Teliospore Germination and Nuclear Behavior of *Zundelula thirumalacharii*

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Abstract

Meiotic nuclear division in the germinating teliospores of *Zundelula thirumalacharii* Pavgi et Giri was followed by emergence of the promycelium. The first phase of the division appeared equational and the second segregational, while development and maturation of sporidia proceeded in a basipetal sequence. Fusion between 2 compatible sporidia apparently occurred after their discharge.

Zundelula thirumalacharii Pavgi et Giri causing an ovariicolous smut disease of a marshy weed *Scirpus supinus* L. was collected and described from Varanasi (Pavgi and Giri 1966). The granulated glomeruli-like spore balls showed layer(s) of sterile cells surrounding an inner core of fertile spores. Their mode of germination and nuclear behavior in the process were studied in sequence; the observations on are presented here.

Materials and Methods

Mature sori of *Zundelula thirumalacharii* Pavgi et Giri in the infected ovaries of *Scirpus supinus* L. were collected, air-dried and stored at room temperature (22—24° C). Spore balls from an unruptured sorus were separated and teliospores teased out of them. The teliospores were adhesized to slides by alternate wetting and drying and induced to germinate by the method described by Thirumalachar et al. (1950, 1953). A dip in acidulated water or "Surfactol — 100" (Rohm & Haas Co. Inc., Philadelphia, Pa., U. S. A.; 1 drop in 50 ml dist. water, 5 min.) considerably enhanced their germination count. Germinating teliospores were killed at the desired stage and fixed in Carnoy's fluid or weak chrom-acetic solution for 1 and 24 hr respectively. The slides were washed in dist. water (15 min) and dipped in a mordant (freshly prepared 1.5% iron alum. soln., 5 min), washed in running water and in dist. water (15 min each). The spore mounts from Carnoy's fluid were directly stained in 0.5% aceto-carmin. Some slides fixed in weak chrom-acetic soln. were stained with Heidenhain's hematoxylin, washed in running warer (5 min) and destained in saturated picric acid soln. for a few seconds, excess stain washed, dehydrated in alcohol series (5 min each) and later through alcohol and alcohol: xylol grades and mounted in canada balsam (Johansen 1940).

Observations

Teliospores from the air-dried sori as well as from freshly harvested sori of *Zundelula thirumalacharii* germinated well but with a higher percent germination in the former lot. The teliospores imbibed water and the germination process initiated after 12 hrs at 25° C. The diploid fusion nucleus divided into 2 nuclei during the first phase of the meiotic nuclear division occurring within the teliospore and the exosporium cracked open with the emergence of a stout promycelium (Fig. 1). Usually one but occasionally 2 promycelia emerged out at 2 random sites in the teliospore indicating absence of predetermined germ points in the exosporium wall (Figs. 2, 10). The openings in the exosporium, however, appeared located in the direction of the metaphase plate (Figs. 2, 3). The 2 nuclei mitotically divided completing the second phase of the meiotic division within the teliospore, producing 4 haploid nuclei. The nuclei invariably became associated in 2 (compatible) pairs, concurrent with gradual elongation of the promycelium (Fig. 3). The spindle axes of the 2 nuclei in the mitotic metaphase were oriented parallel to each other and not in axial line as in the promycelium. The paired nuclei reoriented in the dense cytoplasm and linearly moved into the young promycelium (Fig. 4). Their orientation was infrequently asynchronous; either pair became delayed and the lower pair awaited oriented movement of the upper one (Fig. 5). Association of the 4 haploid nuclei in compatible pairs indicated the first phase equational and the second segregational in the sequence of meiosis.

Vacuolation appeared in the lower half of the teliospore with migration of the haploid paired nuclei and cytoplasm into the elongating promycelium (Fig. 6). Most of the cytoplasm with the paired nuclei moved into the promycelium leaving the teliospore empty (Fig. 7). The promycelium elongated and a thin septum was laid across dividing the promycelium into 2 equal cells separating the 2 nuclear pairs, as soon as the first pair reached the upper half of the promycelium (Fig. 8). The upper cell divided into 2 by a septum enclosing a single nucleus in each cell (Fig. 9). The nuclei in the lower pair moved a little apart and a septum separated them. The haploid nucleus in the basal cell slipped back into the near-vacuolate teliospore and divided mitotically. This nuclear division occasionally stimulated emergence of another (secondary) promycelium in vicinity of the primary one into which a daughter nucleus moved in, while the other moved to the basal promycelial cell. The secondary promycelium remained dwarfed and underdeveloped and eventually aborted in the absence of a compatible nucleus (Fig. 10). Normally a fully developed promycelium became 4-celled, with each cell containing a single haploid nucleus and the basal cell containing the haploid nucleus located in the center. The teliospore cell became empty but the exosporium wall did not crumple

or collapse in any preparation except after processing through the alcohol series (Fig. 11). Each promycelial cell gave rise to small sporidial initials, budding out laterally just below the cross septa, the terminal cell developing the sporidial initial apically (Fig. 12). The cytoplasm moved into the young sporidia, enlarging them in size. The single haploid nucleus also moved towards each sporidium with rest of the cytoplasm in basipetal succession (Fig. 13). As the sporidia further enlarged, the nucleus with rest of the cytoplasm moved into each sporidium leaving the promycelial cell empty and vacuolate. Maturation of the sporidia initiated from the distal rounded end and the short rudimentary sterigmata gradually reduced in length (Fig. 14). Movement of the cytoplasm and nuclei in the sporidia and their maturation also proceeded basipetally. Mature sporidia were violently discharged in the same order of their maturation on the promycelium. They were hyaline, uninucleate, pyriform to obovate, haploid, measuring 3.5—6.5 × 2—3.2 μ (Fig. 15). No fusion between the primary sporidia was noticed in slide cultures, which obviously occurred in nature after their expulsion.

Discussion

The process of teliospore germination in *Zundelula thirumalacharii* appeared normal in the sequence of events characteristic for a species of fam. Ustilaginaceae. The meiotic nuclear division of the diploid nucleus and behavior of the haploid nuclei, however, presented several interesting features during the process. The entire process of meiotic division was completed in the activated teliospore. The site of meiosis has been the promycelium almost uniformly in the members of Ustilaginaceae with the sole exception of *Ustilago hordei* (Persoon) Lagerheim, in which the first phase of meiosis is completed in the activated teliospore and the second phase in the promycelium (Fischer and Holton 1957, Paravicini 1917). The present species adds another

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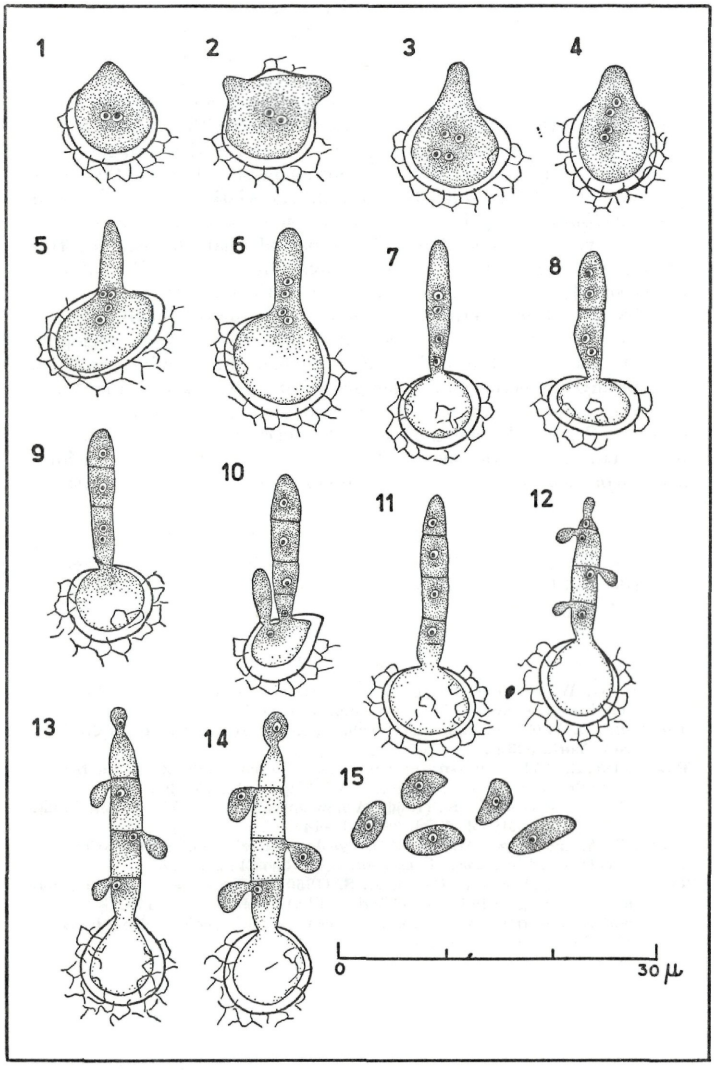
Figs. 1—15. Teliospore germination and nuclear behavior of *Zundelula thirumalacharii*. 1. Two daughter nuclei after first phase of meiotic division of the diploid nucleus followed by emergence of a promycelium. 2. Two promycelia emerging from a teliospore. 3. Promycelium elongates with completion of meiotic nuclear division. 4. Four haploid nuclei moving in pairs into the promycelium. 5. Two nuclei moving into the promycelium followed by the other 2 nuclei. 6, 7. Nuclei moving into the promycelium in compatible pairs. 8. Septation of promycelium separating the nuclear pairs. 9, 10. Septation of the upper and lower promycelial cells separating the 4 nuclei; a haploid daughter nucleus at base of the younger promycelium. 11. Uninucleate 4-celled promycelium. 12. Three lateral and one terminal sporidial buds from each uninucleate parental cell. 13. Haploid, single nuclei moving into sporidia from the top cell downward. 14. Three vacant parental cells each bearing a uninucleate sporidium. 15. Detached uninucleate, mature sporidia.



variation similar to those in the Tilletiaceae (FISCHER and HOLTON 1957, SINGH and PAVGI 1972). The first phase of the meiotic division appeared concurrent with the emergence of promycelium. The pregermination metabolic activation in the teliospore and the meiotic division of the diploid nucleus appeared to be closely associated and interdependent processes and thus were simultaneously initiated within the teliospore.

The cross septa in the promycelium were laid down in the same sequence as the meiotic division, although septation in the lower promycelial cell was slightly delayed. Association of the haploid nuclei in compatible pairs within the teliospore and their migration in the promycelium in similar pairs appeared characteristic for this species. These are rather atypical features of nuclear behavior hitherto not reported in any species in the family Ustilaginaceae.

Absence of predetermined site(s) in the exosporium wall for emergence of the promycelium appeared general despite the enveloping dermata traversing the fertile cells in glomeruli-like teliospore balls. Two promycelia occasionally emerged during germination (Fig. 2), in which a compatible nuclear pair moved into each after the meiotic division. Further development of such a germination type could not be traced. No aberrations in the mode of germination or meiotic division or nuclear movement were observed in any of the preparations.

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