carmine Kano and stained by aceto-carmine. Conventional squashing method was adopted in chromosome slides making of chromosome in root tip cells.

2.3 Chromosome C-banding Disposal

Chromosome C-banding referred to Endo [2] and Li Jilin [3]. After observed using Leica DM6000B microscope, Leica DFC480 was used to process and take picture.

3. 3 Results and Analysis

3.1 Behavioral Observation and Analysis of Chromosome at Metaphase I of hybridism F1 Meiosis of Pollen Mother Cell

As shown in Table 1, the chromosome pairing of 10 random sampling CE-7E "× CS-2C" plants at metaphase I (PMC M I) was analyzed, and 107 pollen mother cells were observed. About 89% of the cells

chromosome pairing was normal, with 21 Ⅱ +7 E Ⅰ +2 C Ⅰ configuration. There were a few abnormal behaviors, with a number of univalent, rod bivalent, trivalent and tetravalent body. Each cell contained an average of 3.88 univalent, 20.19 bivalent (ring bivalent 16.1, accounting for 80.13% of total bivalent, rod bivalent 4.01), 0.51 trivalent and 0.24 four bivalent. The relative disorder coefficient is 0.23. That indicated that the chromosome pairing was in disorder, so setting rate was very low. CE-7E "× CS-2C" metaphase Ⅰ chromosome pairing configuration was 3.88 Ⅰ +16.18 Ⅱ +0.51 Ⅲ +0.23 Ⅳ (Figure 1-A).

3.2 Chromosome Behavior Observation during F1 Hybrid Pollen Mother Cells Anaphase Ⅰ, Ⅱ and the Late Quarter of Spores.

As shown in Table 2, in anaphase I and II, there was a large number of lagging chromosomes, chromosome fragments, chromosome bridges, chromosome adhesion, abnormal splitting etc. In random testing of cells, 89% of chromosomal division was abnormal. Some cells had relatively a large number of lagging chromosomes, and some appeared early separation. Lagging chromosomes can still be observed in the anaphase. It was noteworthy that chromosome behavior was the most complex. In anaphase Ⅰ, anaphase Ⅱ and the late quarter of spores, cells with chromosome fragments accounted for 54%, in complete disorder. There was two or more lagging chromosome, phenomenon with dual chromosome bridges was obvious.(Fig. 1-B, C, D). A large number of micronuclei appeared late in the second anaphase and the late quarter of spores, the number of cells with micronuclei reached 55%. The situation of chromosomal abnormalities was very complex (Figure 1-E, F).

Pollen formation was observed that some pollen had no content material, that's abortive pollen. Micronuclei were of different sizes, some were formed of chromosomal fragments, some of lagging chromosomes. It showed that gametocidal chromosomes can break chromosomes, and induced chromosomal structural variation. Multivalent meiosis, lagging chromosomes, chromosome bridges, micronuclei, abnormal phenomena etc. appeared in F2 meiosis, may be due to chromosome deletions, translocations and other structural variation. In observation of test pollen mitosis, a few cell divisions were abnormal. In mitosis anaphase, appeared chromosome bridges, lagging chromosomes and chromosome fragments, may be due to meiotic abnormalities affecting pollen mitosis. Kynast et al. [4] considered that there is no abnormal behavior of chromosomes in the process of hybrid meiosis of gametocidal chromosome. This study discovered that chromosome behavior was in disorder during PMC, similar as chromosomal abnormal behavior observed by Li Jilin.

3.3 Identification of C-banding

C-banding identified 102 plants of CS-7E "× CS-2C" hybrids, of which plants 7-81 was C-banding identified, the result was that chromatic number 2n = 42, by C-banding analysis, the plants had a unique banding pattern of chromosomes, different from 21 chromosomes in the Chinese Spring standard bands, and lack one normal chromosome 6B. The specific chromosomal banding pattern and 6B chromosome bands had a lot in common; short arm had a deep intermediate zone, proximal zone and end zone. The long arm was similar to chromosome bands of 7E, with the deep middle zone and the proximal zone. Identified by in situ hybridization, it was determined that the plant was translocation of wheat chromosome 6B and 7E chromosome. According to translocation naming rules, this translocation plant will be named as T7EL • 6BS (Figure 2-A, B); Lines 7-87 of the C-band identification, analysis showed that chromosome number is 2n = 41, the plant had a unique banding pattern of chromosomes, different from 21 chromosomes in the Chinese Spring standard bands, and lack one normal chromosome 7A and one normal chromosome 7B. The specific chromosomal banding pattern was similar to the short arm of chromosome 7A, with a side band; But its

short arm was similar to long arm of chromosome 7B , with rich bands, deep Centro mere band and intermediate zone. Identified by in situ hybridization, it was determined that the plants were translocation of wheat chromosome 7A and 7B chromosome. According to translocation naming rules, this translocation plant will be named as T7AS·7BL (Figure 2-C).

Table 1 Chromosome configuration of CS-7E"×CS-2C" F_1 PMC M Ⅰ

Hybrided combination	observed cells	univalent Ⅰ total (amplitude)	average	divalent rod Ⅱ total (amplitude)	average	rang Ⅱ total (amplitude)	average	total total (amplitude)	average	trivalent total (amplitude)	average	tetravalent total	average	RCC
CS-7E" ×CS-2C"	107	415 (1-7)	3.88	429 (1-9)	4.01	1795 (12-19)	16.18	2160 (0-2)	20.19	54 (0-2)	0.51	26	0.24	0.23

*Relative coefficient of disorder =（No. univalent + No. multivalent）/ No. divalent

Table 2 chromosome abnormalities at AI、AII of CS-7E″X CS-2C″ F_1 PMCs

Hybrided combination	No. cells observed	Lagging chromosome	Fragment	Bridge	No. cells observed	Micronucleus tetraspores	No.cells observed	Micronucleus in pollen grains
CS-7E"X CS-2C"	104	61 2.89 0-7	47 6.93 1-10	35 1.98 0-4	97	78 3.53 0-6	88	49 2.42 0-3

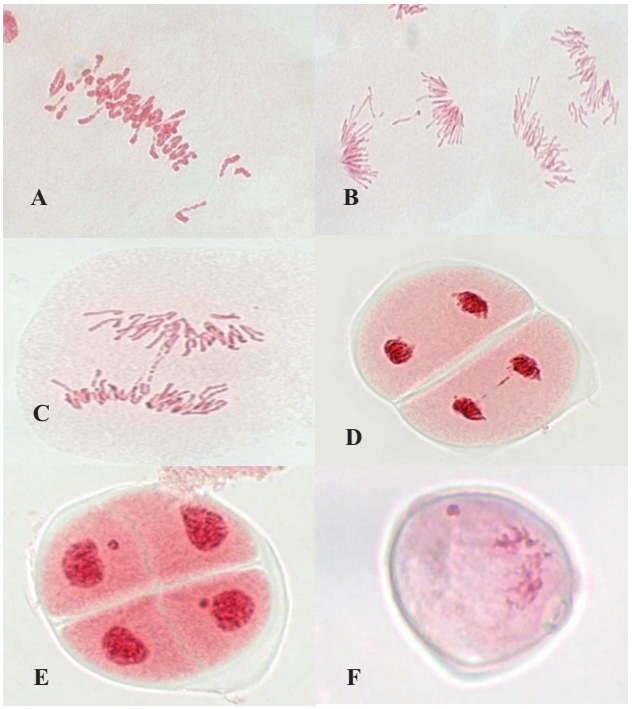

Figure 1 Chromosome unusual behavior of F_1 meiosis in CS-7E″×CS-2C″crossing

A. PMC M Ⅰ，6 rod divalents and univalents; B. PMC Ana Ⅰ，lagging chromosomes; C. PMC Ana I, 2 chromosome bridges; D. PMC Ana Ⅱ, chromosome bridge; E. PMC Tel I, 2 micronucleus tetraspores; F. Micronucleus in pollen grains

4. Discussions

4.1 Discussion during Period of the Role of Gametocidal Chromosome 2C

In this study, may be due to the role of gametocidal chromosome, the chromosome of hybrid offspring caused fracture, structural variation etc., and the infiltration of alien chromosomes inevitably led to an imbalance of genetic material, caused its behavior disorders. Multivalent phenomenon was observed in meiosis, which was due to wheat chromosomes and Agropyron elongatum chromosome have some homological relationship, thus produced by ancestral match and chromosome translocation. By cytological studies, we believe that the role of gametocidal chromosomes maybe effective during the whole process of meiosis: as early as joint line stage and pachytene, as late as the first pollen mitosis. All of this illustrated that the role of gametocidal chromosome was involved in the whole process of meiosis.

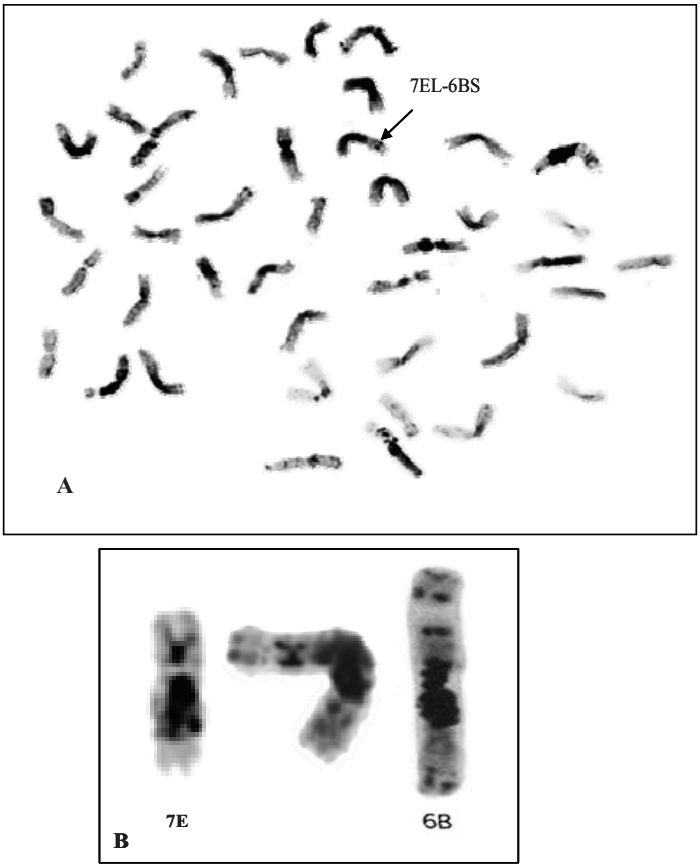

Figure 2 C-banding of F_1 line in CS-7E″×CS-2C″ crossing

A. B. F_1 line 7-81，7EL-6BS chromosome translocation

C. F_1 line 7-87，7AS-7BL chromosome translocation

4.2 Discussion on Chromosome Aberration Frequency induced by Gametocidal Chromosome 2C

Translocation and deletions induced by gametocidal chromosome had high frequency. Endo [6] made use of gametocidal chromosome 3C to induce substitution lines chromosomal aberrations of triticale 1B/1R, 1R chromosome structural variation frequency is 11.5%, of translocation frequency reached 9.6%; Friebe et al [7] obtained the total variance frequency of rye chromosome is 7%; Wang Xianping *et al* [8] made use of gametocidal chromosome 3C to induce Tritileymus chromosome translocation, with 5.08% of total variance frequency; Li Jilin [3] and Qumin et al [9] make use of gametocidal chromosome 2C to induce aberration of Chinese Spring- E. elongatum -5E, 1E disomic addition, with translocation frequencies 5.35% and6.02% respectively. In the study, by using gametocidal chromosome 2C to induce aberration of Chinese Spring- E. elongatum 7E disomic addition, 102 plants of CS-7E "and CS-2C" hybrids offspring were detected, and 7 plants of chromosomal translocation were received. Among them, there was 1 plant of translocation between wheat genome in which the translocation frequency is 6.86%. It can be seen that translocation between wheat and heterologous chromosomes can be induced effectively by using gametocidal chromosomes, with high frequency both in translocation and deletion. Therefore, it is an effective way to induce chromosomal translocation between wheat and it's kindred genus using gametocidal chromosome because it's high frequency, different variety types and simple operation.

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