

## TIMING OF MEGASPOROGENESIS IN *TRIPSACUM* SPECIES (POACEAE) AS RELATED TO THE CONTROL OF APOMIXIS AND SEXUALITY

OLIVIER LEBLANC AND YVES SAVIDAN

**Abstract:** In several pseudogamous aposporous species (*Panicum maximum*, *Ranunculus auricomus*, *Paspalum notatum*), different megasporogenesis time courses in sexuals and aposporous have been reported, making that apomeiosis yields mature female gametophytes more precociously than meiotic development. A main consequence of such difference in the production of mature female gametophyte might be the lost of receptivity of the unreduced egg cell by the time the pollen tube reaches the female gametophyte. In two pseudogamous diplosporous species, *Tripsacum dactyloides* and *Tripsacum zopilotense*, cytoembryological observations showed that meiosis is also delayed when compared to diplospory. Precocious embryony – embryo starts to divide before anthesis – is an effective system to prevent egg cell fertilization that do not occur in all the pseudogamous apomicts. The timing difference in sexual and apomictic developments has been observed across apomicts of different origin and nature, which do not show precocious embryony. Thus it appears to be a fundamental component of apomixis as a cause of the egg cell receptivity lost, making that egg cell fertilization is no longer possible while fusion of one sperm cell and the two polar nuclei still takes place.

**Key words:** apomixis, diplospory, meiosis, parthenogenesis, fertilization failure

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### INTRODUCTION

Gametophytic apomixis differs from sexuality by two basic events, apomeiosis (failure of both chromosome reduction and recombination) and parthenogenesis (embryo development without fertilization). Apomeiosis can be achieved through 1) diplospory when meiosis fails or aborts, or 2) apospory when, beside the legitimate sexual cell line, one or few somatic nucellar cells develop into unreduced female gametophytes. In the second step, the egg cell of the unreduced megagametophyte develops into the embryo parthenogenetically, but polar nuclei fertilization is generally required for seed development (pseudogamy). Studies of the mode of inheritance of apospory in

*Bothriochloa-Dichanthium* (HARLAN *et al.* 1964), *Panicum maximum* Jacq. (SAVIDAN 1982a, 1983), *Ranunculus auricomus* L. (NOGLER 1984a), *Cenchrus ciliaris* L. (SHERWOOD *et al.* 1994), and the *Brachiaria decumbens* complex (DO VALLE *et al.* 1994) have revealed that apospory is controlled by one dominant gene. A few older reports claimed a two-gene control for aposporous apomixis, but interpretations were controversial either because of the origin of the sexual material or mostly because of the relative unreliability of the screening tools used. On the other hand, though MOGIE (1988) showed that the gene(s) controlling diplospory in *Taraxacum* Wigg. are located on one

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Table 1. Number of pistils analyzed per pistil class after bulking by species and reproductive nature.

Class #	0	1	2	3	4	5	6	7	8	9
intervals (mm)	< 0.50	[0.50;0.75]	[0.75;1.00]	[1.00;1.25]	[1.25;1.50]	[1.50;1.75]	[1.75;2.00]	[2.00;2.25]	[2.25;2.50]	> 2.50
Diploid bulks*										
<i>T. zopilotense</i>	12	15	18	22	37	36	24	24	23	27
<i>T. dactyloides</i>	6	13	17	25	33	35	23	30	28	35
Tetraploid bulks*										
<i>T. zopilotense</i>	11	17	15	26	29	35	33	21	22	21
<i>T. dactyloides</i>	13	19	30	41	66	72	52	40	23	26

\* All accessions are from the ORSTOM-CIMMYT collection established at Tlaltizapán, Morelos State, Mexico. *T. zopilotense* bulks: 49-431, 49-1382, 49-1388, 51-470 (diploids); 49-439, 49-292, 7002-1 (tetraploids). *T. dactyloides* bulks: 152-2357 and 154-2426 (diploids); 1-480, 9-569, 16-59 (tetraploids).

chromosome, no credible genetic analysis regarding the control of diplospory has been conducted.

According to NOGLER (1984b), apomeiotic hybrids that lacked parthenogenetic capacity were rarely encountered in *Parthenium argentatum* A. Gray, *Potentilla argentea* L. or *Ranunculus auricomus*. Linkage between the gene(s) controlling apomeiosis and parthenogenesis seems to be strong, given that in most apomicts the presence of the gene(s) responsible for apomeiosis generally results in maternal progenies. Therefore, as SAVIDAN (1982a) postulated, the main components of apomixis, apomeiosis and parthenogenesis, may be viewed as a dominant 'supergene' resulting from a strong genetic linkage.

Observing the timing difference in the formation of mature female gametophytes in aposporous vs sexual *Panicum maximum* accessions, SAVIDAN (1982a, 1989) proposed a second hypothesis for the control of parthenogenesis, saying that it might result basically from aposporous non-reduction. This 'egg cell wall completion' hypothesis, as it is called, holds that, as a consequence of the precocious development of unreduced female gametophytes compared with reduced ones, the egg cell may not be receptive by the time the pollen reaches the ovule, causing the failure of fertilization. Recent ultrastructural observations in aposporous *Cenchrus ciliaris* (JEAN-PHILIPPE VIELLE, University of Texas A & M, personal communication) indeed suggest the presence of a complete cell wall around the egg cell that may act as a mechanical barrier by the time of pollen tube discharge. According to MOGENSEN and NAUMOVA (personal communications), the development of such a wall may be independent of pollen tube growth. However, in the parthenogenetic *hap* barley mutant, male and female gametes remain side-by-side without any apparent cell wall to block fertilization (MOGENSEN 1982), suggesting either a chemical barrier or a specific time window of receptivity.

Developmental timing differences such as that found in *Panicum maximum* have been reported in a few other aposporous materials, such as *Ranunculus auricomus* (NOGLER 1984a) or *Paspalum notatum* Fluegge (MARTÍNEZ *et al.* 1994). Ana-

lysis of megasporogenesis and megagametogenesis in several *Tripsacum* species (LEBLANC *et al.* 1995), have shown that, regardless of species, diplospory occurs in polyploid types whereas diploids are sexual. While working on this study, we observed a difference in timing between the two reproductive pathways which closely mimicked that reported by Savidan in aposporous and sexual *Panicum maximum*. This preliminary observation led us to a more detailed cytoembryological analysis of *Tripsacum dactyloides* (L.) L. and *Tripsacum zopilotense* Hernández Xolocot & Randolph, which data are reported here.

#### MATERIALS AND METHODS

In contrast with apospory, where sexual and apomeiotic developments can occur concomitantly as unreduced female gametophyte(s) are initiated from somatic nucellar cells, meiosis is omitted or aborted in diplosporous types of apomixis. Diplospory in *Tripsacum* is primarily of the *Antennaria*-type, and mature *Polygonum*-type unreduced megagametophytes are directly produced through three or more mitoses after significant megasporocyte enlargement (LEBLANC *et al.* 1995). Such reproductive mechanisms make it impossible to compare sexual and diplosporous developments in a single ovule. The comparison cannot be done in single plants either, because of the low rate of residual sexuality in diplosporous *Tripsacum* accessions (LEBLANC *et al.* 1995). Finally, given that *Tripsacum* spp. are mononeous grasses, ovules cannot be classified by pollen

stage. Faced with these constraints, we chose to use interference contrast microscopy (CRANE & CARMAN 1987) to determine developmental stages in sexual and diplosporous ovules, after sorting pistil length by size. To limit the expression of species' peculiarities and because ploidy level and mode of reproduction are closely related in *Tripsacum* spp., pistils were collected from sexual diploid ( $2n = 36$ ) and diplosporous tetraploid ( $2n = 72$ ) accessions from *T. zopilotense* and *T. dactyloides*. Ten classes were defined (0.25 mm intervals), and bulks for each of the two species were made by mixing pistils of the same reproductive nature and from the same class (Table 1). In addition, one apomeiotic *T. dactyloides* triploid accession ( $2n = 54$ ) was also analyzed, but not bulked. Stages of development in sexual and diplosporous bulks were then recorded, using the same definitions as LEBLANC *et al.* (1995) in their survey of reproductive mode in *Tripsacum*. In this system, both sexual reproduction and diplospory are divided in key steps from megasporogenesis initiation to the end of megagametogenesis (mature female gametophyte production; Table 2). Given that callose deposition has been associated with sexual megasporogenesis but is absent during diplospory (CARMAN *et al.* 1991; LEBLANC *et al.* 1995), we used this characteristic to distinguish between reproductive modes, especially in young megasporocytes.

#### RESULTS

Although we noted a few differences, such as the generation of meiotic products during apomeiotic development, reproductive features in the two

Table 2. Cytoembryological stages used to study megasporogenesis and megagametogenesis (based on LEBLANC *et al.* 1995). MS: megasporocyte; FG: female gametophyte.

SEXUAL REPRODUCTION	DIPLOSPORY
	No nucellar activity
	Young initiated MS: no difference between both developments
Callose deposition in MS cell walls	Callose-free enlarging MS
dyad	
tetrad	
Degenerative tetrad-remaining megaspore	
	2n-FG
	4n-FG
	Young 8n-FG (last mitotic division not yet followed by cytokinesis)
	Mature 8n-FG ( <i>Polygonum</i> -type)

Table 3. Cytoembryological stages in percentages observed in bulks from diploid *T. dactyloides* (first numbers in columns) and from diploid *T. zopilotense* (second numbers in columns) during megasporogenesis and megagametogenesis. MS: megasporocyte; FG: female gametophyte; Y. 8n-FG: young 8n-FG.

Classes	No nuc. act.	Megasporogenesis					Megagametogenesis		
		Young MS	MS	Dyad	Tetrad	Dg. tetrad	2n-FG	4n-FG	Y. 8n-FG
0	71/79	29/21							
1	27/23	73/77							
2	16/6.5	39/25	45/68.5						
3		4.5/-	54/44	54.5/66					
4			16/3	30/34	48.5/60	5.5/3			
5			5.5/-	11/3	41.5/33.5	39/47	3/16.5		
6					21/13	62.5/45	16.5/42		
7					16.5/-	37.5/20	46/70	-/10	
8						8.5/3.5	69.5/67.5	22/29	
9						11/-	52/51.5	29.5/37	7.5/11.5

*Tripsacum* species appeared to be very similar in nature and timing (Tables 3 & 4). The *T. dactyloides* triploid accession also showed important developmental similarities with the tetraploids, except that no meiotic event was recorded during megasporogenesis. Megasporogenesis and megagametogenesis time courses during sexual reproduction and diplospory in *T. zopilotense* were compared (data, Tables 3 & 4; Fig. 1).

Differentiation of hypodermal nucellar cells

into megasporocytes occurred in both developments at the same pistil stages (0-1). Just after initiation, sexual and diplosporous megasporocytes behaved similarly when observed using the benzyl benzoate-dibutyl phthalate clearing procedure, and differed from nucellar cells by a slight enlargement, dense nuclei and nucleoli, and granular cytoplasm. However, cytoembryological differences between both developments were readily apparent, and from stage 1 to stage 2 almost half the

Table 4. Cytoembryological stages in percentages observed in bulks from tetraploid *T. dactyloides* (first numbers in columns) and from tetraploid *T. zopilotense* (second numbers in columns) during megasporogenesis and megagametogenesis. MS: megasporocyte; FG: female gametophyte; Y. 8n-FG: young 8n-FG; M. FG: mature 8n-FG.

Classes	No nuc. act.	Megasporogenesis			Megagametogenesis			
		Young MS	Enl. MS	Nb. of meiotic products	2n-FG	4n-FG	Y. 8n-FG	M. 8n-FG
0	37.5/30.5	62.5/61.5	-/8					
1	-/5	90/74	10/21					
2	-/6	56/76.5	44/17.5					
3		20/44.5	69/37		11/18.5			
4		3.5/18.5	39/50.5	Dyads: 1/1	57.5/30			
5			18/37	Dyads: -/3; Tetrads: -/2	60.5/56	21.5/7		
6			6/17.5		48/60	39/11.5	7/2	
7			-/5	Tetrads: 1/1; Deg. tetrads: -/1	10/52	45/34	30/9	-/15
8					13.5/39.5	41/33	32/13	4.5/13.5
9				Deg. tetrads: 1/1	-/23	35/46	40/15.5	25/25.5

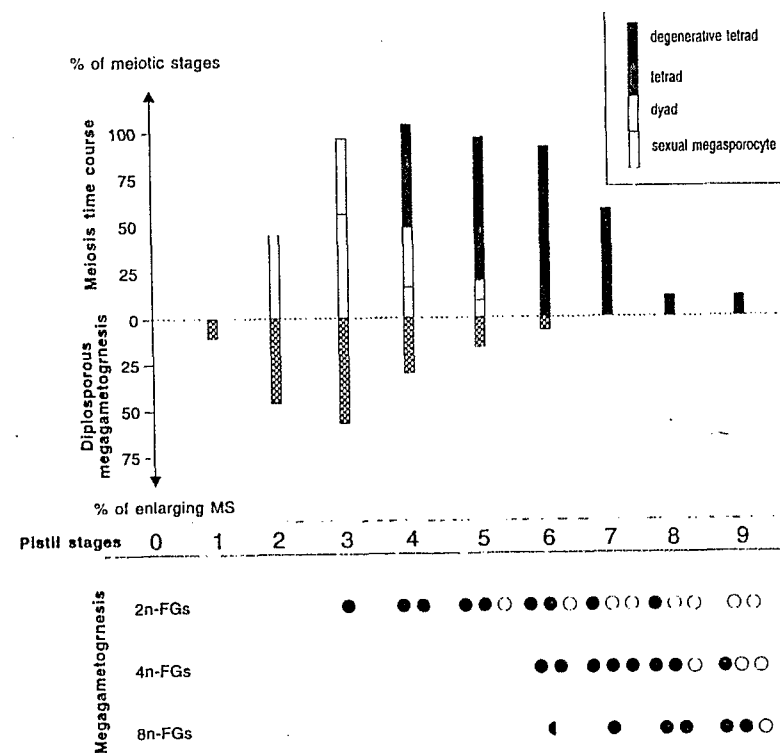


Fig. 1. Relative timing in diplosporous versus sexual development in *T. zopilotense*: meiosis time course and enlarging stage during megasporogenesis, and megagametogenesis. Black and white dots represent diplosporous and sexual materials respectively.

megasporocytes exhibited characteristic reproductive features (callose deposition in sexuals or callose-free enlarging megasporocytes in diplosporous). Megasporocyte enlargement during diplosporous development (stage 2 to stage 6) took longer than usual, but still less than meiosis; as more than 50% of the sexual ovules still showed meiotic products (tetrads or degenerative tetrads) during stage 7. Tetrad degeneration and the remaining chalazal megaspore development accounted for nearly half the duration of meiosis, making this step appears to be the most significant factor in meiosis delay. First mitotic divisions in functional megaspores - i.e. the enlarged diplosporous megasporocyte or the remaining chalazal sexual megaspore - thus occurred earlier in di-

plosporous ovules than in sexual ones. These observations, performed using ovules from different plants and suggesting that megagametogenesis initiation is delayed by meiosis, were confirmed by the rare meiotic stages recorded in ovules from diplosporous accessions, all of which occurred within the meiosis time course observed for sexual accessions (Table 4).

Megagametogenesis in *Tripsacum* yields Polygonum-type, mature female gametophytes, regardless of reproductive development, and seems to take the same amount of time in both reproductive modes. Although FARQUHARSON (1955) reported precocious embryony in a tetraploid strain of *T. dactyloides*, precocious development of egg cells into young embryos neither occurred in ma-

ture unreduced female gametophytes reported in this study nor in LEBLANC *et al.*'s previous work (1995), indicating that fertilization of polar nuclei is required for parthenogenetic egg cell development into embryo and for seed production. Completely differentiated female gametophytes appear earlier in diplosporous materials than in sexuals.

#### DISCUSSION

The results reported here show a strong relationship between a type of reproductive development, i.e. apomixis vs sexuality, and a differential timing in the production of the mature female gametophyte that results from different megasporogenesis time courses. Factors that could cause this difference were not investigated, as the techniques used did not allow such a study, but several remarks may be made in this regard. Unreduced female gametophytes arise through 2 or 3 mitotic divisions (apospory or diplospory of the Antennaria type, respectively), whereas their reduced counterparts result from five (2 meiotic and 3 mitotic) divisions. The differing number of cell divisions, combined with time-consuming degeneration of the tetrad into a mitotically-dividing chalazal megaspore, may therefore bring about the meiosis delay we observed. Whatever the cause, the consequence of apomeiosis is the same in diplosporous *Tripsacum* as in aposporous *P. maximum*: mature female gametophytes are produced sooner during apomictic development than during sexual development, resulting in a gap between unreduced egg cell receptivity and the general floral development (i.e. pollen production, stigma receptivity).

Precocious embryony, where the development of egg cells into embryos precedes anthesis, has been reported in several pseudogamous apomicts, such as *Poa* (TINNEY 1940), *Parthenium* (ESAU 1946), and *Allium tuberosum* Rottl. ex Spreng. (KOJIMA & NAGATO 1992). The obvious consequence of this phenomenon is that, by the time the sperm cells reach the female gametophyte, egg cell fertilization is no longer possible, but endosperm initiation (i.e., fertilization of the polar nu-

clei) still takes place. In our material, as in *P. maximum* and *R. auricomus* (NOGLER 1984a), most embryos start to develop after endosperm initiation. This suggests that the gene(s) involved in the regulation of parthenogenesis is (are) expressed only after the polar nuclei are fertilized. The gap between egg cell receptivity and overall floral development is observed in a wide range of pseudogamous apomicts that do not show precocious embryony (both types of gametophytic apomixis, and in di- and monocotyledonous species), and therefore appears to be a fundamental component of apomixis expression in the species analyzed. Though this gap prevents egg cell fertilization, endosperm development can still be induced for normal seed growth. Whether the failure of fertilization relates to a mechanical barrier, such as a complete egg cell wall, or to a physical-chemical window of receptivity of the egg membrane, remains to be determined. However, the importance of the developmental gap that makes the egg cell unreceptive to fertilization has been astutely confirmed in *Paspalum notatum* (MARTÍNEZ *et al.* 1994), where artificial early pollinations resulted in the production of B<sub>III</sub> seeds (2n + n), whereas the same plant produced only apomictic (2n + 0) progenies when pollination was natural.

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- E. DUBIEL & W. ZIAJA: Contribution to the vascular plant flora of Sörkapp Land (Spitsbergen)
- D. TUMIDAJOWICZ & A. L. DAWIDOWICZ: Algorithm for determination of relative age of above-ground parts of *Rhododendron hirsutum* and *Rh. ferrugineum* (Ericaceae)
- A. NIENARTOWICZ & J. WILKOŃ-MICHALSKA: Numerical syntaxonomy of the Polish heliophilous plant communities
- R. CZAPIK: Chromosome numbers and lack of flowers in *Ornithogallum umbellatum* agg. (Liliaceae)
- W. MULENKO: *Gynerffylla oxalidis* – a new species of Hyphomycetes (Deuteromycetes) for the Polish fungal flora

