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MEIOTIC SEGREGATION OF A RECESSIVE GENE (*w* A2) INCLUDED IN A Dp (II-I) OF *Aspergillus nidulans*

Marialba A.A. de Castro Prado[1] and Tânia M.A. Domingues Zucchi[2]

ABSTRACT

Strains of *Aspergillus nidulans*, bearing a chromosomal segment in duplicate are mitotically unstable. This research deals with the study of an *A. nidulans* mutant with a duplication of the *meth*$^+$ segment translocated from chromosome II to I and inserted in the *paba-y* interval. We present evidence that this segment in duplicate also includes the *w*A2 mutation. Meiotic crosses (hybrid or selfed cleistothecium) of a stable segregant, bearing the duplication and being heterozygous for the markers involved on it, demonstrated segregation of the recessive marker *w*A2. Such segregation is interpreted as the outcome of two different aspects: total or partial deletion in one of the duplicated segments and pairing and crossing-over between them, as evidenced by the *w*A2 segregants.

INTRODUCTION

In fungi, in general and in *Aspergillus nidulans* in particular, strains with duplicate chromosomal segments present mitotic instability (Bainbridge and Roper, 1966; Nga and Roper, 1968). These strains occasionally present deletion or addition of genetic material during vegetative growth, by a process called "mitotic non-conformity" (Nga and Roper, 1969), giving origin to deteriorated and improved sectors, respectively (Azevedo and Roper, 1970). Therefore strains which bear duplicate chromosomal segments, one in the normal position and the other transposed from its

[1] Departamento de Biologia, Fundação Universidade Estadual de Maringá, Av. Colombo, 3690, 87015 Maringá, PR, Brasil.

[2] Departamento de Parasitologia, Instituto de Ciências Biomédicas (II), Cidade Universitária, Caixa Postal 4365, 05508 São Paulo, SP. Send correspondence to T.M.A.D.Z.

original position, when one of these segments is lost, produce improved sectors with phenotypic characteristics and growth rate close to that of normal strains. Sometimes the loss of genetic material includes small chromosomal segments beyond the duplicate one, originating the so-called "hypo-haploids" (Roper and Nga, 1969). It was later demonstrated that in diploids having normal and duplicate segments, the loss could occur not only by deletion, but also by mitotic crossing-over (Case and Roper, 1981).

Unstable haploids resulting from genic duplication can present an increase of mitotic exchanges between the duplicate segments (Zucchi, 1975; Zucchi and Azevedo, 1979). These haploids, in meiotic crosses with normal strains, show segregation of the markers included in the duplication, mainly as a result of crossing-over (Van de Vate and Jansen, 1978).

In this paper we demonstrate that the II-I duplication in the B1 strain (Castro-Prado, 1986) includes the *w*A2 and *meth*$^+$ genes and that there is pairing and crossing-over between the duplicated segments (I and II chromosomes) with segregation of the recessive marker (*w*A2).

MATERIAL AND METHODS

Strains

Following Clutterbuck's proposition (1970) the mutant alleles of the strains used were:

UT 448: *w*A2 (II) white conidia; *ribo* A1, *paba* A124, *bi* A1 (I), with requirements for riboflavin, *p*-aminobenzoic acid and biotine, respectively; *Acr* A1 (II) resistant to acriflavin.

UT 196: *y*A2 (I) yellow conidia; *meth* A17 (II); *pyro* A4 (IV) with requirements for methionine and pyridoxine.

UT 184: *cha* A1 (VIII) "chartreuse" conidia; *pyro* A4 (IV); *s*B3 (VI); *nic* B8 (VII); *ribo* B2 (VIII), with requirements for pyridoxine, sodium thiosulphate, nicotinamide and riboflavin, respectively; *gal* A1 (III), *fac* A303 (V), *lac* A1 (VI), unable to grow in a medium containing galactose, acetate and lactose, respectively, as the sole C source; *sul* A1 (I) and *Acr* A1 (II) resistant to sulphanilamide and acriflavin, respectively.

Media and Solutions

The minimum medium (MM) was Czapeck Dox with 1% (w/v) glucose. Complete medium (CM) contained yeast extract, hydrolysed casein, hydrolysed

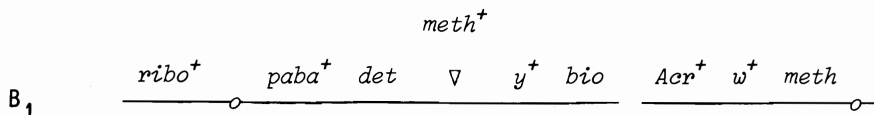
nucleic acids, vitamins, etc (Pontecorvo *et al.*, 1953, modified by Jansen, 1970). The solid medium contained 1.5% agar.

Methods

The general methodology follows Pontecorvo *et al.* (1953). The diploids were prepared by the method of Roper (1952). Allocation of mutant alleles, duplications to their linkage groups by mitotic haploidization (Forbes, 1959) was facilitated by the use of *p*-phenylalanine (Morpurgo, 1961; Lhoas, 1961). Incubation was carried out at 37°.

RESULTS

The B1 mutant is a segregant of the A1 x UT 196 cross (Castro-Prado, 1986). This mutant presents deteriorated morphology, scarce conidiation and bears on chromosome I a segment of chromosome II that is in duplicate and therefore has the *meth*$^+$ phenotype, as shown below.



In crosses with a normal strain (UT 448) this B1 mutant presents normal meiotic behavior.

It is known that in strains with genic duplication, genic balance is restored by deletion of one of the duplicate segments (Azevedo and Roper, 1970).

Analysis of the B1 x UT 448 cross (see diagram below) showed normal recombination frequencies in the chromosomes I and II and normal segregation in the *meth* A17 marker (Tables I and II) which was different from the expected 3 *meth*$^+$: 1 *meth*.

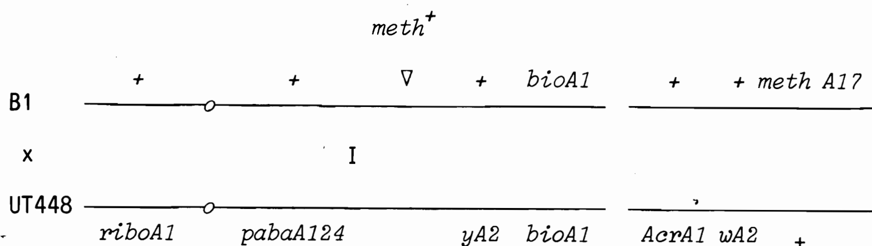


Table I - Meiotic recombination frequencies of the *meth* A17 marker related to some markers of chromosome I and II in the B1 x UT 448 cross.

Linkage interval	Recombination frequency (%)	
	UT 448 x UT 196 (145)	B1 x UT 448 (168)
meth - ribo	46.8	45.2
meth - paba	56.5	54.2
meth - Acr	28.2	24.2
meth - w	2.7	3.0

The number of colonies analyzed is given in parentheses. Control cross: UT 448 x UT 196.

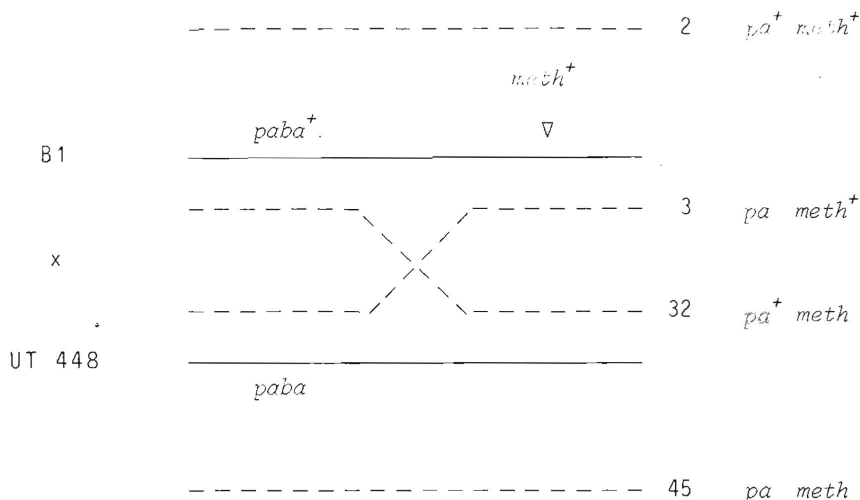
Table II - Meiotic segregation of genetic markers of G.L. I and II in B1 x UT 448 and B2 x UT 448.

	Genetic markers	Segregants B1 x UT 448 (168)
(I)	$ribo^{+}$	87
	ribo	81
	$paba^{+}$	78
	paba	90
(II)	Acr^{+}	73
	Acr	95
	$meth^{+}$	91
	meth	77
	w^{+}	82
	w	86

The total number of colonies analyzed is given in parentheses.

These results may indicate involvement of variants from B1 that have lost the duplication of chromosome I or may represent the loss of the $meth^{+}$ duplication by crossing-over during meiosis (see diagram below).

Meiotic cross: B1 x UT 448 (168 colonies were analyzed).



By considering the w^+ segregants only (the *w* class is closely linked to $meth^+$ and this should mask the expression of the $meth^+$ duplication), it is noted that the classes involving the duplication (*paba-Dp* $meth^+$ and $paba^+$ *Dp* $meth^+$) are reduced in relation to the reciprocal ones.

The B1 mutant was then meiotically crossed with a normal strain (3^{25}) which has the *w meth* on chromosome II. This cross permitted us to determine that a single $meth^+$ gene is present on chromosome I of B1 in a duplicate segment (Figure 1).

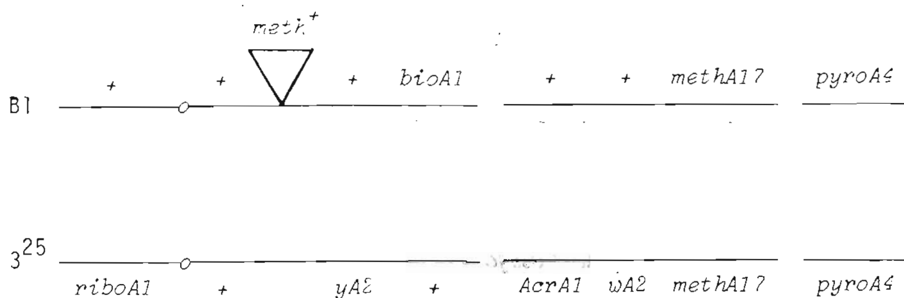


Figure 1 - Genetic markers of chromosomes I, II and IV of strains B1 and 3^{25}.

Segregation of meth$^+$ in a homozygous meth A17 cross

The meiotic recombination frequencies in the B1 x 3^{25} cross were strongly altered, as can be seen in Tables III and IV. Table III shows *paba* segregants from a cross involving two *paba*$^+$ strains (B1 x 3^{25}), including an excess of white segregants (*w*). The *meth* locus is linked to the markers of chromosome I (Table IV) and the recombination frequency in the *meth-w* interval is very high. These results demonstrate the presence of a *meth*$^+$ duplication in chromosome I of B1.

Table III - Meiotic segregation of the markers of chromosomes I and II in the B1 x 3^{25} cross.

	Genetic marker	Segregants
(I)	*ribo*$^+$	156
	ribo	67
	paba$^+$	218
	paba	5
	y$^+$	3
	y	20
	bio$^+$	79
	bio	144
(II)	*Acr*$^+$	72
	Acr	151
	w$^+$	23
	w	200
	meth$^+$	149
	meth	74

Total number of colonies analyzed: 223.

Segregation of w in a homozygous cross for w$^+$

The B1 and UT 184 strains were meiotically crossed and 20 hybrid cleistothecia were isolated from this cross. Six of them were larger and presented an unexpected segregation of *w* when the parental strains were *w*$^+$ (Table V).

Table IV - Recombination frequencies between *meth* A17 and other markers of chromosomes I and II in the B1 x 3^{25} cross.

Linkage interval	Recombination frequency (%)	
	UT 448 x UT 196 (145)	B1 x 3^{25} (223)
meth - ribo	46.8	36.3
meth - paba	56.5	64.6
meth - y	50.0	8.7
meth - bio	48.2	26.5
meth - Acr	28.2	54.3
meth - w	2.7	72.6

Control cross: UT 448 x UT 196. Number of colonies analyzed is given in parentheses.

Table V - Meiotic segregation of markers of linkage groups I and II in the B1 x UT 184 cross.

	Genetic marker	Segregants			
		Cleistothecia			
		1	8	19	7
(I)	*paba*$^+$	82	59	79	74
	paba	66	49	63	50
	bio$^+$	73	53	69	54
	bio	75	55	73	70
(II)	*Acr*$^+$	0	0	0	53
	Acr	148	108	142	71
	w$^+$	88	54	76	124
	w	60	54	66	0
	meth$^+$	148	108	142	80
	meth	0	0	0	44

The cleistothecia analyzed were: no. 1 (148 colonies analyzed), no. 8 (108 colonies analyzed), no. 19 (149 colonies analyzed) and no. 7 (124 colonies analyzed).

Four of these cleistothecia (No. 1, 7, 8 and 19) were analyzed separately. Cleistothecia 1, 8 and 18 showed segregation of *w* and absence of *meth* and Acr^{+} segregants. Cleistothecium number 7 showed the opposite results, i.e., absence of *w* segregants and segregation of *meth* and Acr^{+} (Table V). These results are evidence of the meiotic instability of B1.

Since the *w* and *meth* genes are closely linked (Clutterbuck, 1981) and on the basis of the present results (segregation of *w* in crosses homozygous for w^{+}) we may conclude that the duplication present in chromosome I of B1 must include at least two genes: $meth^{+}$ and *wA2*.

Van de Vate and Jansen (1978) demonstrated that in crosses heterozygous and homozygous for the *Dp* (I-II) duplication, the duplicate segments can pair during prophase I of meiosis with crossing-over and segregation of the recessive markers in the duplication. In this case, the pairing should occur in a quadrivalent configuration. In our experiments the segregation of *w*A2 of the B1 x UT 184 cross should have occurred only when the paired chromosomes were in a quadrivalent configuration in meiosis. Only two pairing possibilities should remain:

a) Chromosome I (B1) with chromosome II (B1).
b) Chromosome I (B1) with chromosome II (UT 184).

Since the *w*A2 obtained in this cross (B1 x UT 184) were $meth^{+}$ *Acr* (Table V) the pairing at meiosis may have occurred as shown in Figure 2 (alternative b). The possibility of the *Acr* mutation being included in duplication was tested and proved correct (in preparation). In the diagram shown in Figure 2, double crossing-over in *paba-Dp* and $Dp\text{-}y^{+}$, followed by normal disjunction and distribution in the meiotic products resulted in segregation of the recessive marker (*w*A2) involved in the duplication; i.e.: segregation of the recombinant chromatid (1) with the non-recombinants (2) or (3).

Segregation of w in the homozygous cross (B1 x B1)

In order to detect meiotic segregation in the B1 strain, we analyzed selfed cleistothecia following the methodology of Van de Vate and Jansen (1978). These cleistothecia showed segregation of *w*, and the progenies were composed of two kinds of segregants: white (*w*A2) $meth^{+}$ *Acr* and green (y^{+} w^{+}) $meth^{+}$ Acr^{+}.

These results confirmed the presence of the *w*A2 mutation in the *Dp* (II-I) duplication. This cross also showed other abnormalities related to the segregation of the markers *ribo, paba,* bio^{+} and *Acr* (results not shown) which are currently under study (Castro Prado *et al*., in preparation).

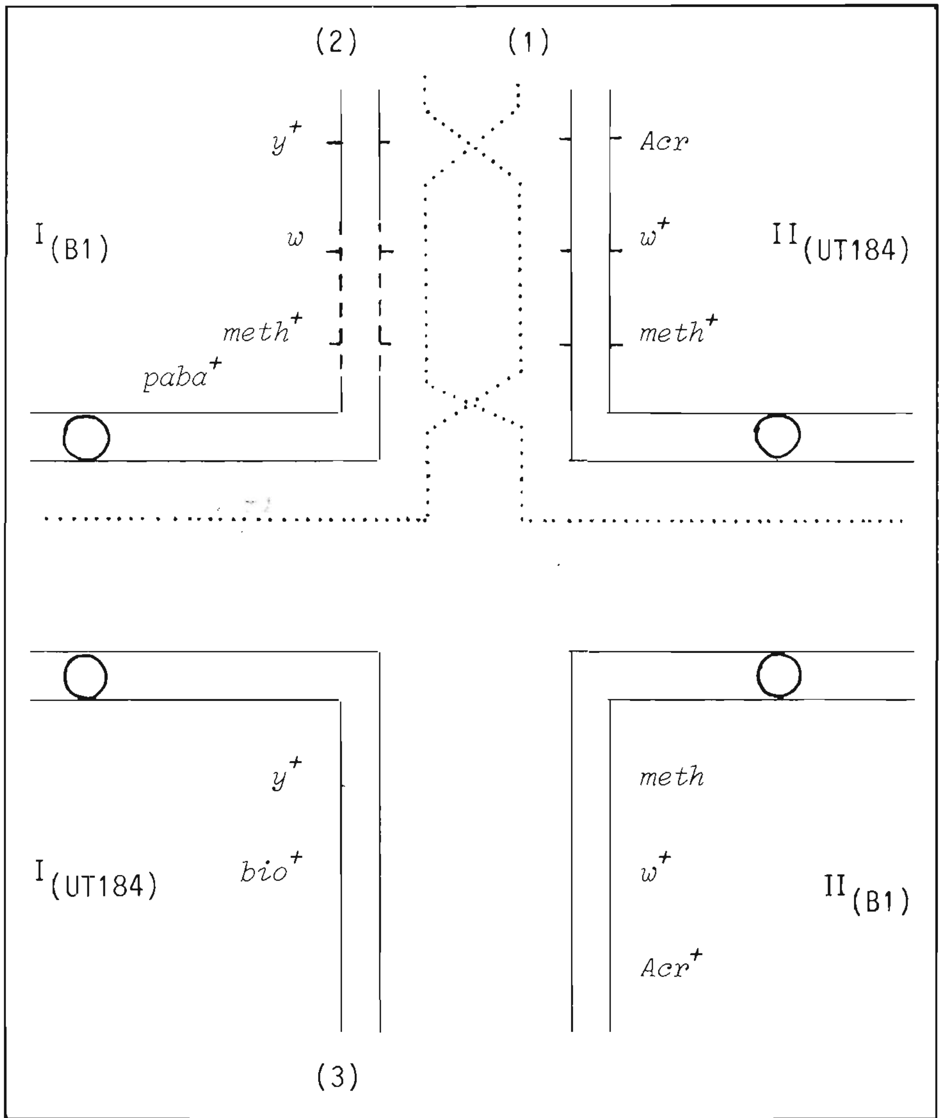


Figure 2 - Schematic representation of B1 x UT 184 in a quadrivalent configuration during meiosis.

Mitotic segregation of the wA2 recessive marker present in the duplicate segment

Although the B1 mutants were mitotically stable, a very rare white sector (*w*A2) was found, confirming once again the presence of this mutation in the *Dp* (II-I)

which, like white segregants from B1 x UT 184 and B1 x B1 crosses, were *meth*$^{+}$ *Acr*, suggesting again that the *Acr* mutation was present in the duplication.

Since *w* is a recessive marker, this white sector can only be originated by a deletion of the segment in the normal position: w^{+} or w^{+} *meth*, on chromosome II of B1.

DISCUSSION

Strains of *A. nidulans* with duplicate chromosomal segments (one in the normal position and the other translocated to another region of the genome) are being used in studies of mitotic "non-conformity" (Nga and Roper, 1969) and for the isolation of recombination deficient mutants (Van de Vate and Jansen, 1978). The approach used for isolation of *rec* (recombination deficiency) mutations is based on the fact that the organisms to be explored must be haploid, because these *rec* mutations may be recessive (Parag and Parag, 1975). In addition, these organisms must be also heterozygous for at least two non-allelic mutations, used for recognizing the *rec* phenotype (Rodarte *et al.*, 1968). In yeasts, these systems have been obtained from disomic strains, having a partially diploid genome that permits detecting the recombination event, but with haploid configuration for the other chromosomes (Roth and Fogel, 1971; Rodarte-Ramon and Mortimer, 1972).

In *A. nidulans*, the existence of strains with chromosomal segments in duplicate, permitted us to obtain a similar system. The B1 mutant, which has a duplicate segment, including genes *w*A2 and *meth*$^{+}$, is an appropriate strain for the study of *rec* mutations. Exchanges between homologous segments of chromosome I (duplication) and II (normal segment, B1 x UT 184), showed the presence of *w*A2 in *Dp* (II-I) through the segregation of this recessive gene (*w*) (Table V).

In crosses heterozygous for the duplication, the chromosomes may adopt the bivalent or quadrivalent configuration in meiosis and, in this case, only the quadrivalent configuration can originate the *w*A2 segregants. The origin of these segregants suggests that the duplication is inserted in a non-inverted position in the acceptor chromosome. Crossing-over between a normal segment and another in the inverted position could hardly produce viable descendants (Sexton and Roper, 1984).

In crosses homozygous for duplication (B1 x B1), analysis of selfed cleistothecia also showed segregation of *w* among the progeny. This occurred in addition to the presence of a white (*w*) spontaneous mitotic sector. B1.1 derived from the B1 mutant by loss of the segment in the normal position (w^{+}, *meth*, chromosome II), confirms the presence of the *w*A2 mutation in the *Dp* (II-I) mutant.

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RESUMO

Linhagens de *Aspergillus nidulans*, carregando um segmento cromossômico em duplicata são mitoticamente instáveis. Esta pesquisa mostra o estudo de um mutante de *A. nidulans* com uma duplicação do segmento $meth^+$ translocado do cromossomo II para o cromossomo I e inserido no intervalo *paba-y*.

São apresentadas as evidências de que este segmento em duplicata inclui também a mutação *w*A2. Cruzamentos meióticos (cleistotécios híbridos ou autofecundados) de um segregante estável carregando a duplicação e sendo heterozigotos para os marcadores envolvidos nela evidenciou a segregação do marcador recessivo *w*A2. Tal segregação é interpretada como tendo dois aspectos diferentes: deleção total ou parcial de um dos segmentos duplicados; pareamento e crossing-over entre eles, como evidenciado pelos segregantes *w*A2.

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