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Rye B Chromosome Behaviour at First and Second Pollen Mitosis and Its Relationship with Anther Maturity

M.J. Puertas, M. Diez and R. Carmona
Departamento de Genética, Facultad de Biología, Universidad Complutense, Madrid (Spain)

Summary. Rye plants carrying B chromosomes with different nuclear and cytoplasmic constitutions have been analyzed at first pollen mitosis. No differences of B chromosome behaviour have been detected. It has been concluded that non-disjunction and preferential distribution are processes controlled by Bs themselves.

At second pollen anaphase, B laggards have been observed. Both non-disjunction and B laggards occurred with higher frequency in younger anthers.

Key words: B chromosomes – Pollen mitosis – *Secale*

Introduction

The main characteristic of B chromosomes is their special transmission mechanism which has been described in many investigations (reviews by Battaglia 1964; Müntzing 1974; Puertas 1975).

The question investigated in this paper is whether this chromosome behaviour is controlled by B chromosomes themselves or if any additional information from the A chromosomes or cytoplasm, or both, is necessary for B non-disjunction and preferential distribution. Thus, rye plants carrying Bs with different nuclear and cytoplasmic constitutions have been analyzed. If B chromosomes were totally responsible for their own behaviour, non-disjunction would be identical in all nucleo-cytoplasmic combinations.

The behaviour of B chromosomes at second pollen mitosis has been also studied.

Material and Methods

Ten rye plants, *Secale cereale* L. 2n = 14, cultivar 'JNK', carrying 2B chromosomes were studied at first and second pollen mitosis. In addition, 14 plants with 2Bs of the second backcross generation of *S. cereale* + 2Bs × *S. vavilovii* were analyzed at first pollen mitosis. Seven plants had *cereale* cytoplasm and seven had *vavilovii* cytoplasm. It is remarkable that in each individual different segments of *cereale* and *vavilovii* genomes could be present. These plants belonged to the generation following that studied by Carmona and Puertas (1977).

Anthers were fixed in acetic alcohol 1:3. They were hydrolyzed in 1 N HCl for 15 min and stained with fuchsin. Preparations were squashed in a drop of acetocarmin. Anthers at second pollen mitosis had to be strongly squashed to break pollen walls; thus, protoplast diffused out and chromosomes became separated, otherwise chromosomes were seen as indistinguishable clumps.

Results

Results of the first pollen mitosis of *S. cereale* and the *S. cereale* × *S. vavilovii* backcross are shown in Table 1. It can be observed that the number of pollen grains at metaphase with n = 7, 7 + 1 or 7 + 2 is very similar in all cases. At anaphase, B chromosome behaviour was variable both in *S. cereale* and in backcrosses, although non-disjunction frequency was over 60% in every case. The morphology of non-disjunction was always identical and Bs were seen to go towards the generative nucleus in all cases.

At second pollen metaphase, the observed numbers of pollen grains with n = 7, 7 + 1, 7 + 2, 7 + 3 or 7 + 4 correspond to those expected according to the observed chromosome constitutions at first metaphase and the non-disjunction frequency at first anaphase ($\chi^2 = 0.044$, $p > 0.95$) (Table 2). χ^2 was calculated with total values.

A 44% of second pollen anaphases showed B laggards (Fig. 1 a,b). If those B chromosomes were the ones which had undergone non-disjunction at first anaphase both frequencies would be similar. However, non-disjunction occurred in 70% of first anaphases in this material. It is therefore clear that not all Bs undergoing non-disjunction lag at second mitosis.

If at first pollen mitosis only 70% of all anaphases

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showed non-disjunction, it can be assumed that the same percentage of pollen grains would show B laggards at second anaphase. In other words, those nuclei with normal disjunction at first division (30%) will do the same at second division, while from the remaining 70% undergoing non-disjunction, a 70% will still be delayed thus showing laggards at second anaphase.

Therefore, the number of expected second anaphases with B laggards can be calculated as the observed number of anaphases with non-disjunction at first mitosis divided by 30 and multiplied by the number of mitosis with 7 + 2, 7 + 3 or 7 + 4 at second metaphase. Under this assumption, observed and expected numbers of second anaphases with B laggards did not differ significantly ($\chi^2 = 1.090$, $p > 0.95$) (Table 2).

Both at first and second division, B anaphase migration is delayed in a percentage of the pollen grains. Thus, we have studied the possible relation between the frequency of delayed Bs and anther stage of maturity.

In 8 anthers at first mitosis all anaphases were ob-

Table 1. First pollen mitosis of *Secale cereale* and *Secale cereale* × *Secale vavilovii* backcross individuals

	Backcrosses																	
Plant no.	*Secale cereale*						*Cereale* cytoplasm						*Vavilivii* cytoplasm					
	Metaphase			Anaphase			Metaphase			Anaphase			Metaphase			Anaphase		
	7	7+1	7+2	D	N.D.	M.	7	7+1	7+2	D.	N.D.	M.	7	7+1	7+2	D.	N.D.	M.
1	–	27	3	6	24	–	1	29	–	1	29	–	1	28	1	4	26	–
2	–	27	3	7	22	1	–	28	2	4	26	–	2	28	–	5	25	–
3	2	26	2	9	21	–	1	26	3	6	24	–	1	29	–	5	24	1
4	2	27	1	8	21	1	2	28	–	7	23	–	–	29	1	7	23	–
5	2	26	2	8	21	1	–	30	–	7	23	–	4	26	–	9	21	–
6	1	27	2	9	20	1	1	29	–	8	22	–	3	27	–	9	21	–
7	2	26	2	8	20	2	1	28	1	9	21	–	1	28	1	11	19	–
8	1	28	1	9	19	2												
9	3	26	1	12	18	–												
10	2	26	2	12	17	1												
Frequency %	5	88.7	6.3	29.3	67,6	3	2.8	95.2	2	20	80		5.7	92.9	1.4	23.8	76.2	0.4

D = disjunction
N.D. = non-disjunction
M = micronuclei
30 cells of each individual were scored

Table 2. Second pollen mitosis of *Secale cereale*

Plant no.	Metaphase								Anaphase			
	7		7+1		7+2		7+3 or 4		Normal		B-laggards	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
1	0	0	5	5.4	22	22.2	3	2.4	17	10	13	20
2	1	1	7	6.3	19	20.4	3	2.3	15	13.9	15	16.1
3	2	2	8	6.5	18	20	2	1.5	18	16	12	14
4	1	2.5	7	5.6	20	20.4	2	1.5	18	14.6	12	15.4
5	3	3	6	5.6	19	19.8	2	1.6	14	15.3	16	14.7
6	3	2	7	6.4	18	20.2	2	1.4	19	16.7	11	13.3
7	3	4	7	5.5	18	19	2	1.5	18	16.7	12	13.3
8	2	2	8	7.7	18	20.4	2	0.8	15	17.3	15	12.7
9	3	3	8	8.7	18	17.6	1	0.7	15	18.6	15	11.4
10	2	2.5	10	9.3	16	16.9	2	1.3	19	19.8	11	10.2
Total	20	22	73	67	185	196	22	15	168	159.3	132	140.7

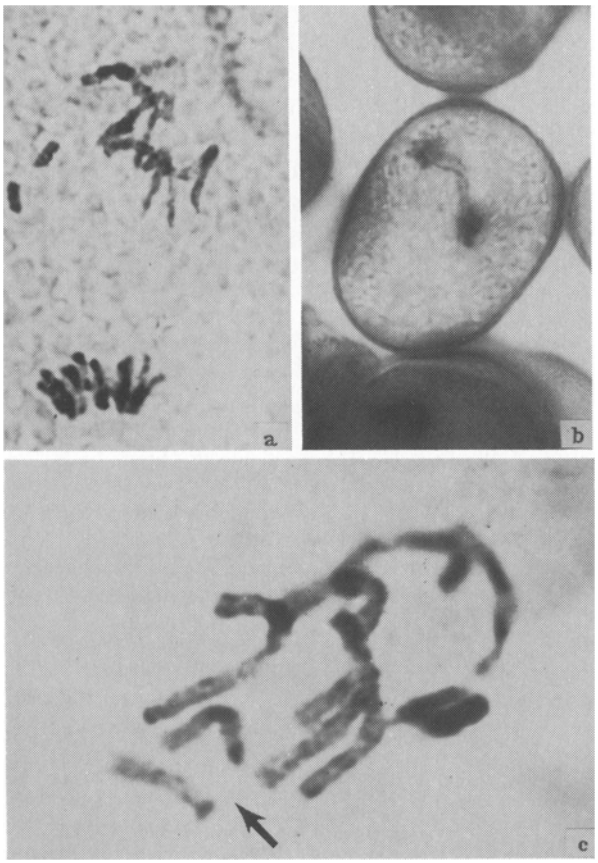

Fig. 1a-c. **a** Pollen second anaphase showing 2 B laggards, **b** B laggards forming a bridge before their complete release, **c** Pollen second metaphase showing 7 + 2 B chromosomes (arrowed)

served and the quotient anaphases with non-disjunction / anaphases with normal disjunction were calculated. Additionally, 100 pollen grains chosen at random were also observed and the quotient mononucleated pollen / binucleated pollen was calculated. This quotient indicates the stage of anther development: the higher the quotient the younger the anther. Both quotients were correlated (r = 0.987 ± 0.06). Using Fisher's transformation, *r* value did not differ significantly from 1 (P = 0.95) (Table 3).

In the same way, 5 anthers at second pollen mitosis were observed and the quotients anaphases with B laggards / normal anaphases and binucleated pollen / pollen with sperm nuclei were calculated. These quotients were correlated (r = 0.945 ± 0.15) and the *r* value did not differ significantly from 1 (P = 0.95) (Table 4).

Discussion

In all plants studied non-disjunction morphology was identical and its frequency was high. In backcrosses there were no differences due to cytoplasm: non-disjunction mean frequency was 80% on *cereale* cytoplasm and 76% on *vavilovii* cytoplasm (Table 1). A similar result was obtained by Müntzing (1970) with the Lindström line (wheat with rye B chromosomes). He obtained indirect evidence of non-disjunction from crosses 0B × 2B plants.

It becomes clear that cytoplasm or genetic background do not alter the transmission mechanism of B chromosomes. Therefore, it seems that non-disjunction and pref-

Table 3. Relation between anther maturation and non-disjunction frequency (first pollen mitosis)

Anther	Mono-nucleated pollen	Binucleated pollen	Mitosis	Anaphase with N.D.	Anaphase with D.	Mononucleated / Binucleated	N.D. / D.
1	8	38	54	11	9	0.21	1.22
2	10	41	49	15	11	0.24	1.36
3	19	29	52	16	10	0.65	1.60
4	20	20	60	11	6	1.00	1.83
5	39	18	43	12	5	2.20	2.40
6	46	12	42	23	9	3.83	2.55
7	44	10	46	15	5	4.40	3.00
8	36	5	54	13	3	7.20	4.33

Table 4. Relation between anther maturation and B-laggard frequency (second pollen mitosis)

Anther	Binucleated pollen	Pollen with sperm nuclei	Mitosis	Anaphase with B-laggards	Normal anaphase	Binucleated / Sperm nuclei	B-laggards / Normal
1	4	46	50	10	18	0.09	0.55
2	6	38	56	18	30	0.15	0.60
3	16	26	58	14	18	0.61	0.77
4	20	18	62	12	14	1.11	0.85
5	29	13	58	25	25	1.38	1.00

erential distribution are processes controlled by Bs themselves. Possibly this is the only qualitative character carried by rye B chromosomes.

From cytological observations delayed anaphase migration of Bs in a percentage of pollen grains is evident both at first and second mitosis.

It is remarkable that Bs are delayed only at first anaphase when non-disjunction occurs; in other words, when B chromosomes separate normally they migrate at the same time that A chromosomes migrate. In addition, the fact that non-disjunction took place with higher frequency in younger anthers (Table 3) may indicate that B's delay is a decisive factor for the occurrence of non-disjunction. However, B's delay is not the only cause of non-disjunction because in somatic mitoses, where non-disjunction does not occur, Bs are also delayed, as demonstrated by Darlington and Hague (1964) and Ayonoadu and Rees (1967).

Assuming that B laggards observed at second anaphase are consequences of non-disjunction, the correlation between anther maturity and the B laggard frequency demonstrated in Table 4 is easily understood.

As far as we know, second pollen mitosis in rye carrying B chromosomes has not been previously reported. From our observations, the following conclusions can be drawn:

1. From the observation of chromosome configurations and non-disjunction frequencies at first pollen mitosis, second pollen metaphase constitutions can be inferred.

2. At second metaphase, the two Bs of n = 7 + 2 pollen grains were seen separated to each other (Fig. 1c); therefore, the 'knob' maintaining B chromatids together in non-disjunctional anaphases must be released during telophase or interphase.

3. A percentage of delayed Bs at first mitosis were also delayed at second anaphase forming laggards and bridge-like structures. These bridges have been interpreted as slow separations of B chromatids; that is, these bridges would be the previous stage to B laggards.

4. At the sperm nuclei stage, pollen with micronuclei were hardly observed, this indicates that B laggards were not lost, but incorporated into telophase nuclei.

5. The question of B laggards being included symmetrically or assymmetrically in sperm nuclei remains open. Therefore, embryo and endosperm may have different numbers of B chromosomes due to an abnormal second anaphase.

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Dr. M.J. Puertas
Dr. R. Carmona
M. Diez
Departamento de Genetica
Facultad de Biología
Universidad Complutense
Madrid 3 (Spain)