# Megasporogenesis in normal and a synaptic-mutant (*sy-2*) of *Solanum commersonii* Dun.

W. A. PARROTT

*Department of Agronomy, University of Georgia, Athens, GA 30602, U.S.A.*

AND

R. E. HANNEMAN, JR.

*U.S. Department of Agriculture, Agriculture Research Service, Department of Horticulture, University of Wisconsin, Madison, WI 53706, U.S.A.*

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Nomarski differential interference contrast microscopy was used to study megasporogenesis in intact ovules of *Solanum commersonii* Dun., following staining with Mayer's hemalum and clearing with methyl salicylate or cedarwood oil. Previous studies in *Solanum* have observed the *Polygonum* type of megasporogenesis, in which a linear tetrad of megaspores is formed. The three micropylar megaspores then degenerate, leaving the chalazal megaspore to divide mitotically to form the egg sac. Contrary to expectation, only 30% of the observed sporads within the same ovary were tetrads. Triads, including some with one deteriorating cell at the micropylar end, were the predominant form. Many dyads, which are an intermediate stage in megasporogenesis, were found with one cell prematurely deteriorating. These observations can be explained if the micropylar daughter cell of the dyad stage, which formed after telophase I, began deteriorating before the second meiotic division, such that it never underwent the second division. The chalazal daughter cell would still undergo the second meiotic division, followed by death of the new micropylar megaspore.

*Key words*: *Solanum*, potato, megasporogenesis, *S. commersonii*, ovule, clearing.

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La microscopie en interférence différentielle de Nomarski a été utilisée pour l'étude de la mégasporogenèse chez des ovules intacts de *Solanum commersonii* Dun., colorés par l'hémalum de Mayer et éclaircis par du salicylate de méthyle ou de l'huile de cèdre. Dans des études antérieures, le type *Polygonum* de la mégasporogenèse a été observé chez *Solanum*; ce type comporte la formation d'une tétrade linéaire de mégaspores. Trois mégaspores dégénèrent du côté micropylaire, alors que la quatrième subit des divisions mitotiques qui conduisent à la formation d'un sac embryonnaire. Contrairement à ce qu'on aurait pu s'attendre, seulement 30% des sporades observées à l'intérieur d'un même ovaire étaient des tétrades. La forme dominante fut celle des triades, dont certaines présentaient une cellule en voie de détérioration à l'extrémité micropylaire. Chez de nombreuses diades, un stade intermédiaire de la mégasporogenèse, l'une des cellules se détériorait de façon prématurée. Ces observations peuvent être expliquées. Si la cellule-fille micropylaire du stade diade, qui est formée après la télophase I, commence à se détériorer avant que survienne la division II, elle ne pourra donc pas participer à la seconde division; la cellule-fille du côté de la chalaze pourrait quand même subir la seconde division méiotique, suivie de la mort de la nouvelle mégaspore micropylaire.

*Mots clés* : *Solanum*, pomme de terre, mégasporogenèse, *S. commersonii*, ovule, éclaircissement.

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## Introduction

Megasporogenesis in *Solanum* is generally regarded as proceeding according to the *Polygonum* type, in which a linear tetrad of megaspores is the end product of meiosis. Jorgensen (1928) was the first to find and describe meiosis during megasporogenesis in the genus *Solanum*. Describing the process in a hybrid between *S. luteum* and *S. nigrum* L., he stated that after the first meiotic division "both nuclei may enter the homotypic division, or else only the chalazal (lower) does, giving rise to megaspores. The innermost of these become functional." The implication is that a tetrad of megaspores is formed in the first case, and a triad in the latter. Most later investigators have exclusively reported the formation of linear tetrads including: Bhaduri (1932) in *S. melongena* L.; Kruger (1932) in *S. nigrum* L., *S. tubingense*, and *S. proteus*; Rees-Leonard (1935) in *S. tuberosum* L. cv. Irish Cobbler; Lamm (1937) in cv. Hindenburg and cv. Up-to-date; Walker (1955) in *S. demissum* Lindl.; and Stelly et al. (1984) in hybrids between *S. tuberosum* Group Tuberosum (2*x* haploids) and Group Phureja.

Arnason (1943) found exclusive tetrad formation in cv. Earlaine and cv. USDA 46000, but said of cv. Netted Gem that, "in a few ovules, only one of the nuclei formed at the end of the first meiotic division divided; in these, three instead of four cells were present at the end of meiosis." Iwanaga and Peloquin (1979), studying megasporogenesis in normal and

FIG. 1. Megasporogenesis in *S. commersonii* Dun. The micropylar end is oriented towards the top. All figures were photographed under Nomarski differential interference contrast microscopy, except for Fig. 1*d*, which was photographed under Kohler bright field microscopy. (*a*) Pachytene in a megaspore mother cell of a synaptic mutant. Note the two nucleoli. (*b*) Metaphase I in a megaspore mother cell of a normal plant. (*c*) Metaphase I in a megaspore mother cell of a synaptic mutant. The spindle is elongated and the chromosomes (arrows) are scattered along the length of the spindle. (*d*) Two-cell stage of megasporogenesis. The micropylar cell is deteriorating. (*e*) Triad of megaspores. Micropylar cells are in varying stages of degeneration. (*f*) Tetrads of megaspores.

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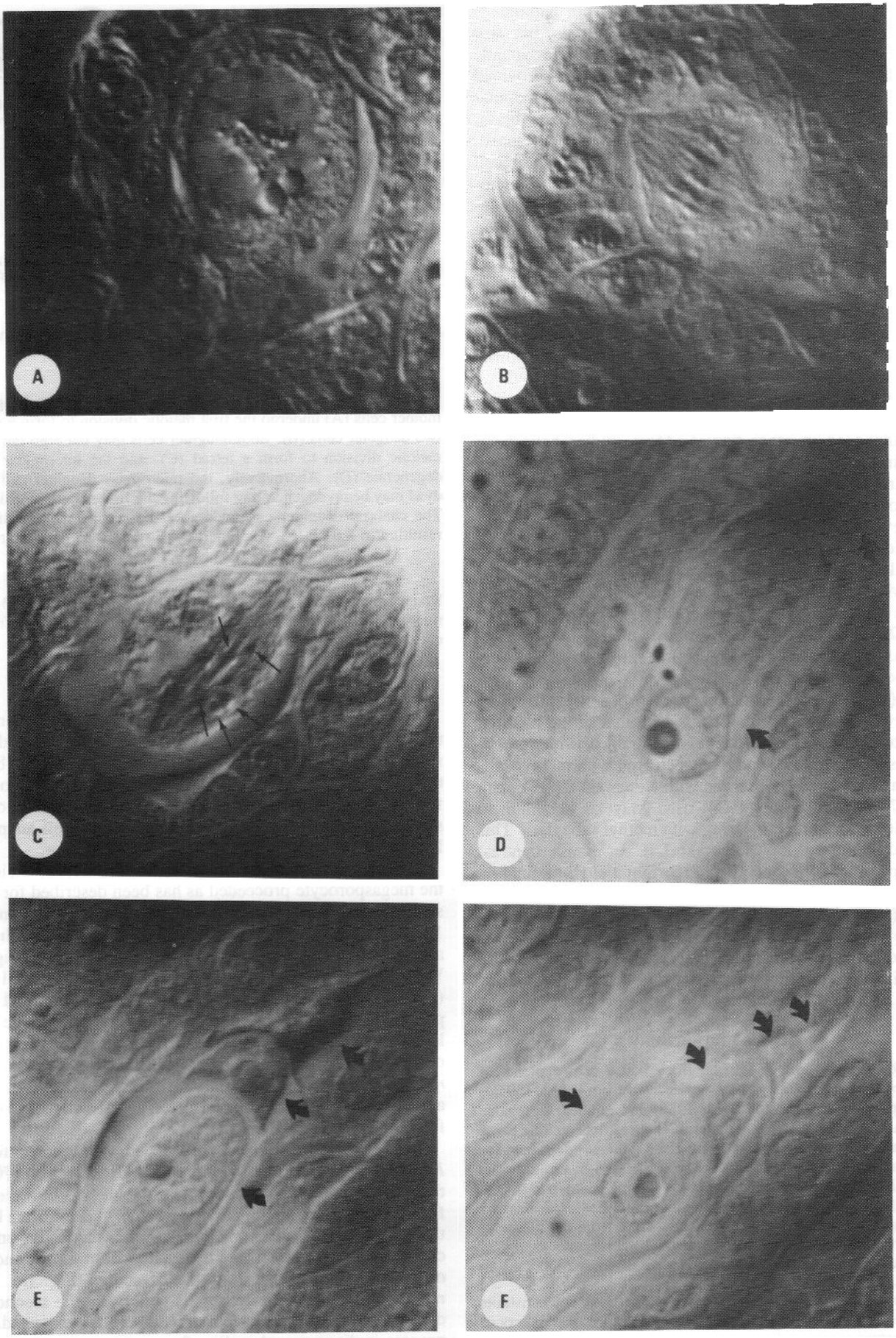
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synaptic-mutant plants derived from Tuberosum haploid Phureja hybrids reported the formation of tetrads, but also found one triad that was attributed to the effect of the synaptic mutation (*sy-1*) on cytokinesis. Jongedijk (1986) has provided the most recent account of megasporogenesis, using a population derived from Tuberosum haploid – Phureja hybrids. While tetrads were the most common result of megasporogenesis, he also stated that, "the micropylar daughter cell which is formed after completion of the first division of meiosis was often found to degenerate before the onset or completion of the second division, giving rise to a triad instead of a tetrad of megaspores." Jongedijk (1986) considered this latter event too frequent to be considered an abnormality, but still considered it to be a random event. Stelly and Peloquin (1986) found triad formation associated with formation of 2*n* megaspores. These could be identified as such because doubling the genetic material in the nucleus (and hence the nuclear volume) resulted in increased nuclear and nucleolar diameters.

The study of megasporogenesis has traditionally been hindered by the difficulties inherent in observing cells that are deeply embedded in other tissues. Most studies have been largely limited to paraffin or resin-embedded sections that both destroy the three-dimensional perspective and limit the number of ovules that can be reasonably examined. Herr (1971) developed a complex clearing fluid consisting of 85% lactic acid, chloral hydrate, phenol, clove oil, and xylene (2:2:2:2:1 w/w) that permitted the examination of megasporogenesis without prior sectioning of tissues, thus greatly facilitating the process (Smith 1973; Rembert 1977; Greene 1978; Herr 1982). The use of cleared, unstained ovules does necessitate the use of phase contrast or Nomarski differential interference contrast (DIC) optics.

Crane (1978) simplified the clearing process by matching the refractive index of the cell walls with that of the medium by substituting methyl salicylate (synthetic oil of wintergreen) for Herr's clearing fluid, and ovules cleared with methyl salicylate can be adequately observed with Nomarski DIC (Young et al. 1979). Stelly et al. (1984) used Mayer's hemalum to stain ovules prior to their clearing with methyl salicylate. This eliminated the need for Nomarski DIC or phase contrast microscopy, requiring only Kohler bright field illumination for adequate observation.

Green (1978) had previously stated the need for a staining technique compatible with Nomarski microscopy. It has been possible to adapt ovule staining (Stelly et al. 1984) for use with Nomarski optics, resulting in an easy and flexible method for the study of megasporogenesis.

## Materials and methods

Flower buds were collected from green house grown plants of *S. commersonii* Dun. These plants were derived from P.I. 243503 as described previously (Johnston et al. 1986) and inluded segregants homozygous for the *sy-2* allele, a mutation that restricts meiotic chromosome pairing. The flower buds were fixed, stained, destained for contrast, and cleared according to Stelly et al. (1984), omitting the xylene infiltration series and the ethanol – methyl salicylate series. Cedarwood oil was substituted for methyl salicylate as a clearing agent and gave excellent results. After dissection from the placenta, ovules were observed with a Zeiss Universal microscope[1] equipped with Nomarski DIC optics. Photographs were taken at 2000× using Kodachrome 25-mm film, a daylight filter, and a Zeiss MC63 Photomicrographic camera. Black and white prints were obtained from the color slides using Kodak Plus-X 70-mm film.

[1]Reference to a specific brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature mentioned.

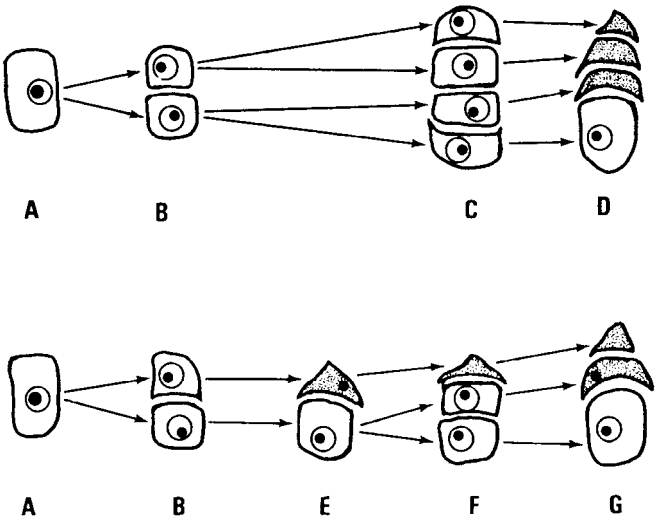


FIG. 2. Megasporogenesis in *S. commersonii* Dun. Megaspore mother cells (A) undergo the first meiotic division to form a dyad of two daughter cells (B). Both daughter cells may undergo the second meiotic division to form a tetrad (C), and the micropylar spores degenerate (D). Alternatively, the micropylar daughter cell of the dyad may begin degenerating following the first meiotic division (E). The chalazal daughter cell undergoes the second meiotic division, resulting in a triad (F). The micropylar spore then degenerates (G).

## Results and discussion

Ovules that had been sufficiently destained for observation under brightfield microscopy were too densely stained to be suitable under Nomarski DIC microscopy and had to be further destained to a point that would not be considered optimal for brightfield microscopy. With this minor modification, the staining technique of Stelly et al. (1984) is well adapted to Nomarski DIC, forming a powerful combination.

Plants homozygous for the *sy-2* allele are sterile. Meiosis in the megasporocyte proceeded as has been described for meiosis in the microsporocytes of the same plants (Johnston et al. 1986). Two nucleoli were usually observed during pachytene, an indication that the SAT-homologues were unpaired (Fig. 1*a*). Whereas normal plants had a defined metaphase I plate (Fig. 1*b*), the synaptic-mutant plants lacked a defined metaphase plate and chromosomes were dispersed along the entire length of the spindle (Fig. 1*c*). This agrees with previous observations of megasporogenesis in synaptic-mutants of *Solanum* (Iwanaga and Peloquin 1979; Stelly et al. 1984). Death and collapse of megaspores occurred shortly after cytokinesis following telophase II.

Meiosis in normal plants proceeded as expected for the *Polygonum* type of megasporogenesis. However, in 70% of the ovules observed, the micropylar daughter cell of the dyad formed by the first meiotic division began to deteriorate before the second meiotic division (Fig. 1*d*). Consequently, only the chalazal daughter cell of the dyad underwent the second meiotic division, forming a triad of megaspores (Fig. 1*e*). In the remaining ovules, both dyad cells underwent the second meiotic division and formed tetrads (Fig. 1*f*) as expected. This process is diagrammed in Fig. 2.

The frequency of triad formation was too high to be considered a random event, and was probably under genetic control. It was easy to observe how triad formation could be derived from the standard *Polygonum* type of megasporogenesis. Furthermore, megagametophytes formed from the functional megaspore of a triad were still monosporic in nature, being no different from megagametophytes derived from a tetrad. The premature death of the micropylar daughter cell suggests that the chalazal end of the megaspore must be predetermined to become the functional megaspore at an early stage.

Triads have been associated with 2*n* gamete formation (Stelly and Peloquin 1986). The 2*n* gametes occur when a cell from the dyad stage fails to undergo the second meiotic division and survives to become a megaspore. They can be distinguished by their doubled nuclear and nucleolar volumes. This is distinctly different from the phenomenon observed in these *S. commersonii* plants, where any cell from a dyad stage that failed to undergo the second meiotic division did so because of cell death and never survived to form a functional megaspore. To further verify the ploidy of the megaspores in the triads, nuclear and nucleolar diameters of the functional megaspores were measured. None had a diameter sufficiently large to suggest that polyploidy (i.e., formation of 2*n* gametes) was occurring.

Triad formation in *Solanum*, as suggested by the literature and this study, is probably not uncommon. The use of cleared whole ovules facilitated the detection of this phenomenon, as it is possible to quickly focus through various planes in an unsectioned ovule, permitting the observation of intact megaspores. The use of ovule staining, clearing, and Nomarski DIC optics provides a simple and flexible technique that should prove useful for further studies on the reproductive biology of angiosperms.

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