

TRANSMISSIONAL BEHAVIOR OF A NEW 9^B TRANSLOCATION IN MAIZE (*Zea mays* L.)

Luiz S. Saraiva

ABSTRACT

A new translocated chromosome in maize involving chromosome 9 and B chromosome was submitted to cytogenetical analysis. The 9^B translocated chromosome carried all of the distal heterochromatic blocks of the B chromosome, as well as most of the euchromatin, and lacked a small terminal segment of the short arm of chromosome 9. The average frequency of female transmission of the 9^B chromosome was 47.6% while the normal 9 was recovered in 52.4% of the eggs. Pollen grains with the 9^B chromosome were unable to germinate and failed to function in fertilization. However, the small Wd ring chromosome complemented the deficiency in the 9^B chromosome and made it transmissible through the pollen.

INTRODUCTION

Accessory, or B chromosomes, have been reported in about 600 species of plants and more than 100 animal species (Jones, 1975).

The B chromosome of maize is the most extensively studied and is responsible for, or participates, in, several cellular effects. The B chromosome increases chiasma frequency and variance (Ayonoadu and Rees, 1968; Ward, 1976) and it also increases intergenic (Hanson, 1961; Rhoades, 1968) and intragenic recombination (Melnyczenko, 1970) in maize.

Himes (1967) concluded that the B chromosome of maize increases DNA and histone content and increases the nuclear volume. It's also responsible for breakage in knob-bearing chromosomes and the consequent loss of the acentric portion of the chromosome arm distal to the breakpoint (Rhoades, *et al.*, 1967).

Roman (1947) was the first to isolate an A-B translocation in maize involving a chromosome of the regular complement (A chromosome) and an accessory chromosome. Since then many A-B translocations have been produced in maize (Be-

ckett, 1967; 1968; 1972; 1975). They have been used mainly to locate recessive mutant genes in the appropriate chromosome arms (Roman and Ullstrup, 1951; Neuffer and Beckett, 1971; 1972; 1973). A series of A-B translocations covering approximately two-thirds of the known genetic linkage map of corn was assembled by Neuffer and Beckett (1971) and more is known now.

The behavior of an isolated new 9^B translocation was investigated through cytogenetical studies of its female and male transmission, in the presence and absence of a Wd-ring chromosome.

MATERIALS AND METHODS

The 9^B chromosome

The translocated chromosome was the result of fusion between a deficient chromosome 9 and a portion of an accessory or B chromosome in a "high-loss" plant (Saraiva, 1979). The break in 9S was proximal to the Yg2 locus and occurred probably in the second chromomere from the end, while the breakpoint in the B chromosome was in the euchromatin near the proximal knob. Thus most of the euchromatic region and all of the distal heterochromatic blocks of the B were translocated to chromosome 9. The translocated chromosome having a chromosome 9 centromere was designated 9^B . The reciprocal B^9 chromosome was not recovered.

Phenotypes of marker genes in chromosome 9

Listed below are the genes on chromosome 9 followed in the experiments. The phenotype of individuals with the dominant allele is contrasted to phenotypes associated with the recessive mutant allele:

Dominant	Recessive
Yg2 = green seedling and plant	yg2 = yellow green seedling and plant
C ¹ = anthocyanin pigment aleurone	c = colorless aleurone
Wx = I2-KI gives blue staining starch in endosperm and pollen grains	wx = I2-KI gives red staining starch in endosperm and pollen grains

¹ A third allele, dominant to C, exists at this locus. The I allele is an inhibitor of aleurone color and gives a phenotype similar to c.

The Wd - ring chromosome

The white deficiency (*wd*) chromosome 9 found by McClintock (1944), lacks the ultimate thread and a part of the last chromomere of 9S 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. McClintock identified a small ring chromosome (Wd-ring) carrying the *Wd* allele. When combined with *wd/wd* homozygotes, the ring supplies the chromatin missing from the deficient chromosomes and viable plants are obtained which may be grown to maturity and bred. Seedlings and plants of *wd/wd* + Wd-ring constitution are green-white striped due to instability of the ring. Mitotic loss of the ring or its *Wd* allele exposes the *wd* deficiency and produces a somatic sector with white tissue. The Wd-ring also carries the *Yg2* and *I* alleles but does not contain more proximal loci of 9S.

Germination of pollen grains on agar

Day-old anthers were removed from the tassels the evening prior to pollen collection, thus guaranteeing only fresh pollen in the collection. Pollen was distributed over plates containing the Cook-Walden (1965) medium in a high relative humidity. Five plates were prepared from each tassel. Observations on pollen germination were made 30 minutes after incubation at room temperature over water in sealed chambers, and repeated, for several hours, at half hour intervals. A solution of I₂KI was then added to identify pollen grains with a normal chromosome 9 (*wx*) and with the translocated chromosome (*Wx*). The grains were scored for red or blue-staining starch, respectively, and the extrusion and growth of pollen tubes (germination) noted for each kind of pollen grain.

Crosses including the Wd ring chromosome

Plants of 9^B *Wx/N9 wx* constitution were pollinated by *wx* plants homozygous for McClintock's male transmissible *wd* deficiency in 9S and carrying the ring with the *Wd* allele. F1 plants of 9^B *CWx/wdCwx* constitution with the Wd ring chromosome were green-white striped because of the instability of the ring chromosome in somatic divisions. They were used as the male parent in crosses with a recessive waxy stock. Since the F1 plants were homozygous *C/C* and the ring carried the dominant *I* allele, the presence of the ring in the endosperm of the test cross progeny could be identified by the mosaic aleurone pattern resulting from ring instability. As mentioned above, presence of the ring in sporophytes of *wd/9^B* constitution could be recognized because they were green-white striped.

RESULTS AND DISCUSSION

Plants heterozygous for 9^B (Def. $Yg2$)/ $N9(Yg2)$ chromosomes were pollinated by tester stock ($yg2/yg2$) and all yellow green seedlings had the 9^B chromosome and all green possessed the $N9$ chromosome. Eight ears were obtained and the data are given in Table I.

Table I - Percentage of female transmission of the 9^B chromosome from $9^B/N9$ heterozygotes.

Family	Seedling				Total
	Green (normal 9)		Yellow green (9^B)		
	Nº	%	Nº	%	
652-1	67	49.6	68	50.4	135
652-2	96	52.5	87	47.5	183
652-4	71	54.2	60	45.8	131
653-1	136	54.6	113	45.4	249
653-4	137	51.9	127	48.1	264
669-1	77	56.6	59	43.4	136
670-2	72	51.1	69	48.9	141
670.3	168	50.4	165	49.6	333
	824	52.4	748	47.6	1572

The average frequency of female transmission of the 9^B chromosome (47.6%) does not differ significantly from the $N9$ (52.4%). So transmission of the 9^B chromosome through the eggs is normal. The presence of the 9^B chromosome in the plants of families 652, 653, 669 and 670 was demonstrated by using them as the male parent in test crosses. Samples of 100 kernels from each of the eight ears were germinated in the sandbench and gave only green seedlings as expected if the 9^B chromosome was not transmitted through the pollen.

Pollen from $9^B Wx/N9 wx$ heterozygotes were normal appearing grains of uniform size with no indication of an impaired development of male gametophytes with the 9^B chromosome despite their inability to achieve fertilization in competition with normal pollen. As expected, since there was no pollen sterility, equal numbers of blue and red-staining grains were found with I_2KI . Because of the linkage of Wx with the breakpoint in 9^B , most of the blue-staining grains had the 9^B chromosome. So, even though pollen with the 9^B chromosome were of normal size they were un-

able to successfully compete in achieving fertilization in competition with normal grains. This functional sterility must be caused by the deficiency in the 9^B chromosome which consists of the distal two chromomeres of the short arm of chromosome 9.

Additional studies were carried out to see if the failure of the normal appearing 9^B pollen to achieve fertilization was caused by their slower rate of pollen tube growth or by their inability to germinate. Fresh pollen grains from 9^B *Wx*/N9 *wx* plants 650-3, -4, -5 and -11 were sown on agar containing the Cook-Walden medium. The extrusion and growth of pollen tubes (germination) was largely restricted to pollen grains that stained red with I₂KI and consequently carried the normal 9. The relatively infrequent blue-staining grains which extruded pollen tubes probably possessed the *Wx* allele in a normal 9 because of crossover. Thus, the failure of 9^B pollen grains to function in fertilization results from their inability to germinate and not because of a slower rate of pollen tube growth.

Since the behavior of the 9^B chromosome during meiosis in P.M.C.'s was completely normal, an attempt was made to see if the postmeiotic (gametophytic) divisions were altered or inhibited by the 9^B chromosome. The cytological examination of mature pollen grains from 9^B/N9 plants stained with carmine showed that all pollen grains contained a vegetative nucleus and two sperm nuclei, demonstrating that the microspore mitoses were not affected by the 9^B chromosome. The defect induced by the 9^B chromosome must be expressed after the second microspore division and the nonfunctioning of 9^B pollen grains was presumably due to the lack of a gene product required for pollen germination. This putative gene(s) must be located close to the tip of the short arm of the normal chromosome 9. It is possible that genes with similar function are present in other chromosomes. Rhoades and Dempsey (1973) described a somewhat different situation involving a chromosome 3 with a terminal deficiency (Df3) of two or three minute chromomeres. The Df3 was not male transmissible and pollen grains with the Df3 were smaller than normal grains. A cytological examination of the small pollen grains disclosed that in some microspores the generative nucleus had divided to form two abnormal appearing sperm cells, while in other the generative nucleus was undivided. Df3 was different from 9^B in that the second microspore mitosis was abnormal or even suppressed while microspores with the 9^B chromosome had two normal appearing sperm cells. They were, however unable to function in fertilization because of their failure to germinate.

The Wd ring chromosome carried the *Yg2* and *I* alleles and consequently covered the distal region of 9S which was deficient in the 9^B chromosome. It seemed possible that pollen grains with the Wd ring and the 9^B chromosome might function, thus permitting male transmission of the 9^B chromosome. In 9^B heterozygotes without the ring there was about 10% of crossing over between the *Wx* locus and the break-point of the translocation. Thus, if the 9^B chromosome was not male functional when combined with the ring chromosome, approximately 10% of the kernels in test crosses onto *wx* silks would be *Wx*. However, if the ring renders pollen grains carrying the

9^B chromosome functional, then the percentage of Wx kernels should be substantially increased. In a total of five test crossed ears, there were 392 Wx kernels and 799 wx . The average percentage of Wx kernels was 32.9% (392/1191), and varied from 25.8% to 35.2% (Table II).

Table II - Percentage of Wx kernels in male test crosses of $9^B Wx/wd wx$ plants with the Wd ring chromosome.

$wx \text{ } \text{♀} \times 9^B Wx/wd wx$ plus ring ♂	Number of kernels			%
	wx	Wx	Total	
495-4 x 515-1	150	75	225	33.3
495-9 x 515-1	197	97	294	33.0
495-2 x 523-1	115	40	155	25.8
500-2 x 528-3	184	100	284	35.2
500-3 x 528-3	153	80	233	34.3
	799	392	1191	32.9

The amount of crossing over between Wx and the translocation point was too low to account for the relatively high frequency of Wx kernels. These data strongly suggested that the 9^B chromosome was male transmissible if combined with the Wd ring chromosome. However, since the Wx locus was not a good genetic marker for the 9^B chromosome because of the amount of crossing over between it and the translocation breakpoint, the experiment was repeated using a marker gene more distally located. A homozygous wd plant with the Wd ring chromosome was found to be segregating for C/c . It was selfed and the homozygous c (colorless) kernels produced some green-white striped plants which had the Wd ring. These were used as the male parent in crosses with $9^B C/N9 c$ plants. The dominant C allele identified the translocated chromosome. The percentage of C kernels had been determined following pollination of c testers by $9^B C/N9 c$ plants without the ring chromosome. It varied from 1.5% to 1.9%. The percentage of C kernels represented the frequency of crossing over between the C locus and the translocation point in the 9^B chromosome since there was no male transmission of 9^B in these plants. Data from the pollination of c silks by $9^B C/wd c$ plants with the ring chromosome carrying I are given in Table III.

The data presented below clearly demonstrate that the small Wd ring chromosome complemented the deficiency in the 9^B chromosome to make it transmissible through the pollen. The average percentage of kernels with the dominant alleles ($C + I-C$) was 23.0%. In several ears the mosaic ($I-C$) and self colored (C) classes of kernels were separated. Plant 434-10 had 61 $I-C$ and 8 C ; plant 389-1 had 20 $I-C$

and 3 *C*; plant 389-8 had 73 *I-C* and only 3 *C* and plant 389-6 had 33 *I-C* and 6 *C*. Clearly, most of the kernels with the *C* allele also had the *Wd* ring. The great majority of these possessed the 9^B chromosome. This was confirmed by growing some *I-C* kernels and sporocytizing the plants for cytological analysis. The 9^B chromosome was present in all eight plants examined. Self colored *C* kernels, without the ring chromosome, came from crossing over between the *C* locus and the translocation point in the 9^B chromosome or from transmission of the 9^B chromosome combined with loss of the ring or its *I* allele during the microspore divisions. The control crosses, without the ring chromosome, gave only 1.7% of *C* kernels and they were all self colored. If the small sample of 207 kernels classified for mosaic vs. self colored aleurone was representative of the total population, then approximately 10% of the combined *C* + *I-C* class should be self colored. This value was 2.3%. The difference between 2.3% and the control value 1.7% might be due to occasional loss of the ring in pollen grains carrying the 9^B chromosome.

Table III - Percentage of male transmission of the 9^B chromosome (*C* or *I-C* kernels) in the presence of the *Wd* ring chromosome.

c ♀ x 9 ^B C/wd c ♂ plus <i>Wd</i> ring (<i>I</i>)	Number of kernels			% of C + I-C
	c	C + I-C	Total	
434-10 x 514-1	128	69	197	35.0
389-1 x 524-2	75	23	98	23.4
389-8 x 524-2	233	76	309	24.6
389-6 x 527-1	173	39	212	18.4
716-38 x 758-1	268	68	336	20.2
716-41 x 758-1	205	72	277	25.9
716-42 x 757-1	241	76	317	24.0
737-4 x 757-1	309	64	373	17.1
737-5 x 759-1	269	82	351	23.4
	1901	569	2470	23.0
(related 9 ^B heterozygotes without the ring)	407	7	414	1.7

It is evident from these two experiments that the *C* and *Wx* markers on the 9^B chromosome were transmitted much more frequently when the ring chromosome was present.

Fresh pollen grains from plant 608-1, a $9^B Wx/wd wx$ heterozygote possessing the Wd ring chromosome, were distributed on agar plates and after several hours were stained with a solution of I_2KI to distinguish Wx grains from wx ones. The Wx locus was not included in the Wd ring chromosome. 21.2% of the pollen grains that germinated had starch that stained blue with I_2KI . No obvious difference was observed between the red and blue staining pollen grains in pollen tube length.

The demonstration that pollen with both the 9^B chromosome and the Wd ring was male transmissible permitted the construction of homozygous 9^B plants with the Wd ring. In such plants it should be possible to test whether 9^B grains without the Wd ring could function when there was no competition other than that offered by 9^B pollen with the Wd ring. A negative answer would be expected since 9^B pollen failed to germinate on agar plates but the experiment was deemed to be of sufficient interest to justify its execution. Accordingly, plants of $9^B C/N9 yg C$ constitution were pollinated by $9^B C/wd c$ plants with the Wd ring carrying the I allele. Mosaic ($I-C$) kernels were planted and green-white striped plants were examined cytologically to identify homozygous 9^B individuals. Three such plants were found: 622-6, 623-1 and 623-5. They were short plants and differed phenotypically from $9^B/wd + Wd$ ring plants. Although the staminate inflorescence was reduced in size and did not yield abundant pollen it was possible to pollinate several $yg2 c$ plants. The data from these crosses are in Table IV.

Table IV - Data from crosses of $yg2 c \times$ homozygous 9^B plants with the Wd ring.

$\text{♀ } yg2 c \times 9^B C/\beta^B C \text{ ♂}$			
+ Wd ring (I)		Origin	Number
Classification of kernels (93.2%)	Mosaic	I-C	789
for aleurone color	Self-colored	C	58 (6.8%)
Classification of C kernels for seedling phenotype	yellow green	Ring lost at the 1st microspore division, present in vegetative nucleus	23
	green- yellow green	Ring lost at the 2nd microspore division, present in only one sperm nucleus	25

The finding that 6.8% of the kernels were self-colored and not mosaic could be taken to indicate the occasional functioning of 9^B grains lacking the Wd ring.

However, additional information came when the 58 *C* kernels were grown in a seedling bench. Of the 48 seedlings obtained, 25 were green-yellow green striped and 23 wholly yellow green. The *C* kernels that gave rise to green-yellow green striped seedlings came from the loss of the Wd ring chromosome from one sperm nucleus at the second microspore division. The sperm nucleus that fertilized the polar nuclei lacked the Wd ring but it was present in the sister sperm that united with the egg. Pollen tube growth of these grains with dissimilar sperm would be unaffected since the Wd ring was in the vegetative nucleus. The essentially equal number of yellow green seedlings coming from *C* kernels could be attributed to loss of the Wd ring chromosome at the first microspore division since it seemed reasonable to assume that loss of the ring would occur with the same frequency at each of the pollen mitoses. In the latter case, the ring was retained in the vegetative nucleus but lost from the generative nucleus.

The presence of the Wd ring chromosome in the vegetative nucleus permitted pollen tube germination and growth of these grains with both sperm lacking the Wd ring.

The data were consistent with the assumption that loss of the Wd ring occurred with the same frequency at the first and second microspore divisions. In all of the 58 *C* kernels, the Wd ring was present in the tube nucleus, although lost from the sperm cell fertilizing the polar nuclei. All of the functional pollen grains possessed the ring chromosome and there was no evidence of transmission of the 9^B chromosome without the ring, even when competition against grains having a normal 9 was eliminated.

The finding that the small Wd ring chromosome supplies the 9S chromatin deleted in the 9^B chromosome allows the construction of homozygous 9^B plants with intact B chromosomes and with the ring. These plants can be used to test whether the large heterochromatic segment at the 9^B chromosome, derived from the B chromosome, acquires the genetic property associated with the terminal knob of 9S, namely the ability to interact with intact B's to undergo the high loss phenomenon.

CONCLUSIONS

Female transmission of the translocated 9^B chromosome was normal, or nearly normal, but pollen grains with the 9^B chromosome failed to germinate. The non-functional grains contained the usual complement of two sperm nuclei and one vegetative nucleus, so the post-meiotic divisions were not eliminated. Starch formation was normal in these grains and their only defect appeared to be the inability to germinate. In the presence of the small Wd ring chromosome the 9^B pollen became male functional; the ring supplies the 9S chromatin deleted in the 9^B chromosome.

RESUMO

Foi analisado o comportamento de transmissão de uma nova translocação em milho envolvendo o cromossomo 9 e o cromossomo acessório B. O cromossomo translocado 9^B carregava todos os blocos contíguos da heterocromatina distal do cromossomo B e era deficiente para um pequeno segmento terminal do braço curto do cromossomo 9. A frequência média de transmissão do cromossomo 9^B através do gameta feminino (47,6%) não foi significativamente diferente daquela encontrada para o cromossomo 9 normal (52,4%). Grãos de pólen com o cromossomo 9^B foram incapazes de germinar e consequentemente não funcionaram na fertilização. No entanto, o pequeno cromossomo Wd em anel complementou a deficiência no cromossomo 9^B e tornou-o transmissível através do pólen.

REFERENCES

- Ayonoadu, U.W. and Rees, H. (1968). The influence of B chromosomes on chiasma frequencies in Black Mexican sweet corn. *Genetica* 38: 75-81.
- Beckett, J.B. (1967). Two new B-type translocations. *Maize Genet. Coop. News Letter* 41: 139.
- Beckett, J.B. (1968). A B-type translocation involving the short arm of chromosome 3. *Maize Genet. Coop. News Letter* 42: 132.
- Beckett, J.B. (1972). An expanded set of B-type translocations in maize. *Genetics* 71: 3-4.
- Beckett, J.B. (1975). Genetic breakpoints of the B-A translocations in maize. *Maize Genet. Coop. News Letter* 49: 130-134.
- Cook, F.S. and Walden, D.B. (1965). The male gametophyte of *Zea mays* L. II. *In vitro* germination. *Can. J. Bot.* 43: 779-786.
- Hanson, G.P. (1961). Alteration of recombination frequencies in A by B chromosomes. *Maize Genet. Coop. News Letter* 35: 61-62.
- Himes, M. (1967). An analysis of heterochromatin in maize root tips. *J. Cell. Biol.* 35: 175-181.
- Jones, R.N. (1975). B chromosome systems in flowering plants and animal species. *Intern. Rev. Cytol.* 40: 1-100.
- McClintock, B. (1944). The relation of homozygous deficiencies to mutations and allelic series in maize. *Genetics* 29: 478-502.
- Melnyczenko, W.I. (1970). The effect of B chromosomes on intragenic recombination. *Maize Genet. Coop. News Letter* 44: 203-205.
- Neuffer, M.G. and Beckett, J.B. (1971). Location of new mutants by A-B translocation method. *Maize Genet. Coop. News Letter* 45: 144-146.
- Neuffer, M.G. and Beckett, J.B. (1972). Location of new mutants by the A-B translocation method. *Maize Genet. Coop. News Letter* 46: 130.
- Neuffer, M.G. and Beckett, J.B. (1973). New mutants located by A-B translocation method. *Maize Genet. Coop. News Letter* 47: 148-149.
- Rhoades, M.M. (1968). Studies on the cytological basis of crossing over. In: *Replication and Recombination of Genetic Material*. (Peacock, W.J. and Brock, R.D., eds.) Australian Academy of Science, Canberra, pp. 229-241.

- Rhoades, M.M. and Dempsey, E. (1973). Cytogenetic studies on a transmissible deficiency in chromosome 3 of maize. *J. Hered.* 64: 125-128.
- Rhoades, M.M., Dempsey, E. and Ghidoni, A. (1967). Chromosome elimination in maize induced by supernumerary B chromosomes. *Proc. Natl. Acad. Sci.* 57: 1626-1632.
- Roman, H. (1947). Mitotic nondisjunction in the case of interchanges involving the B-type chromosome in maize. *Genetics* 32: 391-409.
- Roman, H. and Ullstrup, A.J. (1951). The use of A-B translocations to locate genes in maize. *Agron. J.* 43: 450-454.
- Saraiva, L.S. (1979). Cytogenetical analysis of the mechanism of chromatin elimination induced by B chromosomes in maize. Ph. D. Thesis. Indiana University, pp. 141.
- Ward, E.J. (1976). The effect of accessory chromatin on chiasma distribution in maize. *Can. J. Genet. Cytol.* 18: 479-482.

(Received February 13, 1989)