Teliospore Germination, Cytology and Development of *Entyloma eleocharidis*

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Introduction

Entyloma eleocharidis Pavgi and Singh, parasitic on *Eleocharis plantaginea* R. Br., is widespread in Uttar Pradesh. The nonerumpent sori abundantly develop on the stem as small oval to circular, slightly raised, dull black, rarely coalescent spots surrounded by yellowish discoloration in the tissues. The teliospores are loosely aggregated in the mesophyllar interspaces and are released free on teasing the sorus. The teliospores are light reddish brown, oval to globoid with a smooth epispore (Pavgi and Singh 1968). Relatively few species of the genus *Entyloma* De Bary have so far been ontogenically studied in detail (Das 1949, Kaiser 1936, Lutman 1910, Paravicini 1917, Raciborski 1897, Thirumalachar and Dickson 1949). Typical life cycle patterns and nuclear behaviour in the genus are represented by *E. calendulae* (Oud.) De Bary, *E. nymphaeae* (Cunningham) Setchell and *E. scirpicola* Thirumalachar and Dickson (Fischer and Holton 1957). Observations on germination, cytology and development of teliospores in *E. eleocharidis* are presented here.

Materials and methods

Infected stems bearing well-developed sori were air-dried and stored under low temperature. Mature teliospores from these were induced to germinate on slide mounts at 26–30°C by the methods suggested by Thirumalachar *et al.* (1950, 1953). Germinating teliospores were killed and fixed *in situ* at different stages in modified Carnoy's fluid (30 ml 97% ethyl alcohol : 10 ml chloroform : 10 ml glacial acetic acid) for 4 to 6 hr., brought down to water through serial alcohol dilution grades, treated in Taka Diastase (Parke Davis (India) Ltd., Bombay) soln. (1 tablet in 50 ml dist. water) for 10 min. and mordanted in 0.5% ferric ammonium sulphate 'AnalaR' (iron alum) soln. for 10 min. Excess iron alum was gently washed in running water for 5 min. The slides were rinsed in dist. water, upgraded to 70% alcohol through the alcohol series and stained in Belling's 0.5% aceto-carmin. Germinating teliospores were also killed and fixed in Randolph's modified Navashin soln. (Johansen 1940) for 12 to 14 hr. and stained in Heidenhain's iron hematoxylin for complementary observations. Developmental cytology of the fungus in the host was studied

by killing and fixing sori on infected stems at different stages in Randolph's modified Navashin soln. for 24 hr. and embedded in paraffin (Johansen 1940). Serial sections (6–10 μ) were microtomed and stained with Heidenhain's hematoxylin and counterstained with orange G, where necessary.

Observations

Teliospore germination and meiotic nuclear division

Fresh teliospores did not germinate at all. They were induced to germinate during July–August from collections made in the previous season. The teliospores covered by a film of water absorb moisture, swell and the contents become granular and lighter in color. One or 2 big oil globules in the protoplasm gradually disappear and a deeply stained diploid fusion nucleus becomes evident in the centre (Fig. 1). The fusion nucleus slightly increases in diam. (Fig. 2). Simultaneously a small papillate, hyaline projection emerges from the spore elongating into the promycelium (Fig. 3). The nucleus gradually moves from the centre towards the opening in the exospore and passes into the promycelium without changing its shape, unlike in *Entyloma thirumalachari* Pavgi and Singh (Singh 1968), since the promycelium distends the spore wall wide open (Fig. 4). The nucleus reaches approximately in the middle or slightly towards the tip. Concurrently, cytoplasm in the spore becomes vacuolate or rarely the endospore shrinks along with the cytoplasm, till it reaches the opening of the teliospore. The nucleus enlarges in size, showing the chromatin reticulum and a conspicuous nucleolus entering the interphase and measures 3 to 3.7 μ in diam. (Fig. 5). It continues to enlarge with a prominent nucleolus and nuclear wall entering the prophase. Leptotene and zygotene stages could not be seen in any of the preparations; possibly these merge with late interphase and early pachytene respectively in the continuum. Five elongated paired chromosomes, faintly stained nucleolus and the nuclear wall are seen representing pachytene in the sequence (Fig. 6). The nucleolus and nuclear wall disappear and the chromosomes shorten slightly showing chiasmata at places throughout their entire length representing diplotene (Fig. 7). The position of centromere could not be located in any chromosome. The chromosomes continue to shorten becoming more conspicuous and gradually move to the equatorial plate in metaphase I (Figs. 8, 9). The nuclear spindle generally remains parallel to the axis of the promycelium. The chromosomes remain oriented in different planes on the plate, but are easily countable. The haploid chromosome complement is counted as 5 ($n=5$) (Figs. 8, 9). The chromatids of each chromosome bivalent separate and move poleward during anaphase I. The movement of chromatids is not

* phase I. 10, anaphase I. 11, late anaphase or early telophase I. 12, two daughter nuclei moved apart. 13, anaphase II. 14–17, differentiation of a sporidial whorl and migration of a haploid nucleus in each sporidium. 18–20, different types of conjugation between compatible sporidia *in situ*. 21, dikaryotic secondary sporidia and their germination.

* Figs. 1-21. Meiotic nuclear division and nuclear behaviour during teliospore germination and sporidial development of *Entyloma eleocharidis*. 1-2, teliospores each with a resting diploid nucleus. 3-4, teliospore germination and nuclear migration in the promycelium. 5, interphasic nucleus. 6, pachytene of the prophase. 7, diplotene of the prophase. 8-9, meta-

synchronous (Fig. 10) and the chromatids of a chromosome are not distinguishable due to their small and approximately uniform size. The chromosomes aggregate and lose their identity at both the poles entering telophase I (Fig. 11). The 2 daughter nuclei (Fig. 12) apparently pass through interphase and prophase II and later metaphase II, as these stages have not been observed. Simultaneous occurrence of anaphase II indicates that both the daughter nuclei well coincide in their divisional stages (Fig. 13).

The site of nuclear division during germination in *Entyloma* species differs from species to species. Both the nuclear divisions in *E. microsporum* (Unger) Schröter occur in the teliospore (Das 1949), while the first division occurs in the teliospore and subsequent 2 divisions in the promycelium of *E. scirpicola* (Thirumalachar and Dickson 1949). In the remaining species, it occurs in the promycelium. Wang (1934) reported a diploid number of chromosomes as 4 ($2n=4$) for *E. dahliae* Sydow and the same number has been reported for *E. microsporum* (Das 1949). The number of chromosomes in the haploid complement here is 5 ($n=5$) and the first division is observed reductional followed by an equational one.

Development and cytology of sporidia

Concurrently with the second nuclear division, 2 to 8 tiny papillate projections differentiate at the tip of the promycellium and elongate into a whorl of hyaline, subcylindrical sporidia (Figs. 14 to 18). The 4 nuclei enter the sporidia or undergo a mitotic division in the promycelium increasing the nuclear number to 8. A single nucleus moves in each sporidium and is located approximately in the middle (Figs. 16, 17). Supernumerary nuclei remain in the promycelium (Fig. 18). The nuclear movement is similar to that in *E. thirumalachari* (Singh 1968) and *E. calendulae* (Kaiser 1936) until this stage. Compatible sporidia conjugate *in situ* at maturity. A sporidium sends out a conjugation tube from the base, rarely in the middle, which approaches a compatible sporidium in the whorl. The intervening walls dissolve at the point of contact and the nucleus from one sporidium migrates into the other through the conjugation tube (Figs. 18, 19). Such basal fusion of 2 compatible sporidia through conjugation tube *in situ* has been reported in *E. scirpicola*, parasitic on another host genus of the fam. Cyperaceae (Thirumalachar and Dickson 1949) and in *Urocystis occulta* (Wallroth) Raben. (Stakman *et al.* 1934). Occasionally a conjugation tube is formed between the promycelium and a sporidium and a compatible (supernumerary) nucleus from the promycellium moves into the sporidium through the conjugation tube (Fig. 19). Rarely 2 conjugation tubes are formed between 2 sporidia possessing 4 nuclei each (Fig. 20). The recipient sporidium elongates after conjugation and migration of the other nucleus in it and cuts off several crops of dikaryotic secondary sporidia, until the entire cytoplasm is exhausted. The 2 nuclei in a conjugated sporidium undergo a conjugate division and a pair of nuclei passes into each secondary sporidium. The dikaryotic secondary

sporidia are ellipsoid to falcate, which elongate and send out infection hyphae initiating fresh infections (Fig. 21).

Aberrations in nuclear behaviour

Some interesting variations in the nuclear behaviour were observed during the study of germination cytology, which are broadly grouped into 6 patterns.

1) The meiotic division of the fusion nucleus is completely inhibited even after its migration in the promycelium and differentiation of the sporidia (Fig. 22).

2) Often less than 4 sporidia are formed, while the nuclear number in promycelium becomes 4 after meiosis. In such a case, one nucleus migrates in each sporidium, while the supernumerary remain in the promycelium (Figs. 23, 24). Such supernumerary nuclei in the promycelium have been reported in *E. scirpicola* (Thirumalachar and Dickson 1949) and *E. thirumalachari* (Singh 1968) due to their excessive number formed by mitotic divisions in these species, whereas in the present species, it is due to lesser number of sporidia than the daughter nuclei after meiosis.

3) Infrequently branching of a promycelium or emergence of 2 promycelia from a spore terminating into 2 whorls of sporidia are noticed (Figs. 29 to 32). Out of 4 nuclei, 2 have passed into each branch dividing further mitotically (Fig. 29). Two compatible sporidia conjugate from the 2 whorls, borne on a teliospore. A promycelium shows false septum and rarely the sporidia become septate, separating the daughter nuclei (Fig. 30). Conjugation of sporidia on 2 teliospores has been reported in *U. occulta* (Stakman *et al.* 1934). In others, the nuclei migrate in both the promycelia to feed a single haploid nucleus to each sporidium (Figs. 31, 32).

4) Occasionally asynchronous movement of nuclei into sporidia occurs and as a result the recipient sporidium starts elongation, whereas the non-recipient sporidium awaits entry of a nucleus (Figs. 26, 27). Sometimes the promycelium becomes septate, while the dikaryotized sporidia elongate producing the infection hyphae (Figs. 28, 33). Such aberrant movement of nuclei and formation of infection hyphae have been reported earlier in few *Entyloma* species (Das 1949, Kaiser 1936).

5) Occasionally the nucleus divides after migration into a sporidium and the daughter nuclei are separated by a septum dividing the sporidium into 2 haploid cells (Fig. 35). Sporadic septation of sporidia has been observed in *U. occulta* (Stakman *et al.*, 1934) and regular septation is reported in *E. antennariae* Liro and *E. scirpicola* (Fischer and Holton 1957, Thirumalachar and Dickson 1949).

6) Sometimes haploid secondary sporidia are cut off directly from a primary sporidium (Fig. 25) or after a little elongation (Figs. 34, 36, 37). Often the haploid secondary sporidia are borne in a chain on the primary sporidia (Figs. 36, 38). These secondary sporidia are relatively more variable in size (Fig. 39) than the dikaryotic secondary sporidia formed after conju-

Figs. 22-40. Aberrations in teliospore germination and nuclear behaviour of *Entyloma eleocharidis*. 22, complete inhibition of meiotic nuclear division. 23-24, less than 4 sporidia formed and migration of a haploid nucleus in each. Supernumerary nuclei in the promycelium. 25, direct cutting off of haploid secondary sporidia. 26-27, asynchronous movement

gation and bud out smaller sporidia indefinitely, which serve to propagate and disseminate the fungus in water. Dikaryophase is initiated by fusion between 2 comparable haploid secondary sporidia. A sporidium sends a tube, which approaches a nearby sporidium, intervening walls dissolve and plasmogamy is consummated (Fig. 40). The empty sporidium degenerates later.

Production of haploid secondary sporidia from unconjugated primary sporidia followed by fusion between the compatible ones to initiate the dikaryophase is reported as a normal mode of nuclear behaviour in species of the genera *Doassansia* Cornu and *Tuburcinia* Fries in the fam. Tilletiaceae (Thirumalachar 1946, Woronin 1882).

Development of teliospores

Teliospore development within the host tissues has been critically studied in few species of the genus *Entyloma* (Das 1949, Lutman 1910, Raciborski, 1897, Thirumalachar and Dickson 1949). Serial microtome sections from natural host infections revealed several developmental stages of the sorus, although initial stages of differentiation of and penetration by the infection hypha could not be observed. Young hyphae are branched, intercellular, nonseptate, 0.7 to 1.3 μ in diam., multinucleate with the nuclei remaining paired, arranged in long axis and provided with short lateral sporogeneous cells (Figs. 41 to 43). More such cells are formed sympodially on the hypha, each being furnished with a dikaryon. The mycelium with sparsely septate hyphae ramifies intercellularly between the closely arranged palisade cells (Figs. 44, 45). No intracellular structures or haustoria are observed, indicating direct absorption of nutrition from the host cells. Septation in the hyphae becomes closer and the lateral sporogenous cells start rounding up (Fig. 46). The hyphal mass gives an appearance of compact prosenchymatous cells due to gradual enlargement of lateral cells and close septation, losing identity of individual hyphae (Fig. 47). The dikaryons in the young enlarging cells become more deeply stainable and appear enlarged in size (Figs. 48, 49). The sporogenous cells enlarge further and become oval to spherical, while the 2 nuclei come closer and fuse (Fig. 50). The spore wall thickens and is differentiated into a thicker epispore and a thinner endospore (Fig. 51). Occasionally formation of the spore wall is complete, but the 2 nuclei remain unfused and fusion occurs later in a mature teliospore (Fig. 52). Teliospores are formed singly, but appear in aggregates due to crowding and mutual pressure (Fig. 52). Mycelium invades and produces the teliospores only in the palisade layers, but does not penetrate in the vascular tissues (Fig. 53).

of nuclei and development of sporidia. 28-33, septation of promycelium and direct formation of infection hyphae. 29-32, branching or emergence of 2 promycelia, each bearing a whorl of sporidia. Two compatible sporidia from the 2 whorls conjugating (Fig. 30). 35, septation in primary sporidia. 34, 36-38, direct cutting off of haploid secondary sporidia. 39, haploid secondary sporidia. 40, conjugation of 2 compatible secondary sporidia.

Figs. 41-53. Development of mycelium and teliospores of *Entyloma eleocharidis* in the stem of *Eleocharis plantaginea*. 41-44, intercellular ramification of mycelium and dikaryotic nuclear condition of cells. 45, lateral dikaryotic sporogenous cells. 46-48, stages in enlargement of sporogenous cells; dikaryons coming closer and becoming prominent in each cell.

The teliospore development in this species is essentially similar to that in other species of *Entyloma* (Fischer and Holton 1957). Clamp connections are not observed as in *E. thirumalachari* and the teliospores are devoid of an outer sheath in this species. Nuclear fusion in the spore initial occurs before the spore wall differentiation, whereas in *E. thirumalachari* it occurs usually after the spore wall formation (Singh 1968).

Histopathology

The fungus reduces the mesophyll content at the site of infection, gradually imparting the tissues dark brown discoloration due to teliospore aggregations underneath. The epidermis at the site of infection remains intact without any sign of a bulge-out. The intercellular mycelium pushes the mesophyllar cells increasing the intercellular spaces. Several of the cells are pressed by the development of teliospores in aggregates to lose their identity. The chloroplasts are reduced both in size and number. The nuclei in the host cells become deformed and irregular in shape showing poor stainability. No hypertrophy or hyperplasy of cells in the infected parts occurs as observed in *E. microsporum* (Das 1949) and *E. thirumalachari* (Singh 1968).

Discussion

The parasitic mycelium of *E. eleocharidis* is intercellular and dikaryotic. Teliospores develop by enlargement of the lateral and terminal sporogenous cells, which become oval to spherical and are invested by a thick episporium. The meiotic nuclear division occurs in the promycelium; the first division is meiotic followed by mitotic divisions. Two successive divisions are involved and separation of the homologous kinetophores occurs in the first division of meiosis. There appears to be no difference between the basic features of these divisions and those of other plants except the intranuclear origin of the spindle and small size of the spindle and chromosomes, the disjunction at anaphase being asynchronous. Interphase stages have been of short duration (Olive 1965). The haploid chromosome complement determined at metaphase for this species is $n=5$. Details of chromosomal morphology could not be resolved due to very small size of the chromosomes in the complement and perhaps inadequate staining reactions to bring them out distinctly.

The infected stems wither, fall and disintegrate to release the teliospores from a sorus. The teliospores may lie submerged in water or in wet soil depending on intensity of the first monsoon showers and initiate their germination. The teliospores lying in well-saturated soil germinate and the compatible sporidia conjugate *in situ* to initiate the dikaryophase, which cut off dikaryotic sporidia capable of infecting the host. The teliospores show aberrant

49-50, enlargement of teliospore initials and fusion of dikaryons. 51-52, spore wall thickening on developing teliospores and teliospore aggregates in the mesophyll. 53, distribution pattern of teliospores in the palisade cells. (Scale A: Figs. 41-47; B: Figs. 48-52).

types of germination under certain conditions, among which direct formation of dikaryotic infection hyphae, branching of promycelium, septation of primary sporidia to separate uninucleate cells and cutting off of haploid secondary sporidia either singly or in catenation are common. These aberrations appear an adaptation and are conditioned by variable ecological factors. The occurrence of haploid secondary sporidia is provided probably for prolonged survival and wider dissemination of the pathogen in water. They float in water, mating and plasmogamy being delayed, and are widely disseminated as they move along the flow of water from field to field or pond to pond. A haploid sporidium buds out smaller sporidia indefinitely in the absence of conjugation. Compatible haploid sporidia conjugate on contact and initiate fresh infections at new sites. This wide adaptability may be responsible for fresh infections throughout the growing season of the host.

The representatives of the fam. Tilletiaceae, which have been studied in detail so far exhibit the diplobiontic developmental rhythm of life cycle (Gäumann and Wynd 1952) i.e. conjugation of haploid sporidia occurs *in situ*. *E. eleocharidis* appears well adapted to parasitism on the aquatic host and survival under the conditions and follows both diplobiontic and haplo-diplobiontic life cycle patterns, which are probably governed in nature by the presence or absence of water. The smut species follows a general life cycle pattern typical for the genus and shows close similarity with *E. scirpicola*, parasitic on another member of the fam. Cyperaceae.

Summary

Teliospore germination, cytology and development of *Entyloma eleocharidis* Pavgi and Singh parasitic on *Eleocharis plantaginea* R. Br. have been described. The diploid nucleus migrates in the promycelium during teliospore germination and divides. The first division being reductional, is followed by an equational one. Some of the meiotic divisional stages have been described and the haploid chromosome complement ($n=5$) determined. Several aberrations in the teliospore germination and nuclear behaviour are observed and their repercussions discussed. Sporidial development, conjugation and subsequent development of parasitic mycelium and teliospores in the host indicate that *E. eleocharidis* possesses a diplobiontic and haplo-diplobiontic life cycle pattern. Possible consequences of the ecological factors influencing the life cycle of the pathogen are indicated.

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