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Cytological evidences of SDR-FDR mixture in the formation of 2n eggs in a potato diploid clone

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Summary. Macrosporogenesis and microsporogenesis were investigated in a diploid *S. tuberosum* × *S. chacoense* potato hybrid, characterized by more than 50% 2n egg formation. Fifty-five percent of dyad formation of 2n macrospores is ascribed to two meiotic abnormalities: omission of the second meiotic division, occurring at a frequency of 38%, and irregular spindle axis orientation at metaphase I at a frequency of 16%: These abnormalities give origin to a mixture of 2n eggs, composed of mostly second division restitution (SDR) and a small portion of first division restitution (FDR). Microsporogenesis showed rare dyads of 2n microspores depending on parallel spindles observed in anaphase II.

Key words: Macrosporogenesis – 2n egg – SDR – FDR – Potato

Introduction

2n gametes occur widely in many plant species (Harlan and de Wet 1975) and the genetics and cytology of this phenomenon have been extensively investigated, particularly for 2n pollen production (Ramanna 1974; Mok and Peloquin 1975; McCoy 1982). Cytological mechanisms responsible for 2n egg formation are described in *Datura* (Satina and Blakeslee 1935), maize (Rhoades and Dempsey 1966), barley (Finch and Bennett 1979), potato (Iwanaga and Peloquin 1979; Stelly and Peloquin 1986a; Werner and Peloquin 1987), and alfalfa (Pfeiffer and Bingham 1983). In 2n egg formation, omission of the second meiotic division is the prevalent mechanism, even though two other mechanisms, namely, absence of cytokinesis after anaphase II in the chalazal dyad cell and desynapsis, have been found in alfalfa and potato. Inheritance studies of 2n egg formation identified single genes controlling their appearance (Satina and Blakeslee 1935; Rhoades and Dempsey 1966; Finch and Bennett 1979; Iwanaga and Peloquin 1979; Jongedijk and Ramanna 1988). The genetic consequence of the formation of viable 2n eggs produced by desynapsis is that they carry non-sister chromatids, they are potentially heterozygous and, as a matter of fact, are considered genetically equivalent to the products of a first division restitution (FDR). The 2n eggs produced by the other mechanisms mentioned carry sister-chromatids, and therefore they are potentially homozygous, corresponding to the product of a second division restitution (SDR). The degree of heterozygosis or homozygosis of 2n eggs is clearly influenced by the crossover frequency. Half-tetrad genetic analyses of tuber color and isozymes in potato (Stelly and Peloquin 1986b; Douhes and Quiros 1988) confirm the prevalence of SDR in 2n egg formation. Also parthenogenetic tetraploids are reported to be the product of a mechanism equivalent to SDR (Taylor 1978). Ross and Langton (1974) found a segregation ratio of resistance gene to virus X indicative of FDR 2n egg production. Unreduced egg production was also evaluated in diploid interpecific hybrids from crosses between haploids of *S. tuberosum* and diploid wild species (*S. chacoense, S. phureja, S. tarijense*) by Frusciante et al. (1987) and by Werner and Peloquin (1987).

The purpose of this work was to investigate the cytological events that lead, in a diploid interspecific hybrid, to the finding of more than 50% 2n eggs, as inferred by its seed set after pollination with tetraploid clones. Microsporogenesis in the same clone was also studied.

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Materials and methods

Two diploid genotypes were used, namely, PL1.22, a *S. phureja* clone (2n = 2x = 24) that does not form 2n eggs, and the clone T710, an interspecific hybrid between *S. tuberosum* haploid USW3304 (2n = 2x = 24), selected by S.J. Peloquin, and a *S. chacoense* clone. The tetraploid clone P540, selected in Portici, and other tetraploid genotypes were also used.

The frequency of 2n eggs in clone T710 was estimated in three ways. Firstly, the 2n egg frequency was inferred by the percentage of true seeds over potential seeds after 2x × 4x crosses (potential sees: total seeds potentially produced in a berry, including those aborted). Secondly, the 2n egg frequency was estimated by the ratio in percentage of the average number of seeds per berry after 2x × 4x and 2x × 2x crosses. Thirdly, the 2n egg frequency was determined cytologically by the nucleolar size of female gametophyte nuclei according to Stelly and Peloquin (1986a).

For macrosporogenesis analysis ovaries were collected, fixed in FAA for 24–48 h, stored in 70% ethanol, dehydrated in a graded ethanol series, and carried through the usual paraffin embedding procedure. Serial longitudinal sections were cut at 10–14 μm with a rotary microtome and stained using an acid-Schiff's reagent and fast green combination.

For microsporogenesis immature anthers, fixed in proprionic acid-ethanol (1:3) with a small amount of ferric chloride as mordant, were squashed in aceto-carmine (2%). The pollen viability was estimated by staining with 2% acetocarmine solution. The diameter of nucleoli of megagametophyte nuclei was measured by an ocular micrometer. Mean standard deviation and cumulate frequency distribution of nucleolus size were calculated.

Results

The average number of true seeds per berry, their percentage over the number of potential seeds, and the percentage of seeds obtained from crosses between the diploid clone T710 and tetraploid or diploid clones, used as male parents, are reported in Table 1. The frequency of 2n eggs estimated as true seeds over potential seeds ranged from 73% to 58.5%. The same frequency estimated as a percentage of seed number obtained from 2x × 4x and 2x × 2x crosses ranged from 61.9% to 54.8%.

The nucleolus size was evaluated in T710, in the diploid PI 1.22, and in the tetraploid clone P540. In T710, the nucleolus diameter ranged from 1.66 to 4.44 μm with a bimodal distribution (peaks at 2.44 μm and 3.33 μm). In Pi 1.22 and in P540 the range was 1.33–2.44 μm and 2.22–3.88 μm, respectively, with a monomodal distribution (Fig. 1). Mean and standard deviation of the nucleolus diameter are reported in Fig. 1, where the nucleoli of T710 were considered in two separate groups corresponding to nuclei 1n and 2n. Nucleoli with sizes less than 2.22 μm and greater than 2.77 μm were assigned to nuclei 1n and 2n; nucleoli ranging from 4.21 to 4.44 μm were assigned to nuclei 4n; nucleoli of intermediate size cannot be definitively classified as 1n or 2n. Nevertheless, the minimum between the two peaks in the distribution of T710 was assumed to be on the border between "1n" and "2n" nucleoli.

The cytological analysis of a normal type of megasporogenesis was performed on PI 1.22, a clone that does not form 2n eggs; in a few instances, lagging chromosomes at anaphase I and two adjacent megaspore mother cells (MMCs) in the same ovule at prophase I were observed. The frequency of meiotic abnormalities, as evidenced in the megasporogenesis of the 2n egg-producing clone T710, is reported in Table 2.

At metaphase I 16% of MMCs showed an abnormal orientation of the spindle axis. This axis in normal

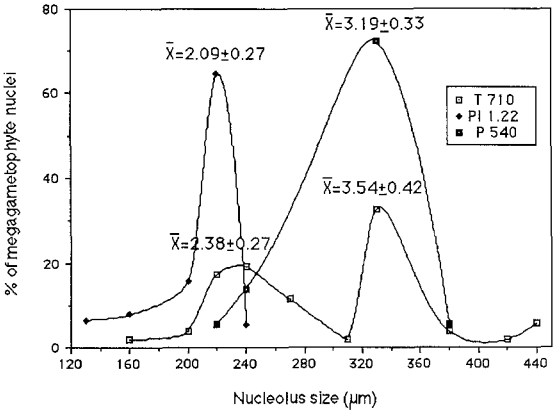

Fig. 1. Distribution of nucleolus size of megagametophyte nuclei in the diploid clones T710 and PI 1.22 and in the tetraploid clone PL540

Table 1. Percentage of true seeds over potential seeds and percentage of seeds in 2x × 2x crosses compared to 2x × 2x involving the diploid clone T710

Type of cross	Male parent	No. of berries considered	Average no. of seeds per berry	Percentage of true seeds over potential seeds	Percentage of seeds in 2x × 4x compared to 2x × 2x crosses
2x × 4x	AVRDC 1287.19	10	56.9	73.0	61.9
	CHIQUITA	15	43.2	58.5	55.2
	R 128.6	9	47,6	67.2	57.6
	W842	25	42.4	60.1	54.8
2x × 2x	PI 1.22	12	35.0	–	–

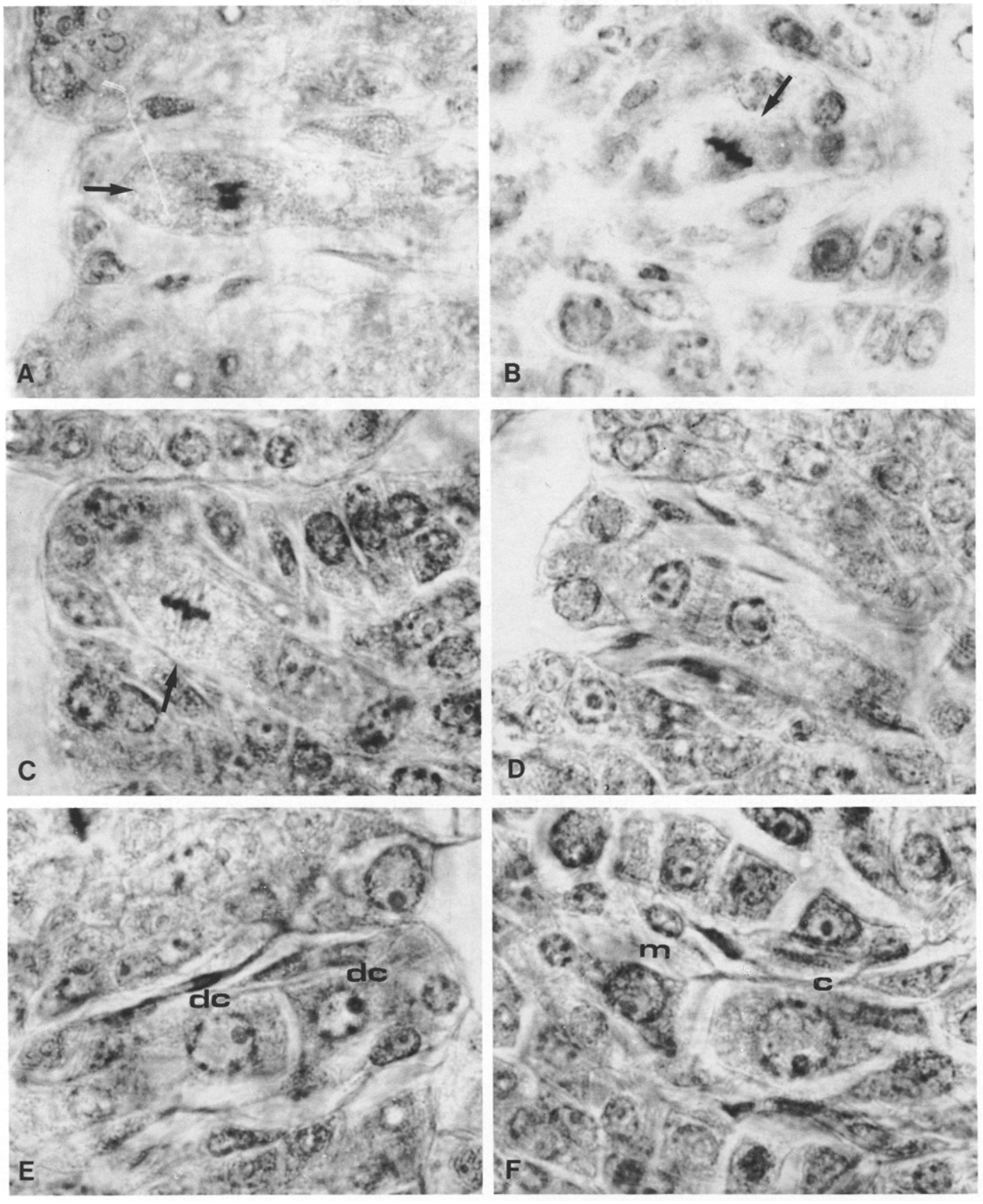

Fig. 2 A–F. Macrosporogenesis in the diploid clone T710. **A–C** Metaphase I. In **A** (1,300×) the spindle axis is parallel to the long axis of the megaspore mother cell (MMC). In **B** and **C** (1,400×) the axis has different orientations. *Arrows* indicate the direction of the spindle. **D** Telophase 1 (1,400×). **E** Two daughter cells (*dc*) after meiosis I at a resting stage (1,400×). **F** Chalazal (2n; *c*) and micropilar (*m*) daughter cells of the dyad in the diploid clone T710 (1600×)

Table 2. Frequencies of meiotic abnormalities found in the megasporogenesis diploid clone T710

Metaphase I		2nd Meiotic division		Sporad stage	
No. of MMCs analyzed	Percentage of abnormal spindle axis orientation	No. of MMCs analysed	Percentage of omission of second division	No. of sporads analyzed	Percentage of dyads
31	16	43	38	72	55

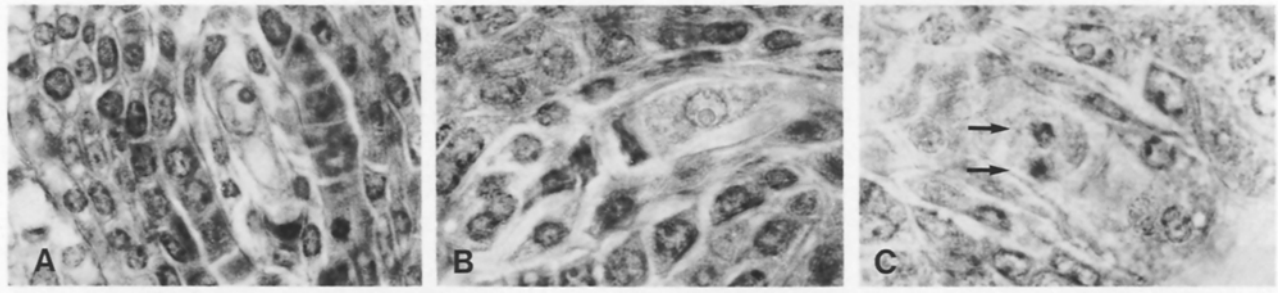

Fig. 3A–C. Macrosporogenesis in the diploid clone T710. **A** Dyad of 2n macrospores (1,050 ×). **B** Tetrad of n macrospores (1,300 ×) **C** Two nuclei (*arrows*) in one chalazal cell (1,450 ×)

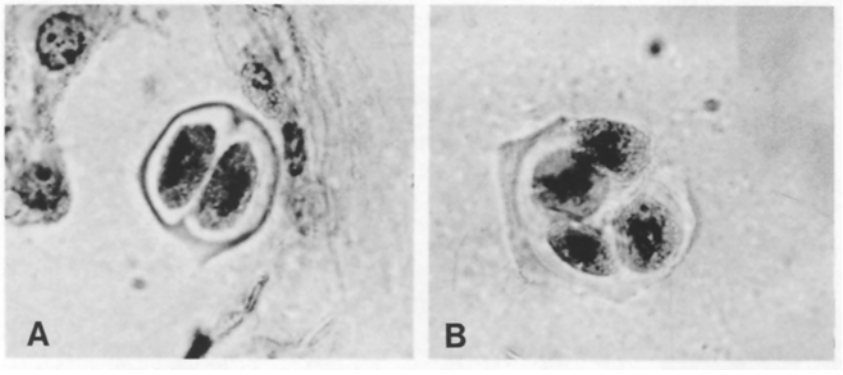

Fig. 4A and B. Microsporogenesis in the diploid clone T710. **A** Dyad of 2n microspores (1,500 ×). **B** Tetrad of n microspores (1,500 ×)

megasporogenesis was longitudinal, i.e., parallel to the long axis of the MMC (Fig. 2A). However it assumed various orientations in the MMCs of the clone T710 (Fig. 2B and C). Thirty-eight percent of the MMCs omitted the second meiotic division: after telophase I (Fig. 2D), the two daughter cells entered into a resting stage, associated with a complete chromosome despiralization (Fig. 2E), which did not occur when the second division took place. The micropylar daughter cell of the dyad began degenerating and the chalazal cell enlarged, becoming a 2n functional megaspore (Fig. 2F). Fifty-five percent of the observed sporads were dyads (Fig. 3A) instead of the usual tetrads (Fig. 3B). The absence of correspondence between the percentage of dyads (55%) and the percentage of omission of the second division (38%) indicated a second mechanism of dyad formation: the irregular spindle axis orientation at metaphase I (16%). Two nuclei at metaphase II in the same chalazal cell and no nucleus in the micropylar cell were evident (Fig. 3C), and this event was considered a consequence of the abnormal spindle axis orientation and also responsible for 2n egg formation.

Spindle axes were oriented at different angles also in anaphase II, thus influencing the linear arrangement of the megaspores. A further deviation from the normal course of megasporogenesis was the phase asynchrony in the second meiotic division between the chalazal and the micropylar dyad cell, which was in metaphase when the chalazal cell had already completed its division; this occurred with a frequency of 25% of the observed MMCs during the second division and it did not seem to be involved in the origin of 2n megaspores.

The acetocarmine staining of the pollen in the clone T710 evidenced a pollen fertility of 80% and rarely 2n pollen grains (0.1%). The microsporogenesis analysis showed MMCs with lagging chromosomes at anaphase I, parallel spindles at anaphase II, and dyads of microspores (Fig. 4A) instead of tetrads (Fig. 4B). These abnormalities occurred at very low frequencies.

Discussion

The megasporogenesis studies conducted with the diploid clone producing only n eggs is in agreement with

earlier megasporogenesis descriptions of cultivated potato, *S. tuberosum* (Rees-Leonard 1935). Development of a surplus of MMCs in the ovule was already observed by Iwanaga (1980) and Jongedijk (1985) in diploid interspecific hybrids and was considered a normal event in megasporogenesis.

Previous studies of Werner and Peloquin (1987) on the 2n eggs producing diploid clone T710 revealed only the omission of the second meiotic division. In this megasporogenesis analysis additional meiotic abnormalities were observed. On the whole, two abnormalities could be related to 2n egg production: omission of meiosis II was the most frequent one, but its occurrence was lower than the frequency of dyads. Abnormal orientation of the spindle axis at metaphase I was also found, which can lead to dyad formation. In fact, it can disturb the correct chromosome movement towards the two poles or the cell plate formation across the phragmoplast. Such abnormalities might give rise to first division restitution (FDR).

A change in spindle morphology was also found by Iwanaga (1980) in meiosis I of megasporogenesis in two *S. phureja* × *S. tuberosum* tetraploid hybrids. Mutations that affect the kinetic apparatus of meiosis were previously described in meiosis II of microsporogenesis of potato (Ramanna 1974; Mok and Peloquin 1975); the fused and/or parallel spindles were also considered causal mechanisms of 2n pollen production. At present, a complete understanding of the functioning of the meiotic kinetic apparatus, in meiosis I or II, is not available and the consequences of its modifications are not clearly predictable. In this sense, however, a careful analysis of the available strains with genetic disturbances of meiosis may help in better defining the normal course of meiotic events.

Finally, it is worth mentioning a methodological issue. When counting the percentage of true seeds obtained over potential seeds in 2x × 4x crosses, the frequency of 2n egg formation may be overestimated due to the difficulties in recognizing all aborted seeds. On the other hand, when the same frequency of 2n eggs is inferred from nucleolus size, underestimated values are obtained. The results presented in this paper reveal a substantial correspondence between the dyad frequency cytologically evaluated and the 2n egg frequency estimated from the seed set of 2x × 4x and 2x × 2x crosses. This supports the adoption of the last method in large screenings for meiotic mutants in potato.

The mutation(s) present in T710 has a partial penetrance because 2n eggs are produced at a 50% frequency; both SDR and FDR mechanisms are active and this situation leads to a higher heterozygosity in comparison with other clones producing only FDR or SDR 2n gametes. On the basis of genetic data, a mixture of 2n megagametophytes, originating mostly by SDR and in a smaller proportion by FDR, was supposed to occur in other diploid interspecific hybrids (Stelly and Peloquin 1986b).

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