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Pollen germination and tube growth in rye (*Secale cereale* L.) homozygous and heterozygous for reciprocal translocations

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Summary. To contribute to the knowledge of the role of reciprocal translocations in rye, a component of fertility was estimated by comparing germination and pollen tube growth in homozygous and heterozygous plants for reciprocal translocations. The results obtained indicate that there are no differences in germination and pollen tube growth rate when homozygous and heterozygous plants as a whole are compared. However, there are significant differences in pollen tube growth between plants carrying different translocations. This suggests that the chromosome constitution of a plant is relevant to these fitness-estimating parameters together with its particular genetic background.

Key words: Rye – *Secale cereale* L. – Reciprocal translocations – Pollen germination – Pollen tube growth

Introduction

An important factor which could determine the transmission of reciprocal translocations could be the influence of the pollen grain chromosome constitution on its own germination ability and pollen tube growth versus the influence of chromosome constitution of the sporophyte which produced the pollen grain. Theoretically, one could expect a double effect of reciprocal translocations on the gamete viability and fertility, namely, i) the formation of unbalanced gametes as a consequence of adjacent co-orientations in meiosis, ii) the alteration of linkage groups which could affect pollen behaviour. In vitro pollen culture techniques have been used to analyze differences in pollen germination ability and pollen tube growth in rye plants carrying different numbers of B chromosomes (Puertas and Carmona 1976).

In the present work, in vitro pollen culture has been used to study those parameters in plants homozygous and heterozygous for reciprocal translocations in a rye cultivar polymorphic for reciprocal translocations in an attempt to elucidate the mechanisms which maintain a rather high frequency of structural heterozygotes (20%)

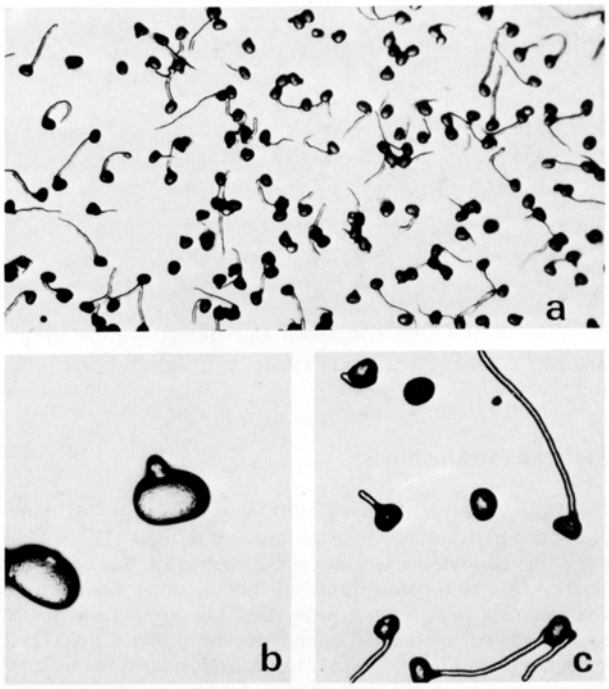

Fig. 1a–c. Pollen grains 15 min after inoculation. **a** General view of the preparation (×47); **b** Germinated and non germinated pollen grains (×187); **c** Pollen tubes of different lengths (×84)

Table 1. Germination and pollen tube growth rate in heterozygous and homozygous rye plants (1980 sample)

	Mother plant	Plant	Germination		Tube growth			% Alternate quadrivalents
			% Pollen grains germinated	Total of pollen grains	Total of pollen tubes	Length mean (μ)	Rate μ/min	
HETEROZYGOTES	8	1	78.8	100	50	61.1±3.9	4.1	80.0
	8	2	66.0	100	50	47.1±2.5	3.2	74.5
	23	1	73.7	114	50	47.0±1.8	3.1	44.9
	23	2	30.6	144	50	69.8±4.9	4.7	64.0
	68	1	44.0	100	50	42.3±2.0	2.8	75.5
	112	1	78.0	100	50	86.3±6.0	5.8	77.3
	146	1	84.0	100	50	51.8±2.0	3.5	85.1
	146	2	56.4	126	52	37.2±3.2	2.5	53.1
	237	1	89.0	100	51	147.5±7.6	9.8	40.0
	237	2	81.0	100	51	67.9±5.4	4.5	53.1
	237	3	81.0	100	50	40.5±2.2	2.7	54.0
	237	4	73.0	233	100	80.5±4.0	5.4	–
	237	5	46.0	100	50	51.8±3.0	3.5	81.6
	385	1	70.1	100	50	42.7±3.0	2.8	68.0
	415	1	68.3	126	50	40.3±3.6	2.7	66.7
	417	1	66.0	100	50	83.9±5.9	5.6	72.0
	456	1	74.2	120	50	70.3±3.6	4.7	–
	456	2	66.0	100	50	53.9±4.3	3.6	–
	$\bar{X}$		67.5			62.4±5.9	4.2	66.0
HOMOZYGOTES	8	3	59.1	230	100	56.2±2.9	3.8	
	8	4	35.0	100	44	50.3±5.0	3.6	
	23	3	87.3	102	50	107.5±5.9	7.2	
	112	2	48.0	100	51	56.4±6.0	3.8	
	130	1	56.0	100	50	48.0±2.4	3.2	
	146	3	75.8	231	100	64.2±2.3	4.3	
	146	4	82.1	224	100	94.3±3.6	6.3	
	237	6	90.8	228	100	97.7±6.6	6.5	
	244	1	77.8	108	52	91.5±3.2	6.1	
	314	1	84.4	109	50	46.4±3.0	3.1	
	385	2	84.0	100	50	48.0±2.6	3.2	
	399	1	93.6	124	50	82.7±4.4	5.5	
	415	2	70.2	218	100	48.4±2.1	3.2	
	417	2	68.7	147	50	87.6±2.9	5.8	
	456	3	83.9	217	100	70.1±2.5	4.7	
	$\bar{X}$		74.5			69.9±5.7	4.7	

in spite of the low fitness of the heterozygous plants (Candela and Lacadena 1975; Candela et al. 1979, 1982).

Materials and methods

The 'Ailés' cultivar of rye, *Secale cereale* L. (2n = 14), polymorphic for reciprocal translocations, was used. The studies were carried out in two successive years. In the first year (1980), 18 heterozygous and 15 homozygous plants were analyzed. The classification into these two groups was previously made on the basis of their PMC chromosome behaviour at Metaphase I (7^{II} or $1^{IV}+5^{II}$, respectively). Such plants were the offspring of 14 different open pollinated structural heterozygotes. In the second year (1981), plants from the same offspring and plants from 7 new heterozygous plants were analyzed. It can be assumed that the 21 parental plants are carriers of different translocations (Candela et al. 1979).

A modification of the Pfahler's method (1965) was used. The medium consisted of 100 ml distilled water, 3.5 g bacto-agar, 30 g sucrose, and 20 mg boric acid, pH 6.5. When anthesis occurred, pollen grains were placed on a slide covered with the culture medium. Fifteen minutes after sowing, preparations were fixed with several drops of acetic-ethanol solution (1:3) (Fig. 1a). A minimum of 100 pollen grains per plant were scored in order to estimate the percentage of germinated pollen (see Fig. 1b). In addition, the length of at least 50 randomly chosen pollen tubes per plant (Fig. 1c) was measured with a micrometer ocular provided with a grid of 35.7 μm.

The egg-cell fertility of the plants analyzed in 1981 was estimated by the proportion of seeds produced in relation to the total number of egg-cells (flowers). In addition, the total number of seeds obtained by open pollination in the offspring of each plant was scored.

The mean values of pollen germination ability of homozygous and heterozygous plants of the 1980 sample were compared by a Student test (t_{31} d.f. = 0.89, $0.4 > p > 0.3$).

Results

Table 1 shows the values of germination ability, length and tube growth rate of pollen grains produced by heterozygous and homozygous plants studied during 1980. Also, the frequency of quadrivalents having alternate coorientation at Metaphase I is included.

In order to quantify the possible effect of the different translocations we studied the second sample (1981) in which all plants were heterozygous for 21 different translocations. The data obtained are shown in Table 2 and in this case, the mean number of seeds and the egg-cell average fertility of the off-spring from each heterozygous parental are also reported.

Tables 3 and 4 show the regression values and the variance analysis, respectively, made with the different parameters estimated in the present work.

The heterogeneity of the data obtained in the two samples can be attributed to their different environmental conditions since the 1980 plants were sown in pots while the 1981 ones were sown in an experimental field.

Considering the alternate co-orientation frequency at Metaphase I of the heterozygous plants analyzed

Table 2. Germination and pollen tube growth rate in rye plants (1981 sample)

	Mother plant	No. of plants	Germination		Tube growth (50 pollen tubes per plant)		Egg cell fertility	Offspring produced
			% Pollen grains germinated	Total of pollen grains	Length mean (μ)	Rate μ/min		
	8	5	77.0	564	111.3±12.9	7.4	51.4± 2.3	621.2± 18.6
	68	2	91.5	211	127.2± 2.9	8.5	60.6± 6.7	848.0± 62.3
	112	3	93.1	321	122.6± 3.4	8.2	67.3± 1.6	867.7± 55.6
	130	3	74.8	385	89.6±12.5	6.0	30.5± 2.2	623.9± 62.3
	146	4	87.8	450	101.8±11.0	6.8	56.4± 5.5	789.8± 60.0
	237	6	93.4	640	109.4± 6.0	7.3	65.3± 3.5	840.0±133.4
	244	2	90.1	222	84.7± 2.6	5.6	57.5± 3.9	517.5± 77.8
	314	5	87.8	542	113.3±24.5	7.6	60.2± 4.0	719.5± 29.1
	385	2	68.3	293	72.4± 1.9	4.8	52.1± 2.6	407.3± 13.5
	399	2	88.5	200	148.2±14.8	9.9	68.7± 3.1	968.1± 25.3
	415	6	88.1	662	89.7± 5.34	6.0	61.1± 4.7	583.5± 43.2
	417	2	75.2	210	53.3± 1.0	3.4	39.0±14.0	266.0± 52.3
	456	3	93.8	322	118.6± 6.2	7.9	59.9± 2.4	738.1± 27.1
	47	4	74.0	503	142.9±13.2	9.5	58.5± 2.9	930.0± 74.8
	59	3	77.4	328	109.3± 8.0	7.3	51.0± 5.1	746.8± 44.7
	62	3	89.3	338	94.9± 3.5	6.3	56.7± 4.4	703.5± 37.9
	92	2	46.4	237	71.6± 0.5	4.8	49.1±11.6	514.9±104.3
	101	3	90.5	326	162.6±17.2	10.9	51.2± 5.6	837.1±110.0
	135	4	80.2	450	107.6± 7.5	7.2	54.1± 4.5	569.1± 44.3
	245	4	84.4	422	107.6± 7.9	7.2	64.4± 3.3	637.0± 32.5
	331	4	81.5	466	94.7±10.8	6.3	62.5± 5.1	723.0± 59.0
$\bar{X}$			82.5		106.3± 5.7	7.1	56.1± 2.0	688.2± 39.1

Table 3. Regression data between different pairs of characteristics in heterozygous and homozygous rye plants

Variable		Sample	b	d.f.	t	*P*
X	Y					
Pollen germination (A.V.)	Tube growth rate	1980	0.1	31	2.8	0.001–0.01
Pollen germination (A.V.)	Alternate quadrivalents (A.V.)	1980	−0.2	13	−0.9	0.3 –0.5
Tube growth rate	Alternate quadrivalents (A.V.)	1980	−1.4	13	−1.4	0.1 –0.3
Pollen germination (A.V.)	Tube growth rate	1981	0.1	19	2.4	0.01 –0.05
Pollen germination (A.V.)	Eggcell fertility (A.V.)	1981	0.4	19	2.9	0.01 –0.05
Pollen germination (A.V.)	Offspring produced	1981	7.8	19	2.5	0.01 –0.05
Tube growth rate	Eggcell fertility (A.V.)	1981	1.4	19	2.3	0.01 –0.05
Tube growth rate	Offspring produced	1981	86.2	19	7.8	< 0.001

A.V.: angular values

Table 4. Analysis of variance

a Pollen grain germination (1981 sample)			
Source of variation	d.f.	S.S.	
Reciprocal translocations	20	1,861.97	N.S.
Daughter plants	51	3,781.87	
b Pollen tube length			
1. 1980 sample			
Source of variation	d.f.	S.S.	
Chromosomal constitutions	1	25,619	N.S.
Plants	31	1,047,044	***
Pollen tubes	1,973	1,793,506	
2. 1981 sample			
Source of variation	d.f.	S.S.	
Reciprocal translocations	20	1,780,914	***
Daughter plants	51	1,380,877	***
Pollen tubes	3,528	5,048,053	

N.S. = nonsignificant values; *** = $P < 0.001$

and, consequently, the corresponding unbalanced gametes produced (Table 1), one could expect, for this kind of plant, a lower proportion of pollen grains able to germinate. However, although the percentage of germinated pollen was slightly higher in homozygous (75.5%) than in heterozygous (67.5%) plants, both values were not statistically different.

Likewise, significant differences in pollen tube length were not found when heterozygous and homozygous plants are compared (Table 4 b–1).

When heterozygous progeny from the 21 different heterozygotes are analyzed, significant differences in pollen tube growth among plants with different translocations and also among the offspring of a particular translocation were found; in the last case the differences were attributed to the different genetic background of the plants (Table 4 b–2).

With respect to the percentage of pollen germination, significant differences among the several translocations were not found (Table 4 a). However, this character was correlated with pollen tube growth rate (Table 3).

Discussion

The fact that both homozygous and heterozygous plants showed very similar percentages of germinated pollen (Table 1) suggests that either there are some mechanisms which buffer the deleterious effect of the quadrivalent adjacent coorientation, increasing the proportion of balanced gametes, or the chromosome constitution (balanced vs. unbalanced, standard vs. translocated) does not influence the germination ability of the pollen grains.

In agreement with this last possibility, we did not find any correlation between the frequency of alternate quadrivalents and both the germinated pollen frequency and pollen tube growth rate (Table 3). Previous data (Figueiras 1980) seemed to suggest the absence of correlation between the frequency of alternate quadrivalents and both the frequency of viable pollen, estimated by its stainability with aceto-carmin in a glycerol solution, and the egg-cell fertility.

Sybenga (1968) suggested the occurrence of a mechanism which originates alternate segregation by means of centromere reorientation. If this is true, this mechanism would explain the absence of correlation between the parameters analyzed in this work.

In *Haworthia browniana,* Brandham (1973) reported that the elimination of the chromosomal unbalanced gametes must occur during the maturation process which precedes anthesis.

Another possibility could be the independence between the germination ability of a pollen grain and its chromosome constitution indicating that either the chromosome segments which are absent or duplicated are not relevant in the pollen maturation process or that the pollen germination has a sporophytic determination. Nevertheless, whatever the actual mechanism which allows a pollen grain to germinate, the fact is that plants carrying chromosome deletions and duplications have been found arising from gametes formed after adjacent co-orientation (Shalev and Ladizinsky 1976; Menzel and Brown 1978).

On the other hand, a plant heterozygous for a reciprocal translocation produces two kinds of pollen grains with respect to their chromosome constitution: standard and translocated, both with the same frequency. If the chromosome constitution of a pollen grain was directly responsible for its pollen tube growth rate, a bimodal distribution for such character would be found in the structural heterozygous plants or at least the variance should be higher than that of homozygotes. However, the observed values for both homozygous and heterozygous plants distribute very similarly, indicating that the character is not significantly affected by the particular chromosome constitution of the pollen grain (Table 4 b–1).

Another source of evidence which is in agreement with this possibility comes from the data shown in Table 1, which indicates that plants having the same female parent have a similar behaviour independent of being homozygotes or heterozygotes.

Obviously, we would expect to be able to estimate differences in fitness parameters attributed to the plant chromosome constitutions only in the case that their fitness contributions were strong enough to minimize the effect of the interplant genotypic variation.

The discrepancy between percentage of pollen germination and pollen tube growth values found when the 1981 sample was analyzed (Tables 4 a, 4 b–2) could be attributed to a lesser influence of the pollen germination ability on the fitness: the pollen tube growth rate being the main component since the pollen grains with the highest growth rate would be the most effective in fertilization. This assumption agrees with the positive correlation found between the pollen tube growth rate and the egg-cell fertility or the number of seeds produced (Table 3).

In addition, it must be taken into consideration that not all the germinated pollen grains would produce a pollen tube capable of fertilization, so one could expect that pollen grains

producing the longest pollen tube should be the most suitable for fertilization.

Summing up, one can conclude that there is an influence of the plant chromosome constitution on pollen tube growth which is only detectable by analyzing a larger number of different plants, and that the different translocations are involved in the establishment of the various pollen grain characteristics.

The fact that some translocations seem to be preferentially transmitted by the male side as a consequence of the higher pollen tube growth rate could contribute, in addition to the recurrent chromosomal mutation, to the maintenance of the interchange polymorphism in 'Ailés', in spite of the lower fitness of structural heterozygotes considered as a whole (Candela et al. 1982).

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