

ANALYSIS OF THE "DELAYED KNOB REPLICATION" HYPOTHESIS INDUCED BY B CHROMOSOMES IN MAIZE

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ABSTRACT

The "delayed knob replication" hypothesis induced by B chromosomes in maize was analyzed through cytological studies of the kinds of structural rearrangements arising in "high-loss" plants when the microspores contained two heterologous knobbed chromosomes.

Strong support for this hypothesis was obtained since the predictable kinds of chromosome aberrations were found. Depending on whether the two bridges rupture on the same or on opposite sides of the spindle plate, fusion of freshly broken ends produced two kinds of structural rearrangements: (1) non-reciprocal translocations, in which only one translocated chromosome was recovered and the zygote after fertilization was partially trisomic for one knobbed segment and deficient for the other; and (2) dicentric chromosomes, in which both knobbed segments were lost. Reciprocal translocations were not expected since the two translocated chromosomes move to opposite poles.

INTRODUCTION

Rhoades *et al.* (1967) discovered an unexpected and dramatic effect resulting from the interaction of accessory or B chromosomes with the heterochromatic knobs of the regular complement chromosomes. This "high-loss" phenomenon induced by B chromosomes is characterized by breaks in knob-bearing chromosomes and the consequent loss of the acentric portion of the chromosome arm distal to the breakpoint. Breaks are induced in knobbed chromosomes at the second microspore division only when at least two B chromosomes are present and all knobbed chromosomes are subject to loss (Rhoades and Dempsey, 1972, 1973).

The genetic data associating a specific loss pattern with a particular arrangement of the knob, marker gene, and centromere for several different knobbed chromosomes led Rhoades and Dempsey to propose a mechanism to account for chromatin loss (Rhoades and Dempsey, 1970, 1972, 1973; Rhoades, 1978). According to the hypothesis of Rhoades and Dempsey (1972, 1973), B chromosomes induce a delayed replication of knobs at the second microspore division resulting in a dicentric bridge at anaphase. Breakage of the bridge between the knob and centromere produces deficient chromosomes and the type of loss pattern depends on the location of the marker gene.

Although breaks can occur anywhere between the knob and the centromere, Rhoades and Dempsey (1973, 1975) found from genetic data that the distribution of breakpoints in the postulated dicentric bridge is nonrandom. Saraiva (1979) analyzed cytologically plants deficient for chromosome 9 induced by B chromosomes and found that 80% of the breaks occurred at the centromere.

Rhoades and Dempsey (1973, 1975), however were unable to demonstrate cytologically the anaphase bridges in the second microspore mitoses postulated in their hypothesis. At this stage, the microspores are surrounded by a thick exine layer, and the cytoplasm contains numerous starch granules; these factors have made the visualization of the loss mechanism technically difficult. In the present investigation, we sought to verify the proposed mechanism in an indirect manner. When simultaneous breaks occur in the postulated dicentric bridges from two different knobbed chromosomes, predictable types of structural rearrangements will be recovered depending on the location of breaks and the fusion of broken ends. A search was made for such aberrations in the belief that they would constitute proof of dicentric bridge formation at anaphase of second microspore mitosis.

MATERIALS AND METHODS

Pollen from plants with several B chromosomes identified as a high loss strain carrying the dominant marker genes *Yg2* (green seedling and plant) and *C* (colored aleurone) on chromosome 9 with a large knob terminating the short arm (9S) was applied to the silks of *yg2* (yellow green seedling), *c* (colorless aleurone) tester plants. Since the *Yg2* locus is close to the terminal knob, selection of exceptional yellow green progeny (*yg2*) plants which have lost this locus screens for deficiencies of various lengths in 9S, as well as for more complex rearrangements. Several other chromosomes of the high loss stock had interstitial knobs on the long arms. Chromosomes 2 and 3 had large knobs and chromosomes 4, 5, and 6 had small knobs. All knobbed chromosomes were subject to loss, but the frequency of loss should be higher with larger knobs than with smaller knobs. Ears with high frequencies of loss for *C* in the endosperm (around 15% to 25%) were selected. Fertilization of egg and polar nuclei by sperm nuclei with the deficient

chromosome is random; an ear with a high frequency of endosperm losses should also give a high frequency of deficient zygotes. The dominant *C* kernels, with no loss in the endosperm, were germinated in a sandbench. Since the primary loss event occurs at the second microspore mitosis, there is noncorrespondence between embryo and endosperm constitutions for chromosome 9. Thus, some *C* kernels should carry a deficient chromosome 9 in the zygote, but all *c* kernels, representing loss in the endosperm, should have a normal 9 in the zygote. The fertilization of an egg cell with recessive *yg2* by a sperm nucleus deficient for the *Yg2* allele produces a yellow green sporophyte. The yellow green exceptionals were transplanted to the field and sporocytes were collected for cytological analysis of the pachytene stage of microsporogenesis through the squashing technique.

RESULTS AND DISCUSSION

A total of 267 exceptional yellow green plants with a deficient chromosome 9 which had undergone breakage were examined cytologically at meiosis to determine if another knobbed chromosome had undergone breakage and the kinds of structural changes that had occurred. The great majority of the structural changes arising at the second microspore division consisted of simple, terminal deficiencies. Twenty-four plants were found, however, with structural rearrangements involving chromosome 9 and another knobbed chromosome.

The constitution of the sperm nuclei or male gametes produced following concomitant breaks in the short arm of chromosome 9 (9S) and in a second knobbed chromosome are shown in Figure 1. For illustrative purposes a chromosome 3 with a knob in the long arm (3L) was chosen.

Sperm nuclei (1) and (2) are produced when breaks in the two anaphase bridges are on the same side of the equatorial plate. Gametes (3) and (4) result following a break in a bridge above the equatorial plate and below in a second bridge. Gamete (1) has a dicentric translocation chromosome that is deficient for segments of 9S and 3L. Sperm nucleus (2) has a normal 9 (N9) and a normal 3 (N3) chromosomes and acentric fragments that can either fuse or not fuse. Gamete (3) has a deficiency for the distal tip of 9S and has a duplicated distal segment of 3L. Sperm nucleus (4) has a deficiency for a piece of 3L and has a duplicated distal end of 9S.

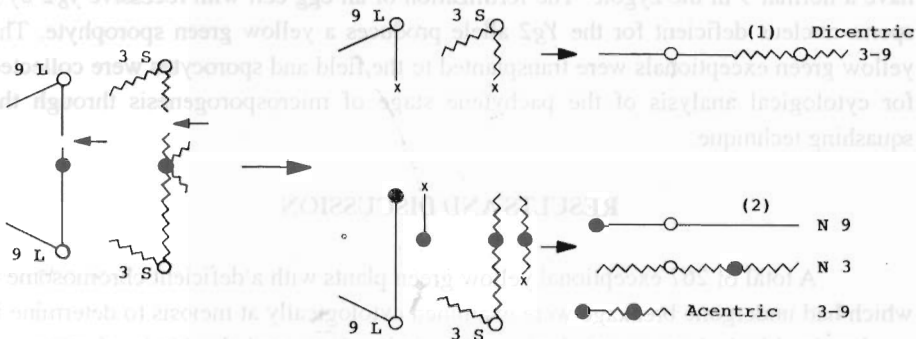
Fertilization of a *yg2* egg by sperm nucleus (1) gives a *yg2* plant deficient for segments of chromosomes 3 and 9 and possessing a dicentric chromosome. This chromosome would be subject to strong selection against its survival in the sporophytic tissue because it would undergo the chromosome type of bridge-breakage-fusion cycle and hence tend to be eliminated. It is unlikely that the population of exceptional *yg2* plants surviving to maturity would include many individuals of this composition.

Anaphase of 2nd
microspore mitosis
(short arrows
indicate breaks)

Telephase constitution
at top and bottom
poles following breakage
and replication of knobs

Constitution of sperm
nuclei or male gametes
following fusion
of broken ends

I. Breaks on same side of equatorial plate



II. Breaks on opposite sides of equatorial plate

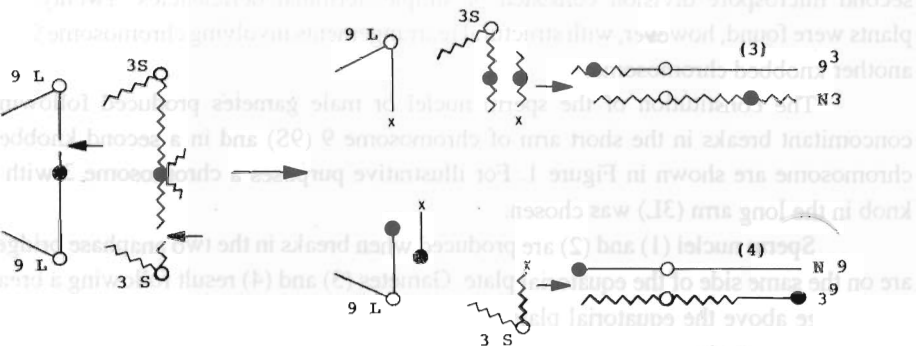


Figure 1 - The predicted classes of sperm nuclei produced following delayed replication of the knobs on chromosomes 3 and 9; breakage of the resulting dicentric bridges at anaphase of the second microspore mitosis and fusion of broken ends (indicated by the letter "X").

A male gamete of class (2) would give a normal *Yg2* zygote with structurally intact chromosomes 3 and 9. The broken ends of the two acentric fragments would presumably fuse to form a translocated chromosome. However they would be eliminated whether they fused or not.

A sperm nucleus of class (3) produces *yg2* sporophytes with the 9^3 chromosome, deficient for the *Yg2* allele but capped by a piece of 3L, a normal chromosome 9, and two chromosomes 3. These plants are trisomic for part of 3L and give chains of 4 at diakinesis.

A male gamete of class (4) yields *Yg2* zygotes with two normal chromosome 9, one normal 3 and the 3^9 translocated chromosome.

The zygotes from the functioning of sperm cells (3) and (4) are complementary. Those from gamete (3) are partial trisomics for chromosome 3 and those from class (4) are partial trisomics for chromosome 9. However, zygotes from class (4) gametes would not be detected in these experiments because they give rise to green plants and loss of the *Yg2* allele was the marker used for selecting plants for cytological study. This type of zygote should be found if pollen mother cells were taken from green as well as from *yg2* plants.

Twenty-four plants were found possessing structural rearrangements (Table I). There were concomitant breaks at the second microspore anaphase in two different knobbed chromosomes. The most common type of structural rearrangement expected in the exceptional *yg2* plants surviving to maturity was the non-reciprocal translocation, which is partially trisomic. Twenty-four nonreciprocal translocations were found, all involving a deficient chromosome 9, and either chromosome 2, 3, 4, 5, or 6, all of which were knobbed in the high-loss male parent. With one exception, the segment of the heterologous chromosome translocated to 9S was knobbed (Table I).

Only nonreciprocal translocations (the recovery of one of the two expected translocated chromosomes) were found although breaks had clearly taken place in two chromosomes. Sister sperm nuclei (3) and (4) each have one of the two expected translocated chromosomes. They were sequestered, however, to opposite poles of the spindle (Figure 1), and there was no opportunity for the fusion of broken ends needed to produce two reciprocally translocated chromosomes in one nucleus. Reciprocal translocations were not found by either Rhoades and Dempsey (1972) or in the present investigation.

Attempts were made to detect genetically reciprocal translocations involving chromosomes 3 and 9. In the high-loss strain used, these chromosomes have large knobs and, consequently, are subject to loss induced by B chromosomes. Crosses were made with pollen from a high-loss strain carrying the dominant *A1* allele in chromosome 3 and the *Yg2 Wx* alleles in chromosome 9. The female parent was recessive for the *al*; *yg2 wx* alleles. The *A1*; *Wx* kernels were planted and the ensuing green plants grown to maturity. Pollen of all plants was scored for sterility, and 78 semi-sterile plants were selected as putative carriers of reciprocal translocations. These were used as male and female parents in crosses with the *al*; *wx* tester stock to test for unexpected linkage between *A1* and *Wx*, resulting from a translocation between chromosomes 3 and 9. In all cases, these two genes

segregated independently, and semi-sterility was not transmitted to the offspring. Thus, semi-sterility did not occur because of a heterozygous reciprocal translocation. This finding is in accordance with the Rhoades-Dempsey hypothesis.

Table I - Cytological analysis of the structural rearrangements found in the progeny of high-loss plants used as the male parent.

Plant no.	Translocated chromosome	Partially trisomic for	Knob present on translocated fragment
1. 166-1	9 ³	3L	yes
2. 128-3	9 ³	3L	yes
3. 253-6	9 ² or 9 ⁵	2L or 5L	*
4. 253-10	9 ³	3L	yes
5. 258-1	9 ⁴	4L	yes
6. 259-2	9 ³	3L	yes
7. 260-1	9 ³	3L	yes
8. 263-2	9 ²	2L	yes
9. 264-3	9 ⁶	6L	*
10. 281-4	9 ⁶	6L	*
11. 283-3	9 ³	3L	
12. 296-3	9 ² or 9 ⁵	2L or 5L	yes
13. 304-3	9 ⁵	5L	yes
14. 335-2	9 ⁵	5L	yes
15. 342-2	9 ⁴	4L	yes
16. 400-5	9 ²	2L	yes
17. 575-2	9 ³	3L	no
18. 577-1	9 ³	3L	yes
19. 584-4	9 ⁵	5L	*
20. 592-3	9 ²	2L	yes
21. 595-3	9 ³	3L	yes
22. 597-2	9 ²	2L	yes
23. 33893-1	9 ²	2L	yes
24. 33878-21	9 ²	2L	yes

* Due to the poor spread of the chromosomes at pachytene, it was not possible to be sure if the translocated segment carried a knob.

Sperm nucleus of class (1), possessing a dicentric chromosome, should be produced but, after fertilization, would give rise to weak plants not likely to reach sexual maturity. Therefore, a different approach was used to demonstrate the generation of dicentric chromosomes. Dominant *C* kernels from the pollination of *yg2 c* silks by a high-loss male parent homozygous for *Yg2 C* were germinated in a sandbench. Exceptional *yg2* seedlings deemed to possess a dicentric chromosome because of decreased vigor were subject to cytological examination. Root tips were excised from 72 slow-growing and off-type *yg2* seedlings, these plants should be good candidates for the desired class of structural rearrangement. The presence of a dicentric chromosome at a somatic mitosis can be detected by the formation of dicentric bridges in those anaphases where the two centromeres carried by each chromatid move to opposite poles. In half of the anaphases, the two centromeres on each chromatid move to the same pole and bridges are not formed. The cytological analysis of the 72 weak seedlings disclosed that four seedlings had a dicentric chromosome and, as expected, bridges were present in about half of the anaphases (Table II). These four plants were transplanted to pots but they died before reaching 30 cm in height.

Table II - Anaphase counting of dicentric bridges in mitosis from exceptional *yg2* seedlings.

Seedling identification	Total number of anaphases examined	Number of anaphases with dicentric bridges
47-1	16	7
52-4	12	5
76-2	21	8
89-6	19	8

Most of the breaks in the knobbed A chromosomes induced by B chromosomes were either at or near the centromere (Saraiva, 1979). When a dicentric translocated chromosome was formed, the two centromeres will generally lie in close proximity and, since a half-twist must occur in the region lying between the two centromeres if they are to pass to opposite poles, they will usually orient to the same pole. Consequently, most of the dicentric chromosomes would not be detected by the formation of bridges in root tip cells. However, the recovery of four seedlings possessing dicentric chromosomes, from male gametes of class (1), constitutes convincing evidence of the existence of this class of structural change as predicted by the Rhoades-Dempsey hypothesis. The

dicentric chromosomes were also identifiable at somatic metaphase in good preparations by the presence of two centric constrictions.

The finding of non-reciprocal translocations, of dicentric chromosomes and the absence of reciprocal translocations are all in accordance with the Rhoades-Dempsey hypothesis, and convincingly corroborate the assumption that bridges are formed at anaphase of the second microspore division. Furthermore, the observation that the breaks resulting in translocated chromosomes, with only one exception, occurred in knobbed arms supports the concept that knobs are responsible for bridge formation.

RESUMO

A hipótese de "replicação tardia de knobs", induzida por cromossomos B em milho, foi analisada através de estudos citológicos dos tipos de rearranjos estruturais surgidos em plantas nas quais os cromossomos B induziram quebras concomitantes em dois cromossomos não homólogos portadores de knobs. Os resultados obtidos confirmaram a hipótese, pois apenas os tipos de aberrações cromossômicas preditos foram encontrados: 1 - translocações não-recíprocas; 2 - cromossomos dicêntricos.

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