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Role of the Generative Cell in Androgenesis in Henbane

Abstract. *When anthers of henbane containing uninucleate pollen grains were cultured, a large number of embryoids originated exclusively from the division of the generative cell. In a small proportion of pollen grains, both generative and vegetative cells contributed to embryoid formation. Embryogenesis by segmentation of the vegetative cell alone was rarely observed.*

When immature anthers of certain angiosperms are cultured at an appropriate stage of development in a defined nutrient medium, the pollen (*1*) may form sporophytic plants with the haploid number of chromosomes. Cell divisions are initiated in a certain proportion of the pollen grains contained in excised and cultured anthers, leading to the formation of embryoids. The latter develop through stages reminiscent of those of true zygotic embryos before they emerge as plantlets outside the anther wall. This phenomenon, known as androgenesis, has been reported to occur readily in plants belonging to Solanaceae (*2*).

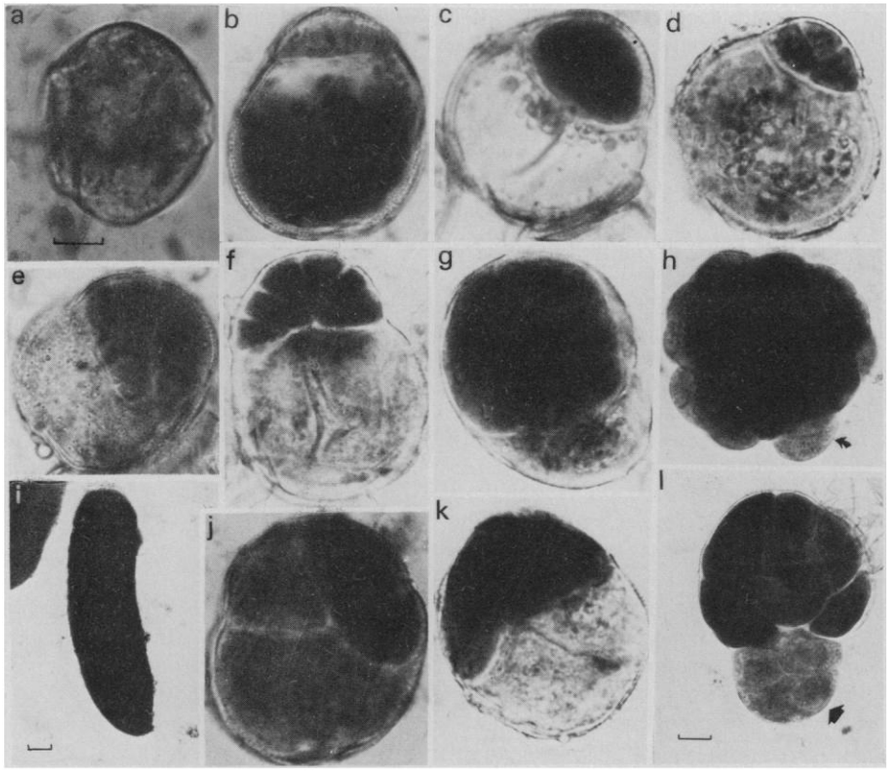

Fig. 1. (a) Uninucleate pollen grain of henbane 24 hours after culture; the nucleus is in division. (b) Large densely stained vegetative cell and a small generative cell with granular nucleus, following the first pollen mitosis. The nucleus of the vegetative cell is not seen; 72 hours after culture. (c) Contrasting staining patterns of the nonembryogenic vegetative cell and the embryogenic generative cell; 72 hours after culture. (d) Division of the generative cell in the embryogenic pathway, forming a quadrant; 96 hours after culture, (e and f) Planes of division of the generative cell to form embryoids. The large vegetative cell has not divided; 144 hours after culture. (g) A globular embryoid originating from the generative cell, still enclosed within the exine. The basal cell is the partially crushed vegetative cell; 168 hours after culture. (h) A later stage of globular embryoid, after release from the exine. Arrow points to the vegetative cell, which persists as a suspensor-like organ; 168 hours after culture. (i) A torpedo-shaped embryoid. No trace of the suspensor is seen; 216 hours after culture. (j) Division of the vegetative and generative cells; 72 hours after culture. (k) An embryoid originating from the generative cell. The vegetative cell has divided once; 120 hours after culture. (l) A globular embryoid originating from the generative cell. The vegetative cell has divided to form a multicellular suspensor-like organ (arrow); 192 hours after culture. The scale (0.1 mm) in (a) refers to (a) to (e), (j), and (k); the scale (0.1 mm) in (l) refers to (f), (g), (h), and (l); the scale in (i) is 0.1 mm.

Several workers (*3*) have established that in cultured anthers, following the characteristic asymmetric pollen mitosis, embryoid formation results from the activity of the large vegetative cell, while the small generative cell gradually degenerates. Not uncommonly, the pollen grain might form two equal cells after the first mitosis, and both cells contribute to the formation of the embryoid (*4–6*). Still a third pathway, which involves fusion between the vegetative and generative cells and their subsequent division in a complex manner, has also been described (*6*). These results indicate that the generative cell has practically no role or only a limited role in androgenesis. This is surprising, since in the normal ontogeny of the male gametophyte the generative cell gives rise to gametes while the nucleus of the vegetative cell degenerates or survives as a vestigial structure in the pollen tube (*7*). Observations presented in this report show that in anther cultures of henbane (*Hyoscyamus niger*, annual variety), continued division of the generative cell alone following the first pollen mitosis may account for a significant proportion of the embryoids formed. Details of culture conditions for obtaining optimum yield of embryoids from anthers of henbane have been described elsewhere (*8*).

Anthers cultured at the uninucleate pollen stage were collected at intervals of 24 hours for 10 days and examined by squashing in acetocarmine. Pollen grains at the late uninucleate stage or in the process of division at the time of culture were generally binucleate by 24 to 48 hours after culture. At this time it was possible to separate a group of densely staining, slightly enlarged, potentially embryogenic pollen from the rest of the population. Observations of anthers on subsequent days showed that many potentially embryogenic pollen grains had become abnormally enlarged and full of starch. The starch-filled grains as well as the majority of the binucleate pollen that stained weakly with acetocarmine were nonembryogenic.

Division of the pollen grain in the embryogenic pathway was observed as early as 48 to 72 hours after culture. After the first pollen mitosis, wall formation occurred, delimiting a small generative cell with granular nucleus and a large vegetative cell with diffuse nucleus (Fig. 1, a and b). The cytoplasm of the large cell also stained intensely with acetocarmine, as it does in a typical vegetative cell (*9*). As Fig. 1c illustrates, the staining reaction of the vegetative cell was transient and it dis-

appeared shortly after the segmentation of the pollen grain. This disappearance was followed by the deposition of starch in the vegetative cell and the acquisition of staining by the cytoplasm of the generative cell. After it was cut off, the generative cell lost its morphogenetic individuation and underwent two internal divisions without intervening growth to form a group of four cells of typical somatic cell size (Fig. 1d). At this stage the embryoid appeared as a compact mass of densely staining cells subtended at the base by a large, relatively colorless cell. Variable planes of division in the cells of the quadrant were observed to give rise to multicellular embryoids (Fig. 1, e and f). Further growth resulted in the rupture of the exine and formation of typical globular and heart-shaped embryoids in which all the cells were derived from the generative cell. The vegetative cell appeared as a colorless, undivided cell at the proximal end of the embryoid giving the effect of a suspensor (Fig. 1, g and h). Globular and heart-shaped embryoids were found in great numbers in anthers sampled 144 to 168 hours after culture. Although bipolar embryoids at more advanced stages were also observed in later collections of anthers (192 to 240 hours after culture), it was not possible to confirm that they originated from the generative cell, since whatever remained of the original vegetative cell was crushed by the growing embryoid (Fig. 1i). No early-division-phase embryoids that seemed to have originated from the generative cell were found in these anthers, however. This indicates that such embryoids would have proceeded to form typical bipolar embryoids and rules out the possibility of a developmental arrest or degeneration of embryoids that originated from the generative cell.

In a slight variation of the segmentation pattern described above, after the generative cell had formed an embryoid of two or more cells, the vegetative cell divided several times. The cells originating from the vegetative cell stained weakly with acetocarmine and formed a multicellular, suspensor-like structure in the globular embryoid, which as before was formed entirely by the division of the generative cell (Fig. 1, j to l). The connection of the suspensor to the embryoid appeared relatively weak and no late stage embryoids with attached suspensors were observed. About 50 percent of a total of 230 embryoids counted in randomly selected anthers in a single experiment developed from the generative cell in one of the two ways described above (Table 1).

In a small percentage of the densely staining embryogenic pollen the first mitosis was not followed by wall formation.

Table 1. Number of different types of embryoids formed in anther cultures of henbane. Pollen grains that had undergone one or more divisions in the embryogenic pathway as described in the text, as well as all embryoids whose origin could be assigned to one of the segmentation patterns, were counted. (A) Generative cell forms embryoid; vegetative cell does not divide. (B) Generative cell forms embryoid; vegetative cell forms a multicellular suspensor. (C) Both generative and vegetative cells contribute to embryoid formation. (D) Vegetative cell forms embryoid; generative cell does not divide. Unclassified types refer to multicellular embryoids whose origin could not be determined.

Anther (No.)	Embryoids (No.)	Embryoids (No.) assigned to segmentation pattern				Unclassified types (No.)
		A	B	C	D	
1	23	6	5	4	1	7
2	16	8	2	1	0	5
3	48	8	13	1	2	24
4	36	12	2	6	1	15
5	13	2	4	4	0	3
6	30	12	3	8	0	7
7	16	6	2	2	1	5
8	22	8	5	2	0	7
9	26	11	2	0	0	13
Total	230	73	38	28	5	86

The nuclei formed were morphologically different, one being more granular and densely staining (generative nucleus) than the other (vegetative nucleus). Further nuclear divisions were accompanied by wall formation and cells derived from both generative and vegetative nuclei contributed to the formation of embryoids. Occasionally, the vegetative cell formed after an asymmetric mitosis was also found to divide and form an embryoid in which the generative cell could be recognized as a nondividing entity. This pathway accounts for only a very small proportion of the total number of embryoids formed in henbane anthers, however (Table 1).

These results indicate that, contrary to the well-documented reports on other plants (*2, 3*), the generative cell plays a predominant role in androgenesis in henbane. In a report of work on anther cultures of *Nicotiana tabacum*, Devreux *et al.* (*10*) mention the occurrence of one albino plant, presumed to have originated from the generative cell. The basis for this claim is genetical and neither morphological description nor figures are given in support. In *Nicotiana sylvestris*, Rashid and Street (*5*) traced the segmentation pattern in a few pollen grains up to the stage of formation of several free nuclei from the generative nucleus, but no later stages were observed to determine whether this is a pathway in androgenesis. As far as I am aware, this is the first documented report in which a large number of embryoids, which constitute a significant proportion of the total yield from an anther, are shown to originate exclusively from the generative cell of the pollen grain.

The results provide proof of the totipotency of yet another specialized cell of the angiosperm. Since the generative cell is already programmed for DNA synthesis in preparation for the formation of gametes, the rapidity with which embryogenesis is initiated in henbane pollen is probably related to the passage of the generative cell into a mitotic state with relative ease. This contrasts with the long induction period (12 to 14 days) required for the initiation of embryogenic division in the vegetative cell, where the DNA synthetic machinery is normally shut off and a program for nuclear disintegration is also very likely set in motion. Some evidence has been presented (*11*) that suggests that during the long induction period a new pattern of cytoplasmic organization necessary to return the vegetative cell to a mitotic condition is established. Further work is necessary to determine how information transfer mechanisms of the vegetative and generative cells, normally conditioned for terminal differentiation, are channeled into pathways resulting in renewed cell divisions and growth.

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