Proc. 4th Inter. Wheat Genet. Symp. (1973), pp. 225–229

M. Feldman

RECENT STUDIES ON VARIATION OF CHROMOSOMES IN CALLUS TISSUES FROM TETRA-5A, -5B, AND -5D OF CHINESE SPRING WHEAT

M. OKAMOTO[1], H. ASAMI[2], T. SHIMADA[3], AND N. INOMATA[4]

Nara Agricultural College
Sakurai, Nara-ken, Japan

SUMMARY

The original numbers of chromosomes of seeds from tetra-5A, -5B, and -5D remained the modes of chromosome numbers at the fourth successive culture.

Dicentric chromosomes were observed in callus cells from tetra-5B, and one callus cell from tetra-5D had a chromosome each arm of which had a satellite. No abnormal chromosomes were found in callus cells from tetra-5A.

INTRODUCTION

Considerable variation of chromosome constitution of callus cells has already been reported by many authors (FOX, 1963; NISHIYAMA and TAIRA, 1966; SHIMADA *et al.*, 1969; KAO *et al.*, 1969; ASAMI *et al.*, 1972). We have studied chromosomes in callus tissues from various aneuploids of *Triticum aestivum* var Chinese Spring in an attempt to answer the following questions:

(1) Is a particular chromosome responsible for chromosome variation?
(2) Will chromosome constitution be changed during successive cultures?
(3) Will action of centromeres be specific in callus tissues?

The present report is concerned with the effect of extra dosage of chromosomes of 5A, -5B, and 5D on chromosome constitution of callus.

MATERIALS AND METHODS

The materials used for the experiment were seeds of tetra-5A, -5B, and -5D of *T. aestivum* var. Chinese Spring which came from Dr. Sears.

[1]Present address: Abteilung für Pflanzengenetik der Gesellschaft für Strahlen- und Umweltforschung, 8059 Grünbach üb. Erding/Obb., W. Germany.

[2]Shiga Agricultural College, Japan.

[3]Kyoto University, Japan.

[4]University of Osaka, Japan.

The seeds were rinsed in 70% ethyl alcohol for one minute, washed several times in sterilized distilled water, and then left in sterilized distilled water overnight. Then the soaked seeds were sterilized in 9% calcium hypochlorite for 60 minutes and rinsed five times in sterilized distilled water.

To induce callus from sterilized seeds, they were placed on agar slant medium in test tubes (18 x 180 mm), each containing 10 ml of the medium. The medium used was RM-64 basal medium (LINSMAIER and SKOOG, 1965) which contained 3.0 mg/l of 2,4-D (2,4-dichlorophenoxy acetic acid). The medium had been adjusted to pH 5.8 with 1N HCl or 1N KOH before autoclaving for 15 minutes at 1.2 kg/cm^2 of pressure.

Calluses which formed on growing roots of germinating seeds were successively subcultured at intervals of two months on the same medium.

For observations of chromosome constitution, calluses one to two weeks after beginning of subculture were used. They were treated in water at 0^0 C for 24 hours, fixed in Farmer's solution (3 parts ethanol:1 part glacial acetic acid), and hydrolyzed with 1N HCl for 10 minutes at 60^0 C. After staining with Feulgen solution for about one hour, they were squashed for microscopical observation.

EXPERIMENTAL RESULTS

Table 1. Chromosome constitution of callus cells from tetra-5A, -5B, and -5D

Chrom. no.	No. cells observed		
	T-5A	T-5B	T-5D
36	-	1	-
37	-	1	-
38	2	1	-
39	-	-	1
40	-	1	2
41	2	3	-
42	1	6[1]	2
43	4	-	3
44	38	31[2]	36[4]
45	1	-	3
47	-	-	2
55	-	1	-
65	-	-	1
73	1	-	-
80	1	-	-
83	-	2	-
85	-	2[3]	-
88	-	1	-
Total	50	50	50

[1] 1 cell with 1 dicentric chromosome.

[2] 1 cell with 1 telo.

[3] 2 cells with 2 dicentric chromosomes.

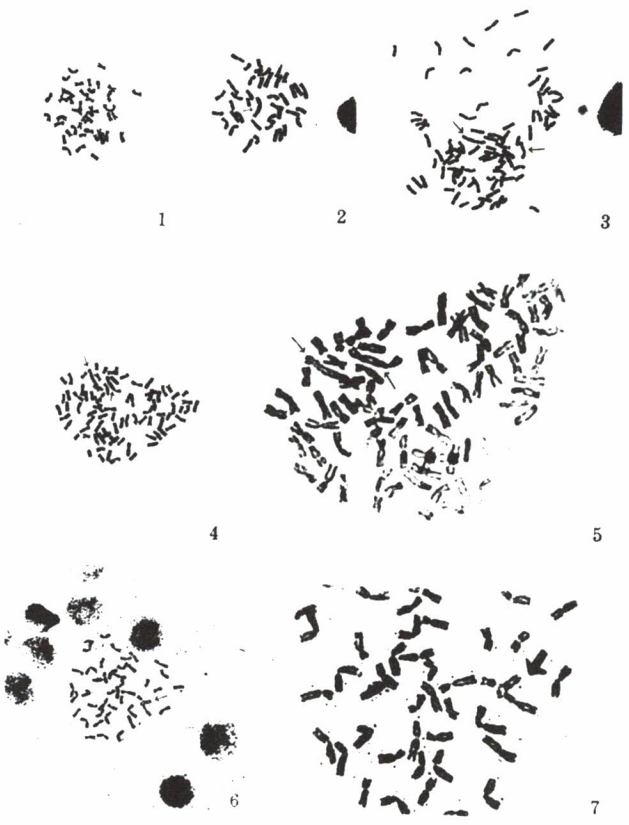
[4] 1 cell with a chromosome each arm of which had a satellite.

Cytological observation of callus tissues was made at the fourth successive culture. Table 1 shows the chromosome constitution of 50 cells observed of callus tissues from tetra-5A, -5B, and -5D.

Chromosome numbers of callus cells from tetra-5A varied from 38 to 80, with 76% of the cells having 44 chromosomes. No morphological changes of chromosomes were observed.

Chromosome numbers of callus cells from tetra-5B ranged from 36 to 88, with 62% of the cells having 44 chromosomes. Figure 1 shows a cell with 44 chromosomes with no morphological changes. Figure 2 shows a cell with 42 chromosomes, in which a pair of chromosomes was found to be united end to end. Figures 4 and 5 show one of two cells with 44 chromosomes which had two giant chromosomes, both of which were dicentric. Furthermore, a cell with 42 chromosomes was found to have one telocentric chromosome.

Chromosome numbers of callus cells from tetra-5D varied from 39 to 65, with 72% of the cells having 44 chromosomes. One of the cells with 44 chromosomes was found to have a chromosome each arm of which had a satellite (Figure 6, 7).



Figures 1-7. Chromosomes of callus cells. (1) Tetra-5B with 44 chromosomes. (2) Tetra-5B with 42 chromosomes including a dicentric chromosome (arrow). (3) Tetra-5B with 85 chromosomes. Two chromosomes (arrow) seemed to be fused end-to-end. (4) Tetra-5B with 85 chromosomes. Two dicentric chromosomes (arrow). (5) Enlargement of Figure 4. (6) Tetra-5D with 44 chromosomes. Both arms of one had a satellite (arrow). (7) Enlargement of Figure 6.

DISCUSSION

Chromosome Number

The fact that the original chromosome numbers of seeds from tetra-5A, -5B, and -5D were the modes of chromosome numbers of callus cells at the fourth successive culture fits the results obtained from previous experiments on variation of chromosome constitution in callus tissues from the long-arm ditelos of 5A, 5B, and 5D, nulli-5B tetra-5D, and normal Chinese Spring wheat (ASAMI *et al.*, 1972), and from unstable telocentric chromosomes of chromosome 3B (STEINITZ-SEARS, 1966; INOMATA *et al.*, in press).

Morphological Changes of Chromosomes

Dicentric chromosomes observed in three callus cells from tetra-5B in the fourth successive culture were not found in any previous experiments with callus tissues from wheat. These dicentric chromosomes are tentatively explained as due to end-to-end fusion of the chromosomes. Whether this dicentric-chromosome formation is due to the effect of the extra dosage of chromosome 5B remains to be decided in the future.

Occurrence of a chromosome each arm of which had a satellite in the fourth culture of calluses from tetra-5D may be explained as due either to isochromosome formation after centromere misdivision or to misdivision of two sat-chromosomes and subsequent reunion of the sat-arms. Which of the above possibilities is correct cannot be decided at present.

No telocentric chromosomes were observed in 50 callus cells of tetra-5D. In a previous experiment on variation of chromosomes at the fifth subculture of calluses from nulli-5B tetra-5D, 10 of 50 cells had 1 telocentric chromosome each and 1 cell had 2 telocentrics (SHIMADA *et al.*, in press). This considerable number of telocentric chromosomes was explained as due to either (1) absence of chromosome 5B, (2) extra dosage of chromosome 5D, or (3) interaction between absence of 5B and extra dosage of 5D. The first hypothesis assumes inhibition of misdivision of chromosomes by chromosome 5B, and the second assumes enhancement of misdivision by the chromosome 5D. Since no telocentric chromosomes were observed in callus cells from tetra-5D, the second hypothesis becomes somewhat less tenable.

Plans for the Future

Only one chromosome, 5B, has been tested for the effect of its absence on variation of chromosomes in callus tissues, leaving 20 still to be tested.

The complete series of dosages of chromosome 5B, ranging from 0 to 4 or more, must be tested for their effect on chromosome variation in callus tissues. Thus far, only 2 and 4 doses have been tried without the complication of extra dosage of 5D.

The experiments were confined to variation of chromosomes in callus tissues only. Attention should also be given to variation of chromosomes in somatic tissues and to misdivision of univalents at meiosis of plants with varying dosage of chromosome 5B.

ACKNOWLEDGEMENT

The authors are extremely grateful to Dr. and Mrs. Sears for supplying all the seeds necessary for the experiment and for their valuable suggestions.

LITERATURE CITED

ASAMI, H., T. SHIMADA, N. INOMATA, and M. OKAMOTO 1972. Preliminary report on variation of chromosome constitution in cultured cells of different lines of Chinese Spring wheat. Wheat Info. Serv. 35:10.

FOX, J. E. 1963. Growth factor requirement and chromosome number in tobacco tissue cultures. Physiol. Plantarum 16:739-803.

INOMATA, N., M. OKAMOTO, and H. ASAMI 1973. Chromosome constitution in callus cells from unstable telocentric chromosomes of Chinese Spring wheat. (In press).

KAO, K., R. A. MILLER, C. L. GANOURG and B. L. HARVEY 1970. Variation in chromosome number and structure in plant cells grown in suspension cultures. Can. J. Genet. Cytol. 12:297-301.

LINSMAIER, E.M., and F. SKOOG 1965. Organic growth factor requirements of tobacco tissue cultures. Physiol. Plant. 45:363-364.

NISHIYAMA, I., and T. TAIRA 1966. The effect of kinetin and indole acetic acid on callus growth and organ formation in two species of *Nicotiana*. Jap. J. Genet. 41:357-365.

SHIMADA, T., N. INOMATA, M. OKAMOTO, and H. ASAMI 1973. Telocentric chromosomes obtained from calluses of nulli-5B tetra-5D. WIS (in press).

SHIMADA, T., T. SASAKUMA, and K. TSUNEWAKI 1969. *In vitro* culture of wheat tissues. 1. Callus formation, organ redifferentiation and single cell culture. Can. J. Genet. Cytol. 11:294-304.

STEINITZ-SEARS, L. M. 1966. Somatic instability of telocentric chromosomes in wheat and the nature of the centromere. Genetics 54:244-248.