

Mechanisms of 2n potato pollen formation in dihaploid *Solanum tuberosum* L. x *S. chacoense* Bitt. hybrid clones*

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ABSTRACT

The backcrosses of dihaploid *Solanum tuberosum* with wild species hybrids generating tetraploids progenies require the formation of non-reduced pollen. In this work, the mechanisms responsible for the formation of 2n pollen in 28 dihaploid *Solanum tuberosum* x *Solanum chacoense* hybrids were studied. Four mechanisms were found: parallel spindles (**ps**), fused spindles (**fs**), premature cytokinesis-1 (**pc-1**) and premature cytokinesis-2 (**pc-2**). The **ps** mechanism was the most frequent, being found in 23 of the 28 assessed clones. The **ps** and **fs** mechanisms led to the formation of dyads by first division restitution (**FDR**), transferring about 80% of the heterozygosity to the progenies. The **pc-1** and **pc-2** mechanisms also led to the formation of dyads, but they were genetically equivalent to second division restitution (**SDR**), transferring only 40% of the heterozygosity to the progenies. Occurrence of **FDR** and **SDR** were shown to be associated in 12 clones, indicating that the clones can produce non-reduced microspores by more than one mechanism. However, only one mechanism is functional in a single pollen-grain mother-cell. Clones 9-2, 9-3, 9-6 and 15-15 are recommended for use in 4x x 2x matings.

INTRODUCTION

Cultivated potato $2n = 4x = 48$ (*Solanum tuberosum* L.) is an autotetraploid species (Lunden, 1960; Gottschalk, 1984) with tetrasomic inheritance (Iwanaga and Peloquin, 1982), giving it the possibility of having multiple allelism, producing heterosis by inter-allelic and non-allelic interactions. Potato has a narrow genetic base, partially explained by geographical isolation and genetic erosion and thus breeders have sought to increase its genetic variability and germplasm base (Hawkes, 1978; Simmonds, 1979).

The genetic basis of the potato can be broadened by matings with wild or cultivated diploid

species ($2n = 2x = 24$). The production of *S. tuberosum* dihaploids ($2n = 2x = 24$) that cross easily with the wild species because of having the same ploidy level is desirable. Iwanaga and Schmiediche (1989) reported that more than 70% of the tuber-bearing *Solanum* species, where the chromosome number is known, are diploids. This makes the application of this type of crossing much easier in breeding programs, as there are many options from which to choose the diploid species to introduce desirable alleles into the population.

Hybrids with wild species, in spite of being vigorous (Peloquin, 1979), usually have many undesirable traits derived from the wild parents. These characteristics limit their direct use, and backcrosses to the cultivated species are necessary. For this to be possible, the hybrid must produce non-reduced gametes (2n pollen) to generate tetraploid progenies.

* Part of a thesis presented by M.N.O. to Universidade Federal de Lavras (UFLA) in partial fulfillment of the requirements for the Master's degree. Departamento de Biologia, Universidade Federal de Lavras, Caixa Postal 37, 37200-000 Lavras, MG, Brasil. Send correspondence to L.C.D.

The non-reduced gametes (2n) are produced by aberrations in the meiotic process and transfer a large part of the allelic and non-allelic interactions to their descendants.

Several mechanisms are involved in 2n pollen formation, resulting in variable heterozygosity levels and consequently different degrees of vigor in the progeny. Among them, the most important are parallel spindles (**ps**) and fused spindles (**fs**), genetically equivalent to first division restitution (**FDR**), and premature cytokinesis (**pc-1** and **pc-2**), genetically equivalent to second division (**SDR**), which transfer 80% and 40%, on average, of the heterozygosity to the offspring, respectively (Mok and Peloquin, 1975a; Ramanna, 1979).

The breeder's knowledge of the mechanisms acting on the 2n gamete formation is important for the maximization of the heterozygosity in the progenies, which is desirable because tuber yield needs several heterozygous loci for full expression (Mendoza and Haynes, 1974).

The objective of this study was to assess 2n pollen formation mechanisms in dihaploid *S. tuberosum* x *S. chacoense* (2n = 2x = 24) hybrids, and to indicate those that through greater transference of heterozygosity to the progenies, increase the chances of success in breeding programs.

MATERIAL AND METHODS

The chromosomic behavior at meiosis and the flowering traits of 28 hybrid clones of *S. tuberosum* x *S. chacoense* producing 2n pollen were assessed. The evaluation was conducted in a greenhouse of the Biology Department of Federal University of Lavras (UFLA), Lavras-MG, Brazil, from September to December 1992. A randomized-complete-block design with three replications was used. Each experimental unit was a single plant growing in pots containing organic vegetal substrate for seedlings (Plantimax Hortaliças).

Flower buds were collected and fixed in 3:1 ethanol-acetic for 24 hours, and stored in 70% alcohol at 5°C until use. The meiocytes were stained with 1% propionic carmin prior to slide preparation. At least 250 meiocytes per clone slides were assessed, following the methodology of Mok and Peloquin (1975b).

Quantity, frequency, and viability of n and 2n pollen were observed. Anthers were collected three days after the flowers opened, stored in 70% alcohol at 5°C until use and pollen grains were stained with 2% acetic carmin. At least 200 pollen grains per clone were

counted. The amount of pollen in the anthers was determined according to the following scores: 1 = very little pollen, 2 = little pollen, 3 = normal amount of pollen and 4 = abundance of pollen. Pollen grains with n or 2n chromosome numbers were determined according to the size classification of Quinn *et al.* (1974).

RESULTS AND DISCUSSION

In 12 of the 28 clones assessed, the mode of formation of 2n pollen was **FDR** associated with **SDR** (Table I). Clones that were homozygous for more than one locus responsible for the formation of 2n pollen (**ps**, **fs**, **pc-1** and **pc-2**) can produce non-reduced microspores by more than one mechanism. However, only one of the mechanisms is functional in each mother-cell of the pollen grains (Mok and Peloquin, 1975b). Twelve of the 28 clones assessed showed 2n pollen formation by **FDR** associated with an **SDR** mechanism. The microspore frequency among the different 2n pollen formation mechanisms (Table I) varies within the same clone, showing that the genes responsible have incomplete penetrance and variable expression. This variation in the frequency of the mechanisms also occurred within single families. In family 15 (clones 15-4, 15-9, 15-12, 15-15, 15-16) and family 18 (clones 18-3, 18-11, 18-12) all four 2n pollen formation mechanisms acted. No epistatic effect was observed for the genes responsible for the production of non-reduced pollen. Figures 1a, 1b, 1c and 1d show the mechanisms **ps**, **fs**, and **pc-1**, respectively. The **ps** mechanism occurred most frequently, being found in 23 of the 28 clones assessed.

The correlations among the mechanisms of 2n pollen formation (**ps**, **fs**, **pc-1** and **pc-2**) and their meiotic products were low [$r(\text{ps/dyad}) = 0.357$; $r(\text{fs/dyad}) = 0.246$]; $r(\text{pc-1/dyad}) = 0.490^{**}$; $r(\text{pc-2/dyad}) = 0.075$. Higher correlation coefficients would allow prediction of the 2n pollen formation mechanisms frequency from the frequency of assessed dyads.

These coefficients were lower than those found by Watanabe and Peloquin (1993), probably because they worked with clones which showed only one 2n pollen formation mechanism. When the correlation between clones and the meiotic product (dyad) involved only those clones with a single reduction mechanism (clones 4-10, 9-2, 15-15, 18-3, 18-12, 26-5, 27-9, 35-2B), the coefficient was much higher than those found earlier ($r = 0.776$).

The data for the Spring 1991 and Fall 1992 plantings were assessed by Cunha *et al.* (1994). The means of the 2n pollen formation frequencies for Spring

Table I - Mechanisms, mode of formation, microspore frequency, and meiotic products of the different 2n pollen formation mechanisms of 28 dihaploid *Solanum tuberosum* x *Solanum chacoense* hybrid clones.

Clone	2n pollen formation mechanism*	2n pollen formation mode**	Frequency (%)							
			Meiocytes					Meiotic products		
			ps	fs	pc-1	pc-2	Normal	Dyad	Triad	Tetrad
4-6	ps, fs, pc-2	FDR/SDR	1.8	8.1	0.0	62.7	27.2	21.8	37.5	40.6
4-10	pc-2	SDR	0.0	0.0	0.0	6.6	93.3	2.3	0.0	97.6
9-2	ps	FDR	5.1	0.0	0.0	0.0	94.8	0.0	0.0	100.0
9-3	ps, fs	FDR	19.7	14.5	0.0	0.0	65.7	3.2	0.2	96.5
9-6	ps, fs	FDR	23.0	1.7	0.0	0.0	75.2	3.5	3.9	92.4
10-2	ps, fs, pc-2	FDR/SDR	15.3	14.2	0.0	1.0	69.3	19.6	0.0	80.3
10-4	ps, fs	FDR	10.5	16.9	0.0	0.0	72.4	24.9	3.6	71.3
13-8	ps, fs	FDR	15.6	1.9	0.0	0.0	82.3	15.4	4.6	79.8
13-9	ps, fs	FDR	5.2	31.5	0.0	0.0	63.1	76.2	9.5	12.1
13-11	ps, fs, pc-1	FDR/SDR	7.6	35.9	1.5	0.0	56.2	16.8	0.0	83.1
15-4	ps, pc-2	FDR/SDR	2.5	0.0	0.0	2.0	95.2	4.3	0.0	95.6
15-9	ps, fs	FDR	3.7	0.7	0.0	0.0	95.5	4.7	0.9	94.2
15-12	ps, pc-1	FDR/SDR	3.7	0.0	0.3	0.0	95.8	0.0	0.0	100.0
15-15	ps	FDR	13.1	0.0	0.0	0.0	86.8	0.0	0.0	100.0
15-16	ps, fs, pc-1	FDR/SDR	0.0	5.0	6.4	0.0	88.5	35.7	0.0	64.2
17-5	ps, fs, pc-2	FDR/SDR	1.3	14.4	0.0	21.0	63.1	52.4	0.0	47.5
18-3	pc-1	SDR	0.0	0.0	33.8	0.0	66.1	35.6	2.1	62.2
18-11	ps, fs, pc-2	FDR/SDR	10.8	16.6	0.0	3.6	68.8	16.0	1.0	83.0
18-12	ps	FDR	3.7	0.0	0.0	0.0	96.2	0.0	0.0	100.0
21	ps, fs	FDR	6.3	0.9	0.0	0.0	92.6	2.8	0.0	97.1
26-5	ps	FDR	5.0	0.0	0.0	0.0	94.9	0.0	0.0	100.0
26-6	ps, fs, pc-1	FDR/SDR	4.8	0.6	3.8	0.0	90.6	37.8	0.0	62.1
26-7	ps, fs	FDR	10.0	0.4	0.0	0.0	89.4	2.3	0.0	97.6
27-6	ps, pc-1	FDR/SDR	2.8	0.0	40.0	0.0	57.1	58.7	2.5	38.6
27-9	pc-1	SDR	0.0	0.0	59.0	0.0	40.9	8.9	0.0	91.0
28	pc-1, ps	FDR/SDR	4.8	0.0	7.3	0.0	87.8	10.5	0.0	89.4
35-28	pc-1	SDR	0.0	0.0	66.6	0.0	33.3	72.2	0.0	27.7
36	pc-1, fs	FDR/SDR	0.0	1.8	87.2	0.0	10.9	47.8	0.0	52.1

***ps** (parallel spindles), **fs** (fused spindles); **pc-1** (premature cytokinesis-1), **pc-2** (premature cytokinesis-2).

**FDR (first division restitution); SDR (second division restitution).

1991 and Spring 1992 were lower than that for the Fall 1992 (Table II). Cunha *et al.* (1994) suggest that temperature could be responsible for the low mean in the Spring planting.

In the Spring 1992 planting the mean viability of 2n pollen was higher than in other plantings (Spring 1991 and Fall 1992) (Table II). The clones showed between 30% and 100% 2n pollen viability. To obtain useful information for selection of clones capable of increasing the chance of successful 4x x 2x crossings and of transferring high heterozygosity to the progenies, the 2n pollen frequency results should be associated

with pollen viability and mode of pollen formation. Thus, clones 9-2, 9-3, 9-6 and 15-15 deserve special attention as they combine 2n pollen frequencies higher than 5%, high 2n pollen viability (greater than 50%), and the **FDR** mode of pollen formation (Table I).

The production of n pollen grains is as important as that of 2n, to maintain the diploid level during the processes of genetic recombination and improvement of these populations. Clones with higher 2n pollen frequencies generally produce n pollen with less viability (Table II), as indicated by the negative correlation coefficients obtained between 2n pollen

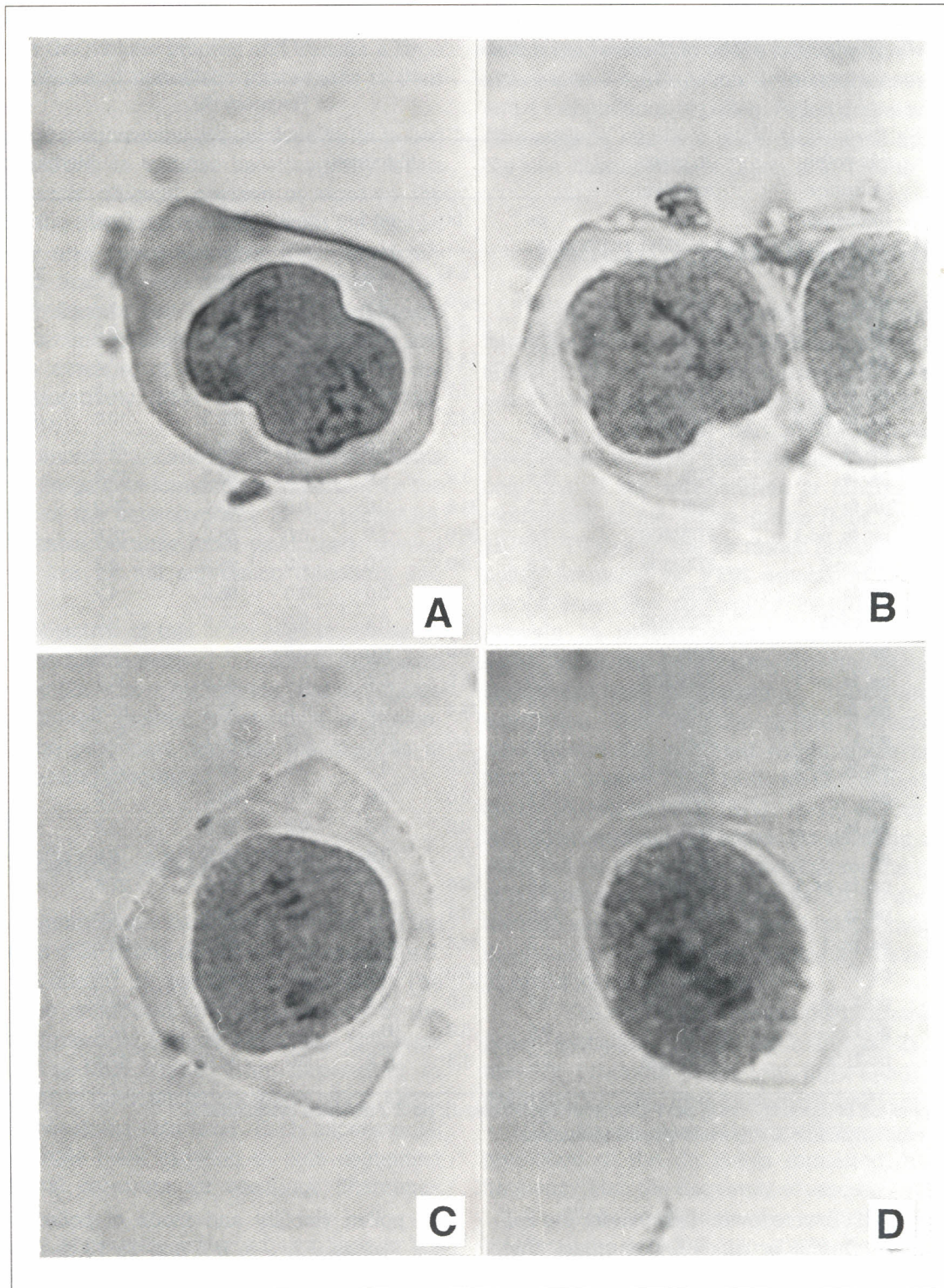


Figure 1 - Mechanisms of 2n pollen formation. A - Parallel spindles (**ps**). B - Fused spindles (**fs**). C - Premature cytokinesis-1 (**pc-1**). D - Premature cytokinesis-2 (**pc-2**). Magnification: 2048X.

frequency and viability in three evaluation periods (r Spring/91 = -0.56; r Fall/92 = -0.22; r Spring/92 = -0.62). A probable unequal distribution of the gene products in the presence of the 2n pollen formation genes leads to low viability of the cells producing reduced pollen

grains. This result should be taken into account when the breeder wants to use clones with both characteristics for crossing with a tetraploid species for tetraploidy restoration or for maintaining the diploid level, to improve the population.

Table II - Means of 2n pollen frequency, 2n pollen viability, quantity of pollen on anther, and pollen viability for 28 dihaploid *Solanum tuberosum* x *Solanum chacoense* hybrid clones.

Clones	Frequency of 2n pollen (%)			Viability of 2n pollen (%)			Quantity of pollen on anther*			Viability of n pollen (%)		
	Spring/91**	Fall/92**	Spring/92	Spring/91**	Fall/92**	Spring/92	Spring/91**	Fall/92**	Spring/92	Spring/91**	Fall/92**	Spring/92
4-6	7.2	5.2	-	85.6	79.4	-	2.7	3.0	-	56.9	55.6	-
4-10	1.3	26.1	-	43.3	47.7	-	3.7	3.0	-	75.5	41.7	-
9-2	12.7	25.4	9.1	81.6	76.1	100.0	2.0	2.7	3.0	41.5	51.3	84.3
9-3	18.0	12.9	25.9	92.7	60.3	92.5	1.3	1.7	2.0	40.4	24.8	22.8
9-6	0.0	6.9	9.5	0.0	33.3	90.4	1.3	1.0	2.6	9.0	12.1	57.3
10-2	0.0	18.1	8.5	0.0	79.4	62.5	1.0	1.0	1.3	36.6	23.8	42.3
10-4	11.6	10.3	4.7	80.0	68.8	45.4	1.7	2.0	1.6	13.0	36.9	44.8
13-8	0.0	6.1	-	0.0	54.6	-	3.3	1.9	-	90.3	49.7	-
13-9	0.0	8.9	9.4	0.0	52.5	30.0	2.3	1.7	1.0	57.9	45.0	3.4
13-11	2.4	6.9	1.5	55.6	50.0	40.0	1.7	1.7	2.0	30.7	49.0	51.8
15-4	0.3	8.1	3.2	33.3	88.4	71.4	2.7	3.0	3.0	63.6	80.8	81.7
15-9	0.0	12.2	1.7	0.0	97.0	90.0	3.7	4.0	2.3	58.7	57.3	74.9
15-12	0.0	16.3	4.1	0.0	78.7	58.3	4.0	2.7	3.0	79.2	54.6	72.8
15-15	1.4	17.2	6.4	66.7	84.4	78.1	2.7	3.7	3.0	77.9	65.6	65.7
15-16	4.8	27.9	9.9	48.2	57.9	48.1	1.0	1.3	1.0	34.2	23.8	41.5
17-5	16.9	4.9	4.5	30.4	29.3	42.8	1.0	1.7	1.0	14.4	26.0	20.4
18-3	5.3	0.7	2.6	89.1	33.3	66.6	2.3	1.7	1.3	60.9	37.3	60.5
18-11	50.9	-	17.9	88.9	-	76.9	1.0	-	2.0	10.1	-	7.4
18-12	0.4	31.5	2.6	41.7	81.8	100.0	2.3	2.1	1.3	81.8	19.7	83.2
21	15.8	10.8	4.7	66.0	50.1	65.2	1.7	1.0	2.0	11.4	26.0	72.9
26-5	0.3	15.2	2.0	33.3	60.7	70.5	3.0	3.0	2.3	80.6	70.3	81.0
26-6	5.8	6.9	5.9	91.1	67.8	42.8	3.3	2.0	3.0	66.0	74.3	69.2
26-7	0.0	10.4	4.4	0.0	30.4	96.5	3.3	4.0	2.3	77.8	63.3	69.0
27.6	0.0	8.3	4.0	0.0	80.6	100.0	2.7	3.0	3.0	29.3	46.1	65.6
27-9	0.0	8.7	2.5	0.0	77.8	88.2	3.0	1.3	2.3	90.5	33.1	79.1
28	0.2	11.8	3.5	33.3	83.8	79.1	1.7	3.0	2.0	71.9	69.2	75.1
35-28	7.5	15.8	7.6	83.3	52.2	68.7	2.7	2.0	2.0	22.8	20.5	31.9
36	-	1.1	6.8	-	63.4	68.7	-	2.7	2.3	-	51.8	46.7
Média	6.0	12.0	6.9	42.3	63.7	70.9	2.3	2.3	2.1	51.2	44.8	56.2

*Grades from 1 to 4 (4 most).

** Data obtained by Cunha *et al.* (1994)

- Flower buds were produced but they did not develop into flowers.

RESUMO

Os retrocruzamentos de híbridos dihaplóides de *Solanum tuberosum* com espécies selvagens gerando progenies tetraplóides necessitam da formação de pólen não reduzido. Neste trabalho, os mecanismos responsáveis pela formação de pólen não reduzido (pólen 2n) em 28 híbridos de dihaplóides de *S. tuberosum* com *S. chacoense* foram estudados. Quatro mecanismos foram encontrados: **ps** (parallel spindles), **fs** (fused spindles), **pc-1** (premature cytokinesis-1) e **pc-2** (premature cytokinesis-2). Destes,

o mecanismo **ps** foi o mais freqüente, encontrado em 23 dos 28 clones avaliados. Os mecanismos **ps** e **fs** levam à formação de díades geneticamente equivalentes a **FDR** (first division restitution), transmitindo em torno de 80% de heterozigose às progênies. Os mecanismos **p-1** e **pc-2** também levam à formação de díades, mas geneticamente equivalentes a **SDR** (second division restitution), transmitindo em torno de 40% de heterozigose às progênies. Os modos de formação **FDR** e **SDR** apresentaram-se associados em 12 avaliados, mostrando que os clones podem produzir micrósporos não reduzidos por mais de um mecanismo. No entanto, somente

um mecanismo é funcional em uma célula-mãe dos grãos de pólen. Recomenda-se o emprego dos clones 9-2, 9-3, 9-6 e 15-15 para a realização de cruzamentos 4x x 2x.

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(Received May 24, 1994)