No. 32
M. Feldman

Seiken Zihô ***10:*** *49~56* (1959)

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Reprinted from SEIKEN ZIHÔ, Report of the
Kihara Institute for Biological Research
No. 10. Dec. 1959

財団法人　木原生物学研究所　**生研時報**　第10号　別刷
昭和34年12月30日発行

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A Preliminary Note on Cytological Abnormalities in Barley

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In barley a relatively small number of cytological abnormalities has been reported though such phenomena have been often studied in other plants. However, as a result of the recent progress in cytogenetic studies of barley, various abnormalities in the cytological behavior have been observed also in this plant. They may be roughly classified into two categories: mitotic and meiotic aberrations.

Reports on mitotic abnormalities in barley are scarce, f. inst. formation of fused or giant cells resulting from the failure of cytokinesis or cell wall formation was reported (TAKAHASHI *et al.* 1954, 1955). Also, various division abnormalities have been found in irradiated materials (CALDECOTT & SMITH 1952a, b, and others).

Meiotic abnormalities found in microsporocytes may be further divided into 1) various types of asynapsis or desynapsis (EKSTRAND 1932; BERG 1936; BURNHAM 1946; MCLENNAN & BURNHAM 1948; MOH & NILAN 1954; NYBOM 1954; TSUCHIYA 1955b), 2) formation of syncytes or multiploid sporocytes (SMITH 1942; MOH & NILAN 1954; TSUCHIYA 1955a), and 3) chromosome fragmentation (NYBOM 1954).

In the course of his cytogenetic studies started in 1947 the present author observed several types of abnormalities of meiotic chromosomes. He has classified them primarily into the following two types, 1) failure of chromosome pairing at meiosis and 2) syncyte formation in the microsporocytes.

The meiotic chromosomes were investigated exclusively in microsporocytes. Anthers were fixed with 1 : 3 acetic alcohol. Preparations were made by acetocarmine squash method according to the author's procedure (TSUCHIYA 1957).

I. Failure of chromosome pairing at meiosis

1. Partial asynapsis in Slender trisomics

The present author in 1953 obtained all 7 possible typess of primary simple trisomics and other chromosomal types (TSUCHIYA 1954, 1958, 1959) in the offspring of autotriploids (cf. TSUCHIYA 1953) of *Hordeum spontaneum nigrum* (var. *transcaspicum* VAV.). Failure of chromosome pairing at meiosis was observed in the following 7 plants all of which showed the characteristic slenderness of Slender in all plant parts (TSUCHIYA 1958, 1959).

1) 3 plants[1] with 2n=15 ($1_{III}+6_{II}$),
2) 1 plant with 2n=15+1 fragment ($1_{III}+6_{II}$),
3) 3 plants with 2n=16 ($2_{III}+5_{II}$).

At diakinesis and first metaphase of those plants failure of chromosome pairing was observed. The behavior of chromosomes at meiosis in the asynaptic sporocytes was different from the other cases of asynapsis or desynapsis in barley previously reported. The frequency of sporocytes showing asynapsis was very low; about 1 percent ranging from 0.7 to 1.5 percent. At MI a varying number of univalents was observed. Most frequent was complete asynapsis showing 15_I in simple and 16_I in double trisomics.

Fig. 1. Meiosis in Slender, a primary simple trisomic type. ×810.
a and b, Normal sporocytes at diakinesis (a) and MI (b) showing $1_{III}+6_{II}$. c, Asynaptic sporocyte showing 15 univalents.

The univalents in the asynaptic sporocytes oriented themselves on the equatorial plate at MI and showed longitudinal splits as shown in Figure 1c. Their behavior reminds of the so-called "pseudo-homotypic" division. The phenomenon was observed not only in the simple trisomics with 2n=15 chromosomes but also in Slenders with 2n=15+1 fragment and double trisomics with 2n=16 chromosomes.

The univalents were shorter than those of autotriploids, tetraploids, trisomics and X-ray induced asynaptic plants (Fig. 2) and showed a clear split as Figure 1c shows.

Since the other 6 types of primary simple trisomics showed no asynapsis, the failure of pairing in Slender may be ascribed to the specific effect of its extra chromosome which is one of the three longest chromosomes of the complement.

2. Desynapsis in an X_2 plant

Dormant seeds of a wild two-rowed diploid variety of barley, *Hordeum spontaneum nigrum* were irradiated by X-rays at 180 kVp., 3 mA, distance 13 cm, no filter by MATSUDA's Type KXC-17 apparatus. The doses were about 13,500 r units. One X_1 semi-sterile head obtained from those seeds produced an X_2 plant which exhibited desynapsis at meiosis.

The desynaptic plant showed a complete failure of pairing at diakinesis and MI. From a detailed study of early prophase, it was ascertained that this was a case of desynapsis or precocious separation of chromosomes at pachytene or early diplotene stage (cf. BEADLE 1933). At zygotene and early pachytene stages the homologous

1) The same asynaptic phenomenon as found in those 3 Slender plants was observed in many Slender trisomics which were obtained in progenies of other triploids and these 3 Slender plants.

chromosomes associated with each other. At diplotene, however, almost all chromosomes appeared as univalents with a few exceptions which showed bivalents associated by true chiasmata.

At diakinesis all 14 chromosomes appeared as univalents of which two touched the nucleolus by a large satellite (Fig. 2a). At MI all the univalents were irregularly scattered on the spindle and/or in the cytoplasm. However, some chromosomes showed the appearance of "pseudo-bivalents" as shown in Figure 2d. The meiotic process was somewhat similar to that of *Datura* (BERGNER *et al.* 1934) and was very irregular throughout the two successive divisions.

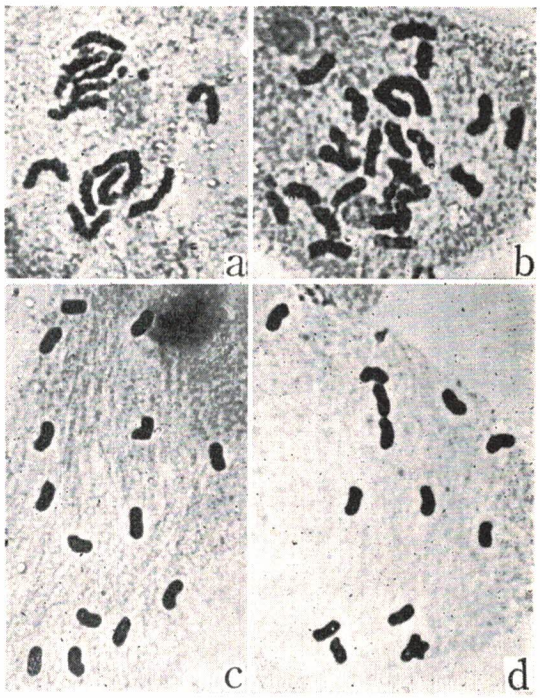

Fig. 2. X-ray induced desynapsis. ×1,000.
a and b, Diakinesis. a, A chain of four and ten univalents; two univalents attached to the nucleolus with large satellites. b, A syncyte with 28 chromosomes almost of which are univalents. c and d, MI. c, 15 univalents. d, One pseudo-bivalent and 12 univalents.

At MI some univalents which were situated on and/or near the equatorial plate formed a nuclear plate and the remaining ones remained scattered on the spindle. Later all univalents regardless of their position on the spindle showed longitudinal splitting. The halves of the univalents which were situated on the plate went to the poles, while those which were scattered freely on the spindle went through despiralization in this position and formed *in situ* resting nuclei whose size and shape varied widely owing to different numbers of chromosomes contained.

As a result of the irregular behavior at first meiotic division, interkinesis nuclei were very abnormal. The nuclei in a sporocyte were variable in number and shape as found by KIHARA (1946) in *Triticum-Aegilops* hybrids. The second meiotic division was also very irregular: laggards were common, sticky chromosomes were also met with. The tetrads were abnormal. The pollen grains were abortive, empty or disintegrated if they had some stainable cytoplasm. Complete ovule sterility was also observed in the desynaptic plant; no seeds were obtained, either from selfing or dusting the stigmas with normal pollen. Some of the results will be reported in another paper.

3. Asynapsis in multiploid sporocytes

In some multiploid sporocytes which will be mentioned in the next paragraph various types of asynapsis were observed. In almost all of the syncytes a varying number of univalents appeared. The most extreme case was found in a diploid hybrid F_1 from the cross Robust (a trisomic type)× Brachytic (a linkage tester); about 45 univalents were counted at MI in a syncyte which seemed to consist of 3 nuclei. Next to it were syncytes of a double trisomic Slender which showed in the normal sporocytes asynapsis as described above (p. 50).

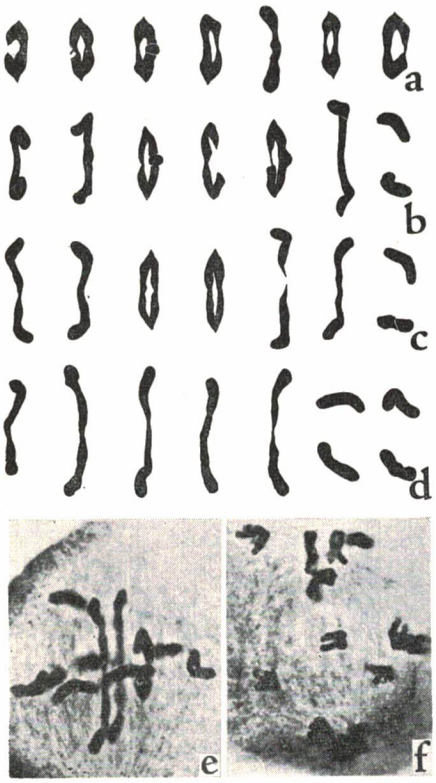

Fig. 3. "Long" chromosomes and partial asynapsis. ×900.
a~e, MI. a, Normal configuration of 7_{II}; 6 ring and 1 rod bivalents. b, $6_{II}+2_I$; showing 3 ring, 1 rod and 2 "long" bivalents. c, $6_{II}+2_I$; showing 2 ring and 4 "long" bivalents. d, $5_{II}+4_I$; showing 5 "long" chromosomes. e, $3_{II}+8_I$ showing 1 ring and 2 "long" bivalents. f, AI showing 5 lagging chromosomes which are splitting at the equator.

4. Partial asynapsis in some pure lines subjected to cold

Indian barley, Indian barley (striped), Olli, Tainan-Zairai and some other varieties which are typical spring form have headed in early or middle March, 1958, due to the warm weather in the winter of 1957/58. The materials entered meiosis as early as February and early March, while the weather has become very cold. Meiotic abnormalities could be observed in March and April. At metaphase I many "long" chromosomes and univalents were observed in many sporocytes (Fig. 3). The chromosome behavior was very similar to that of BURNHAM's "long" chromosomes (BURNHAM 1946; MCLENNAN & BURNHAM 1948). The shape of univalents was peculiar; they were very long in comparison with those of triploids, trisomics, tetraploids and other asynaptic types (Fig. 3a~e). At anaphase I many lagging chromosomes were observed frequently (Fig. 3f).

II. Syncyte formation in microsporocytes

1. Tetraploid sporocytes in X-ray induced desynaptic plants

At diakinesis and MI of the X-ray induced desynaptic plants mentioned above, several sporocytes were observed consisting of two nuclei. In these syncytes like

in other asynaptic sporocytes all 28 chromosomes appeared as univalents (Fig. 2b).

2. Syncyte formation in some trisomic plants and their progeny

Four different types of syncyte formations were observed in some materials of *H. spontaneum nigrum* and its hybrids with some *H. sativum* linkage testers. The four

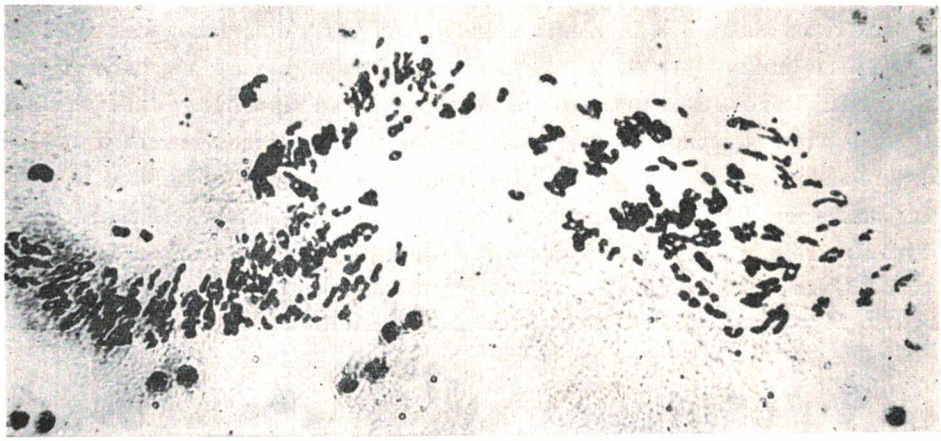

Fig. 4. Syncyte formation, Type I. ×340.
About 400 bivalents at MI in a large plasmodium.

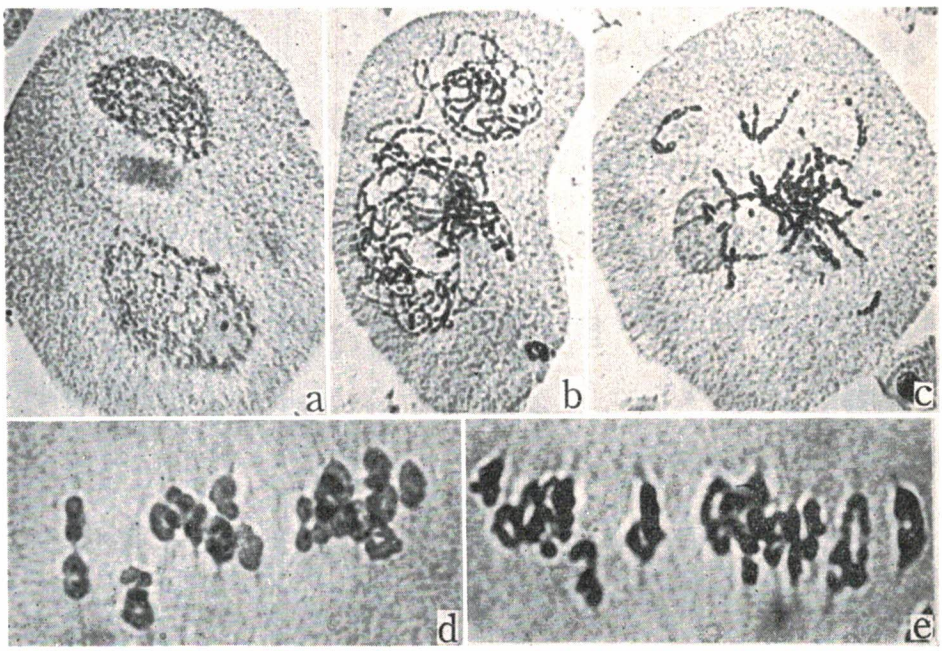

Fig. 5. Syncyte formation, Type II.
a~c, Prophase. ×600. a, Very early prophase showing 2 nuclei in a syncyte. b, Pachytene showing 2 or 3 nuclei in a syncyte. c, Early diakinesis cell consisting 3 nuclei. d and e, MI. ×1,200. d, Hexaploid nucleus having 21_{II}. e, Octoploid nucleus with one or more quadrivalents.

types have the following cytological characters.

Type I. This extremely abnormal type was observed in some tillers of a diploid derivative (F_1) from the cross Robust[1] × Brachytic.[2] At prophase many nuclei were freely scattered in a large plasmodium. At MI large nuclear plates were observed which consisted of various numbers of chromosomes; the maximum number observed in a plate was about 400 bivalents (Fig. 4). A very similar case was reported for barley (SMITH 1942). At AI all bivalents in a plate almost regularly separated simultaneously; in one case about 140 bivalents separated regularly. Multivalents were rarely met with. Uncoiled chromosomes similar to those found in inbred rye (REES 1955) were also observed. Sometimes the direction of the spindles differed in different nuclear plates within a plasmodium. Microspores differed in size and shape.

Type II. This type was observed in one diploid hybrid plant of the cross Pseudo-normal[1] × Colsess V.[2] About 40 percent of the sporocytes were syncytes consisting of 2 (with 14_{II}) to about 10 nuclei (with $\pm 70_{II}$). Multivalents occurred in about 20

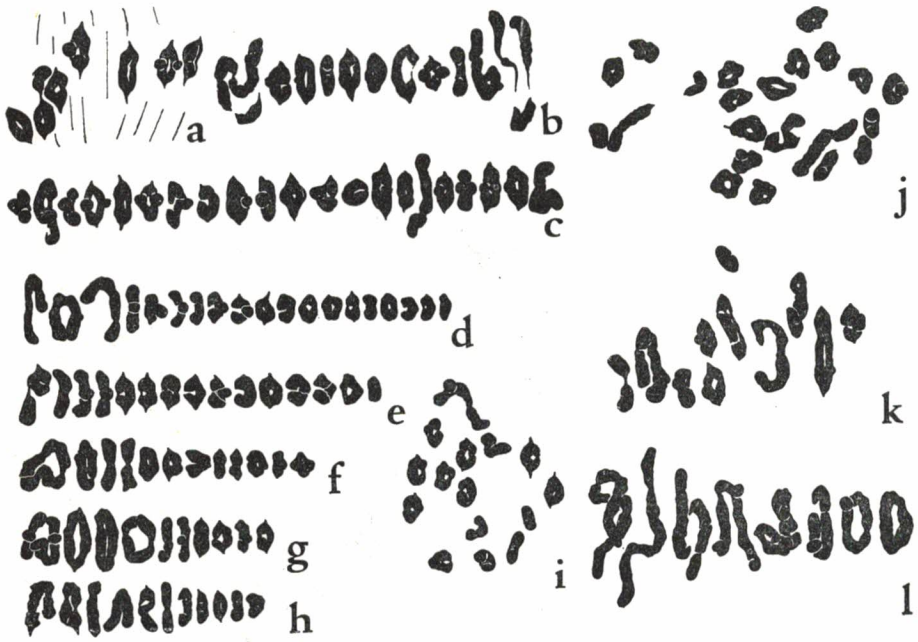

Fig. 6. Syncyte formation, Type III. ×810. All MI configurations.
a~c, 54-87-11. a, Normal sporocyte having $1_{III}+6_{II}$. b, A syncyte of tetraploid nucleus showing $1_{VI}+2_{IV}+8_{II}$. c, Hexaploid nucleus having $3_{III}+18_{II}$. d~i, Various types of syncytes in Pale 2-6. d, A syncyte of hexaploid nucleus having $2_{IV}+1_{III}+15_{II}+1_I$. e~i, Tetraploid nucleus each having $1_{III}+13_{II}+1_I$ (e), $1_{VI}+1_{IV}+10_{II}$ (f), $1_{VI}+3_{IV}+6_{II}$ (g), $3_{IV}+2_{III}+6_{II}$ (h), $1_{III}+13_{II}+1_I$ (i). j~l, Syncytes in Pale 4-2. j and k, Hexaploid nucleus having $3_{III}+18_{II}$ (j) and $4_{IV}+6_{II}+2_I$ (k). l, Various types of multivalent chromosomes; hexavalents (the first 5) and quadrivalents (the last 3).

1) Primary simple trisomic type.
2) Linkage tester with 2n=14 chromosomes.

percent of multiploid sporocytes. A large (fused) nucleus among some separated nuclei was observed at various stages of meiotic prophase; from the former the multivalents may have originated. In this type various sorts of extreme abnormalities have been observed which included: uncoiled chromosomes, extremely shortened chromosomes, "long" chromosomes, and others.

Type III. The following 3 simple trisomic plants showed this type of multiploid sporocytes.

1) Two primary simple trisomic plants of Pale (2n=15); Pale 2-6, Pale 4-2.

2) A trisomic F_1 hybrid (2n=15) from the same cross as Type I, Robust×Brachytic.

About 10~15 percent of the sporocytes were syncytes of nearly the same size as type II; the syncytes consisted of 2 to about 10 nuclei. Multivalents, asynapsis and other cytological irregularities occurred more frequently in the multiploid sporocytes of this type than in Type II. The lower frequency of the syncytes and the cytological extreme irregularities are the main differences of Type III from Type II which showed much more syncytes and less irregularities in the meiotic chromosome behavior.

Type IV. The following 5 plants showed this type of syncyte formation.

1) Three double trisomic plants (2n=16, $2_{III}+5_{II}$) with Slender character; the same plants as those which showed failure of pairing at meiosis (see p. 50).

2) One plant with 2n=14+1 fragment ($1_{III}+6_{II}$) which was obtained in an auto-triploid progeny.

3) One diploid plant (2n=14, 7_{II}) from the progeny of a primary simple trisomic, Purple.

The frequency of syncytes was very low in all plants ranging from 0.7 to 1.5 percent of the sporocytes observed. The syncytes usually consisted of 2 nuclei. Multivalents or other irregularities have never been observed except for the trivalents characteristic of trisomics. The absence of multivalents in this type showed that the syncytes might have been formed by fusion of two microsporocytes after the zygotene stage or two nuclei might have been separated from each other until they passed the zygotene.

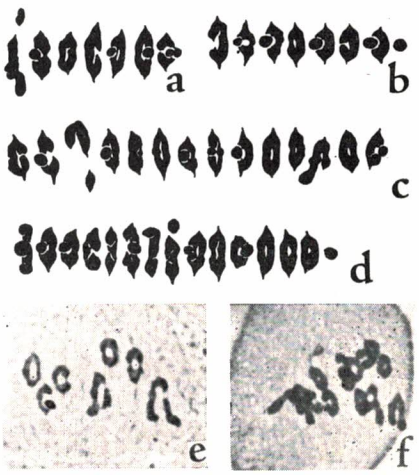

Fig. 7. Syncyte formation, Type IV. a~d, 54-22-1 having 2n=14+1 fragment. ×800. a and b, Normal sporocyte having $1_{III}+6_{II}$ (a) and $7_{II}+1_I$ (b). c and d, Syncytes of tetraploid nucleus having $2_{III}+12_{II}$ (c) and $14_{II}+2_I$ (d), respectively. e and f, Double trisomic Slender, 53-6-7. ×750. e, Normal sporocyte having $2_{III}+5_{II}$. f, A tetraploid syncyte having $4_{III}+10_{II}$.

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Printed by
KASAI PUBLISHING & PRINTING CO.
(*PAN-PACIFIC PRESS*)
Minato-ku, Tokyo, Japan