

## GENETIC EVIDENCE OF AN INTERNAL DELETION INDUCED BY B CHROMOSOMES IN MAIZE (*Zea mays* L.)

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### ABSTRACT

B chromosomes were used to induce deficiencies in the short arm of chromosome 9 of maize (*Zea mays* L.). One chromosome with a small terminal deficiency also possessed an internal deletion whose presence was revealed by several genetic tests. Although internal deletion is not predicted by the high-loss mechanism, breaks in the A chromosomes are frequent and occasional recovery of unusual structural modifications may occur.

### INTRODUCTION

Rhoades *et al.* (1967) discovered the "high-loss" phenomenon that was characterized by breaks induced by B chromosomes in knob-bearing chromosomes, and the consequent loss of the acentric portion of the chromosome arm distal to the breakpoint. Breaks were induced only in knobbed chromosomes at the second microspore division when at least two B chromosomes were present and all knobbed chromosomes were subject to loss (Rhoades and Dempsey, 1972, 1973). Additional observations have revealed that larger knobs undergo loss more frequently than smaller knobs, and that B chromosomes of several strains of maize (*Zea mays* L.) were able to induce loss from knobbed A chromosomes (Rhoades and Dempsey, 1970).

The association of a specific loss pattern with a particular arrangement of the knob, marker gene, and centromere led Rhoades and Dempsey (1972, 1973) to propose a mechanism to account for chromatin loss. According to their hypothesis, B chromosomes induce a delayed replication of the knobs at the second microspore

division, resulting in a dicentric bridge at anaphase. Breakage of the bridge between the knob and centromere produces chromosomes with terminal deficiencies, and the type of loss pattern depends on the location of the marker gene.

Although Rhoades and Dempsey hypothesis explains only the occurrence of terminal deficiency, genetic evidence of internal deletion induced by B chromosomes in maize is presented in this paper.

## MATERIALS AND METHODS

The marker genes used in chromosome 9 included the dominant *Yg2*, located close to the knob, which causes green seedlings and plants, and the recessive *yg2*, which produces yellow green seedlings and plants. The dominant *C* allele causes anthocyanin pigment in the aleurone, whereas the recessive *c* allele has colorless aleurone. The *I* allele, dominant to *C*, is an inhibitor of aleurone color and gives a phenotype similar to *c*. The dominant *Wx* allele gives blue staining starch in endosperm and pollen grains with  $I_2$ -KI, while the recessive *wx* gives red staining. The white deficiency (*wd*) on chromosome 9 was found by McClintock (1944) and lacks the ultimate thread and a part of the last chromomere of the short arm of chromosome 9, including the *Wd* allele. The *wd* chromosome is normally transmitted by female and male gametophytes, but homozygous *wd* plants have a lethal white phenotype and do not survive beyond the seedling stage.

Pollen from plants with several B chromosomes, carrying a large knob terminating the short arm of chromosome 9 (9S) and homozygous for the dominant *Yg2C Wx* alleles, was applied to the silks of *yg2 c wx* tester plants and gave exceptional yellow green progenies. Those that had a deficient chromosome 9, due to a breakage induced by B chromosomes and consequent loss of the *Yg2* allele, were selected.

## RESULTS AND DISCUSSION

Yellow green exceptional plant 270-2 had no pollen abortion. Cytologically, the terminal deletion included three or four of the terminal chromomeres. An average of 1.7% recombination was found for the C-breakpoint interval. Plant 270-2, however, was less vigorous in comparison with other *yg2* exceptional plants but, having deficiencies of a similar size and of the same genetic background. When pollinated by *c* tester stock, plant 270-2 produced a small ear with 40C kernels and 87c kernels. The reduced frequency of female transmission of the *C* marker on the deficient chromosome (31%) was unexpected, because the deficiency was very small.

To check for reduced transmission of the deficient chromosome, twenty C kernels from the ear on plant 270-2 designated as family 910, were planted. Plants of family 910 were weak and only seven of the fifteen that survived to maturity produced

an ear. Although the ears were small, the seven ears had reduced transmission frequency of the deficient chromosome 9, average of 34.3% (Table I), and the chi-square test made with the pooled data was significantly different (at the 0.01 level) from the expected 50%.

Table I - Percentage of female transmission in test crosses of the deficient chromosome from plant 270-2, which was marked by the *C* allele. The normal 9 had the *c* allele.

Family #	Number of kernels			% of colored (C) kernels
	colorless ( <i>c</i> )	colored ( <i>C</i> )	Total	
910-2	84	43	127	33.9%
910-5	41	27	68	39.7%
910-6	102	58	160	36.2%
910-8	81	38	119	31.9%
910-9	69	32	101	31.7%
910-14	32	13	45	28.9%
910-15	17	11	28	39.3%
Pooled	426	222	648	34.3%

The true female transmission frequency of the deficient chromosome should be corrected for those crossovers which place the *C* allele in a normal 9. These, however, are infrequent.

Plants from additional *C* kernels were used as the female parent in crosses with homozygous *w d c* individuals carrying the *Wd*-ring with the *I* allele. Self colored (*C*) and mosaic (*C-I*) kernels from this cross were germinated in a sandbench. Almost all of the seedlings were white or green-white striped, demonstrating that plant 270-2 had a deficiency that included the *Wd* locus.

The low frequency of transmission of the deficient chromosome, together with reduced vegetative vigor, suggested that the 270-2 chromosome had an internal deletion in addition to a short terminal deletion. Cytological analysis of several plants heterozygous for the 270-2 chromosome failed to disclose a small buckle, which would be diagnostic of an internal deletion. According to Stadler and Roman (1948) and Mottinger (1970), however, short internal deletions do not give buckles when nonhomologous pairing occurs. Rhoades (1968) also observed very few buckles in cells containing a N9 and a Tp9 chromosome, where a piece of 3L inserted into the short arm of chromosome 9 increased the length of 9S by 20%.

A sensitive test for internal deletions is the decrease in recombination values between flanking markers or in neighboring regions. The recombination value between the *C* and *Wx* genes was determined using the deficient plants as the male parent. Although the deficient chromosome (*df*) is not male transmissible and only one parental (*c wx*) and one recombinant (*c Wx*) class are obtained, the data allow an accurate estimation of recombination. Plants of *Df 270-2 C Wx/N9 c wx* constitution were used to pollinate *c wx* tester plants. The results are presented in Table II.

Table II - Percentage of crossing over between *C* and *Wx* in *Df 270-2 C Wx/N9 c wx* plants. Female parents were *c wx* testers.

Male parent	Number of kernels			% Crossing over between <i>C</i> and <i>Wx</i>
	<i>c wx</i>	<i>c Wx</i>	Total	
745-6	219	24	243	9.9%
745-1	244	33	277	11.9%
910-2	212	28	240	11.7%
910-6	310	46	356	12.9%
910-9	204	16	220	7.3%
910-7	213	26	239	10.9%
745-8	192	40	232	17.2%
910-8	184	17	201	8.5%
910-8	174	18	192	9.4%
910-1	209	29	238	12.2%
910-4	263	22	285	7.7%
910-2	279	22	301	7.3%
745-6	260	26	286	9.1%
Pooled	2963	347	3310	10.5%
209-8 (control)	204	57	261	21.8%

Plant 209-8, with a deficiency of a size similar to that of plant 270-2 but without the postulated internal deletion, was used as a control and gave 21.8% of recombination between *C* and *Wx*. Analysis of the data of Table II shows that 12 of 13 ears had reduced crossing over values ranging from 7.3% to 12.9%. This suggests that plant 270-2 not only had a terminal deletion responsible for the loss of *Yg2* but also had an internal one which produced the decreased *C-Wx* recombination. One plant (745-8), however, gave 17.2%

of crossing over, a value similar to the control. This probably represents a case in which the terminally deficient chromosome no longer carried the internal deletion, following a crossover between the postulated internal and terminal deletions. This hypothesis could not be tested because the ear on plant 745-8 had inviable kernels due to a fungal infection.

A definitive test for the putative internal deletion in plant 270-2 was made by introducing the small Wd-ring chromosome, carrying the *Yg2* and *I* alleles but not containing more proximal loci. The same combination of alleles also was introduced into four different exceptional yellow green plants, with deletions cytologically similar to that of plant 270-2, to determine if the Wd-ring complements each of the deficiencies sufficiently to make them male transmissible. Heterozygotes of each exceptional *yg2* of *Df9 C/N9 c* constitution were used as the female parent in crosses with plants having the Wd-ring and homozygous *wd c*. Plants arising from mosaic kernels (*C-I*), which have a deficient 9 with the *C* allele and the *I*-bearing Wd-ring, were used as the male parent in test crosses. The data are presented in Table III.

Table III - Percentage of transmission of various deficient chromosomes by the male parent when combined with the Wd-ring

Male parent	Number of chromomeres missing in terminal deletion	% of colored kernels ( <i>C</i> ) (without ring)	% of colored ( <i>C</i> ) plus mosaic ( <i>I-C</i> ) kernels (with ring)
920-1 (from 270-2)	3	1.7%	1.6%
919-3 (from 291-1)	4	0.5%	5.9%
918-5 (from 197-1)	2	6.3%	25.1%
917-2 (from 298-8)	2	3.6%	18.4%
916-4 (from 309-3)	3	1.7%	11.6%

It is clear that the only deficient chromosome not made functional in the presence of the ring was that from plant 270-2 (Table III). Thus, plant 270-2 had not only a terminal deletion but also an internal deletion. The internal deletion is responsible for the nonfunctioning of pollen grains containing the deficient chromosome and the ring.

There is experimental evidence to support the hypothesis that Df 270-2 has two deficient segments, one is a short terminal and the other an internal. The simplest explanation for the origin of this chromosome requires three breaks. These presumably occurred during bridge rupture at the second microspore division in the high-loss line.

Internal deletions are not expected to occur according to the Rhoades and Dempsey (1972, 1973) hypothesis of the high-loss mechanism, but these could be infrequent cases where more than one break arose. McClintock (1951) studied controlling elements and found a structural alteration including an internal deletion. Rhoades and Dempsey (1972) surveyed the types of loss events occurring in chromosome 3 and detected genetically four instances of internal deletions. Similar to the high-loss cases, McClintock's aberration was generated following formation of a dicentric bridge, and it also required more than one break for its inception.

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### RESUMO

Cromossomos B foram utilizados para induzir deficiências no braço curto do cromossomo 9 de milho (*Zea mays* L.). Um cromossomo com uma pequena deficiência terminal também possuía uma deleção interna, cuja presença foi revelada através de vários testes genéticos. Embora deficiência interna não seja esperada pelo mecanismo de quebra cromossômica induzido pelos cromossomos B, quebras nos cromossomos A são frequentes, podendo ocorrer a recuperação ocasional de modificações estruturais incomuns.

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