Cytology of *Ravenelia breyniae* Sydow

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Sydow (1907) first described *Ravenelia breyniae* Sydow parasitic on *Melanthesa rhamnoides* B. (*Breynia rhamnoides* Muell.) (Euphorbiaceae) based on a collection from Pusa, Bihar. The rust was described for its telial stage only (Sydow and Butler 1907) but recently it is reported to occur in all its spore-forms namely, pycnial, aecial, uredial and telial stages in the vicinity of Varanasi (Pavgi and Singh 1969, Singh 1971). Knowledge concerning the nuclear behaviour in teliospores, basidia and basidiospores of rust fungi dates back since the first observations by Allen (1933) in species of *Puccinia*. Later several other genera of Uredinales are included under such studies. However, species of the genus *Ravenelia*, predominantly distributed in the tropical and subtropical belt, remained cytologically unexplored so far. The present observations deal with the nuclear cytology inside teliospores, basidia and basidiospores in *Ravenelia breyniae* Sydow.

Materials and methods

Teliospores from the material stored under low temperature conditions are mounted and stuck on to the slides in a drop of mucilage of *Tradescantia tricolor*. The smears are air dried and wetted frequently. Alternate wetting and drying appear conducive to spore germination. The slide mounts are covered with a wet cotton toweling and incubated at room temperature (25-27°C) for germination (Thirumalachar and Narasimhan 1953, Thirumalachar and Pavgi 1950). The teliospores germinate (about 20%) after 5 days of incubation.

Germinating teliospores are fixed *in situ* at the desired stage for an hour in modified Carnoy's fixing fluid (6, 95% ethyl alcohol: 4 glacial acetic acid: 4 chloroform) and transferred to 70% ethyl alcohol. The slide mounts may be retained herein for any length of time desired. They are passed through a descending series of 4 alcohol concentrations and finally to dist. water. Thorough washing is done to remove alcohol traces and the slides are transferred to aqueous 0.5% Taka diastase for 10 minutes to remove soluble carbohydrates. The slides are washed thoroughly to remove traces of enzymatic deposits, upgraded through the dilution series to 70% alcohol, mordanted in 0.5% aqueous iron alum for 2-3 minutes, washed again in water to remove the excess of iron deposit and flushed with 0.5% aceto-carmine in 45% glacial acetic acid. Immediate observation is necessary to avoid drying and darkening of the chromatin material (Singh and Pavgi 1966).

Observations

Teliospore germination: Termination of dikaryotic condition as a sign of physiological maturity seems essential for germination. A stout germ tube emerges out of the apical germ pore of the teliospore (Fig. 1). This gradually elongates into a long tubular basidium (Figs. 2, 3). The basidium immediately after emergence characteristically tends to coil towards the telial head. Cytoplasm gradually moves in the basidium leaving the teliospore almost empty. However, a little amount of cytoplasm remains adhered to the endospore wall. The cytoplasm gradually condenses somewhere in the center of the basidium which finally forms a cross septum after the first meiotic nuclear division dividing the basidium into 2 cells (Fig. 10). Two more septa are laid down in the process and the basidium becomes a 4-celled structure (Fig. 13). Small papillae develop on the terminal cell and the process continues in the remaining cells in basipetal succession (Fig. 14). The papillae gradually elongate, taper, and give rise to sterigmatic processes. A small vesicle or basidiospore develops at the tip of the sterigma, wherein the cytoplasm moves through the sterigma, evacuating the basidial cell. Basidiospores develop, mature and finally eject in basipetal succession. They are expelled from the sterigmata on the water surface for final maturation. Mature basidiospores are round to oval, hyaline, smooth and thin walled and measure 7.7 to 12.1 μ in diam. They germinate readily *in vitro* by formation of 1 germ tube normally. The germ tube develops at random showing nonpolarity and absence of predetermined site on the spore. No budding or secondary spore formation is noticed as reported in species of *Coleosporium* (Olive 1942, 1949a) and *Gymnosporangium* (Berliner 1954).

Meiotic nuclear division: Perpetuation of dikaryotic condition from uredial to telial stage terminates in the teliospores. The 2 haploid nuclei fuse as the teliospore matures. The 2 nucleoli also fuse during the nuclear fusion. Presence of 2 unfused nucleoli after nuclear fusion is observed in *Puccinia malvacearum* Bert (Allen 1933) and in species of *Coleosporium* (Olive 1942, 1949a, Sanwal 1953).

The diploid fusion nucleus gradually squeezes out in the basidium through the distended germ pore and enlarges during interphase preparatory to meiotic division (Fig. 2). The nucleus becomes elongated while squeezing through the germ pore. Elongation of nucleus in the basidium is observed in *Gymnosporangium clavipes* (Olive 1949b). The chromatin expands into a fine thread-like coil beaded at short intervals showing the leptotene stage (Fig. 3). The chromatin reticulum and the deeply stained nucleolus appear clearly defined here. The chromatin reticulum appears more beaded at this stage corresponding to the "Granular nucleus" in *Phytophthora infestans* (Mont.) De Bary as described by Marks (1965). This enlarged nucleus appears as a diffused mass except for nucleolus which is distinct and thus resembles the interphasic nuclei of higher plants. Such nuclei are designated as "homogenous or dotted type" in higher plants (Serra 1955). Pady (1935) also observed similar nuclei in the teliospores of *Hyalopsora aspidiotus*. The nuclear membrane and the nucleolus persist during this stage. Transition from interphase to leptotene is not observed. Olive (1949a) and Sanwal (1953) considered prefusion nuclei equivalent to leptotene. Zygotene and pachytene have eluded observations

Figs. 1-15. 1, diploid fusion nucleus in a teliospore. 2, diploid fusion nucleus in the young basidium. 3, expanded diploid nucleus in interphase or leptotene. 4-6, stages in diplotene. 7, 8, stages of metaphase I. 9, anaphase I. 10, nuclei in interphase nearing prophase. 11, metaphase II. 12, anaphase II. 13, 4 haploid nuclei being separated by cytokinesis. 14, sterigmata formation. 15, a to f. 6 chromosomes of the haploid complement showing some morphological features.

probably due to rapidity of occurrence in the sequence. The diplotene stage exhibited by coiling and shortening of the fibrillar bivalents is observed in the continuum. Two to 4 chiasmata are formed in each bivalent. Persistence of nuclear membrane and nucleolus attached with the nucleolar organizer in one of the bivalents is observed throughout the prophase (Figs. 4-6). This stage is described as "phase of elongation" by Olive (1949a) in *Coleosporium vernoniae* B. and C. Diakinesis leading to thickening, shortening and chiasmata formation in the chromosomes is not observed in any of the preparations. Berliner (1954) and Payak (1957) observed diplotene and chiasmata formation in *Gymnosporangium clavipes* Cooke and Peck and *Scopella gentilis* (Syd.) Mundk. and Thirum. respectively, while zygotene, diplotene and diakinesis are reported earlier in *Puccinia sorghi* by Pavgi *et al.* (1960).

The condensed and thick chromosomes move along the spindle fibres towards the equatorial plate during metaphase stage (Figs. 7, 8). The nuclear membrane and nucleolus disappear. Each chromosome is distinct and separate enough and not enclosed as in species of *Puccinia* (McGinnis 1956). The metaphase spindle fibres usually orient parallel to the vertical axis of the basidium. The spindle plate is large enough to accommodate all the chromosomes over it. Rod and ring type of spindle is evident in *Puccinia thwaitesii* (Payak 1962) and in *Hyalopsora aspidiotus* (Pady 1935). Astral rays or polar caps are not discernible in any of the preparations. They are reported to occur regularly in *Thakopsora hydarngae* Magn. (Olive 1943). Presence of centrioles is not discernible. Similar bodies are reported to occur occasionally in *Puccinia malvacearum* (Allen 1933) and also in *P. sorghi* (Pavgi *et al.* 1960). Anaphase starts in the continuum of the process. The spindle fibres attached to the centromere are evident. The chromosomes divide lengthwise into 2 halves and each set of diads moves towards opposite poles during anaphase I (Fig. 9). Movement of the chromosomes appear synchronous. The chromosomes immediately after reaching the poles fuse into aggregated masses and lose their identity. The cytoplasm condenses around the spindle plate and a cross septum is laid down. The basidium thus becomes bicelled, each cell containing a premature haploid nucleus. A delicate and indistinct nuclear membrane is formed around the 2 nuclei. No nucleolus becomes discernible. The 2 nuclei immediately enter into interphase (Fig. 10). Suppression of cytokinesis after I meiotic division is reported in *Gymnosporangium clavipes* (Olive 1949b). Interphase quickly merges into metaphase II. This metabolically active stage is not clearly discernible. The prophase passes very quickly. Neither the nuclear membrane nor the nucleolus is discernible. The chromatin reticulum appears deeply stained. Relatively few reports are available on the interphase giving details of the nucleus. The chromosomes are formed synchronously to move on the 2 spindles in the II phase of meiosis. They move on to the equatorial plate in metaphase (Fig. 11). Precocious or lagging chromosomes are not noticed. Individual chromosomes can be distinctly resolved at this stage. The chromosome count done at this stage shows 6 chromosomes ($n=6$) again representing the haploid complement for the species (Fig. 15). The chromosomes split longitudinally and the halves move apart towards opposite poles at anaphase II. Occasionally lagging chromosomes are noticed during the movement (Fig. 12). Anaphase II persists for a very short time. The chromo-

Figs. 16–26. 16, 17, suppression of meiotic nuclear division. 18, suppression of cytokinesis. 19–21, suppression of II meiotic nuclear division, bifercation of basidium and formation of thick tubular sterigmata. 22–24, 26, II meiotic nuclear division in one of the daughter nuclei and enclosing 2 haploid nuclei in basidial cells. 25, precocious mitotic divisions of the haploid nucleus in the sterigma.

somes lose their identity soon aggregating into diffuse masses. Appearance of nuclear membrane and nucleolus in each nucleus follows quickly. Two more septa are laid down and the basidium becomes 4-celled, each cell containing 1 haploid nucleus (Fig. 13). The haploid nucleus along with cytoplasm moves through sterigma into the basidiospore leaving the basidial cell usually empty. The nucleus becomes elongated while moving through the narrow sterigmatal process. The haploid uni-nucleate basidiospores soon mature and are expelled in the vicinity.

Recently a detailed account of the morphology of chromosomes in some *Ravenelia* species including *R. breyniae* is reported by Singh (1972).

Deviations from the normal nuclear division are observed at various stages in this species. Suppression of meiotic nuclear division is evident in several cases (Figs. 16, 17). Suppression of cytokinesis after the first stage of meiotic nuclear division encloses the 2 nuclei in 1 cell (Fig. 18). Not infrequently II stage of the meiotic nuclear division is suppressed after cytokinesis enclosing a nucleus in each cell, from which sterigmata are produced eventually. These cells are situated in midway with the terminal and basal cells being anucleate (Figs. 19–21). Partial suppression of the II stage results in 3 nuclei enclosed in 3 cells or 2 and 1 nuclei in 2 cells (Figs. 22, 23). Often 2 nuclei are enclosed in the same cell after meiotic nuclear division (Figs. 24, 26). Precocious division of the haploid nucleus takes place in a basidial cell or in enlarged sterigma (Figs. 22, 25). Most of these aberrations are associated with thickening of sterigmata into hyphal branches and failure in basidiospore production.

Mitosis in basidiospores: Mitotic nuclear division occurs in the expelled basidiospores normally prior to their germination. The resting haploid nucleus is round to oval (Fig. 27), gradually enlarges and the chromatin reticulum appears clearer and beaded (Fig. 28). The nuclear division proceeds under normal moisture and environmental conditions and interphase in the nucleus nearly merges into metaphase through the evanescent prophase. Nuclear membrane and nucleolus disappear and metaphase proceeds rapidly. Six chromosomes move on to the spindle and become oriented on the equatorial plate. The spindle fibres are seen attached to the centromeres of the chromosomes. The chromosomes are identical individually in morphological details to those of the metaphase I and II of the meiotic nuclear division described earlier (Fig. 29). The chromosomes split lengthwise and the 2 sets move towards the opposite poles during anaphase. Few chromosomes lag behind during movement (Figs. 30, 31). Eventually the chromosomes aggregate into dense masses. They are not identifiable individually at this stage. The nuclear membrane and the nucleolus reappear in each nucleus (Fig. 32). A small protuberance develops on the basidiospore wall which gradually elongates into a germ tube. The nuclei move into the germ tube along with cytoplasm leaving the spore almost empty (Figs. 33–36).

Germination of basidiospores prior to nuclear division is observed in some basidiospores. The germ tubes elongate leaving the nuclei behind in the spore (Fig. 37). The nucleus occasionally undergoes mitosis in the spore after germination (Fig. 38). The single or the 2 mitotically divided nuclei in the spore are delayed

Figs. 27-42. 27, mature basidiospore with a haploid nucleus. 28, enlarged interphasic nucleus or prophase. 29, metaphase. 30, 31, anaphase stages. 32, 2 haploid nuclei in the basidiospore. 33, germinating basidiospore. 34-36, migration of nuclei in the germ tube. 37, germination prior to mitotic division. 38, enlargement of the nucleus preparing for mitosis after basidiospore germination. 39-41, delayed migration of the single or 2 haploid nuclei in the germ tube. 42, single nucleus in tip of a bulged germ tube.

moving in the germ tube, while the latter elongates considerably (Figs. 39–41). The nucleus moves in and is located at the tip of the germ tube where it enlarges. The mitotic division of haploid nucleus takes place later in the germ tube (Fig. 42). Degeneration of 1 or more nuclei and extension of chromatin in the cytoplasm during the division are reported in the basidiospores of *Coleosporium* species (Olive 1942, 1949a) and *Coleosporium tussilaginis* (Ashworth 1934) respectively. None of these features is observed in the basidiospores of *Ravenelia breyniae*.

Discussion

Teliospore germination in *R. breyniae* is principally conditioned by physiological maturity of teliospores, optimum temperature of microclimate and amount of condensed moisture around the teliospores on slide mounts. Relatively few teliospores germinate with great difficulty as compared to other species (Singh 1967).

Barring few anomalies, the nuclear events observed in some of the developmental stages, are in conformity with the normal steps of meiotic cycle of diploid fusion nuclei in rust fungi. Zygotene, pachytene and typical diakinesis stages lurked during the observations probably due to rapidity of meiotic cycle. Furthermore, centrioles and astral rays are not discerned in any of the preparations. These stages are reported to occur in species of *Coleosporium*, *Puccinia* and *Thekopsora*.

The chromosome complement consists of 6 chromosomes ($n=6$). Observations on details of their morphology and individual distinctive characters have yet been inadequate, primarily due to their small size and insufficient differential stain reactions.

Variations in the environmental conditions specially the amount of moisture condensing over the teliospore and temperature possibly head to aberrant teliospore germination as well as deviations in the stages of the meiotic nuclear division and differentiation of the basidium and the basidiospores. Various types are observed such as suppression of the nuclear division at I or II stages, failure in cytokinetic septation and failure in basidiospore development. Most of their abnormal development are associated with thickening of the sterigmata into hyphal branches and subsequent failure in basidiospore production. Since the teliospores propelled individually by the cyst stick on to the leaf surface on the host, such thickened sterigmatal processes may serve as the infection hyphae directly in case the normal meiotic nuclear division is not upset at one stage or another.

The basidiospores when developed normally do not present much of variation in the sequence of development and the mitotic division of the single haploid nucleus in them. The occasional dialation of the germ tubes at the tips leads to a tendency of development of secondary basidiospores. This only supports the primitiveness of the rust genus in the phylogenetic sequence and exhibits a close phylogenetic relationship with the members of Ustilaginaceae where budding is a characteristic feature.

Teliospores in species of *Ravenelia* are characteristically cemented firmly in an orbicular to suborbicular teliospore head borne on a compound pedicel appended with bulbous cysts ventrally on the teliospores. The teliospores are observed

germinating in the teliospore head only while the mechanically separated ones do not germinate at all. Probably the physiological confluence among the teliospores is disturbed by mechanical separation inhibiting further growth as indicated by germination. Recently, Rajendran (1970) in his excellent paper erected a new genus *Kernkampella* typified by *K. breyniae* (Syd.) Rajendran synonymising *R. breyniae* Syd. characterized by loosely adhered teliospores in the teliospore head. If this statement is true, the individual separated teliospores should normally germinate exhibiting their physiological independence after maturity. However, during the present studies none of the separated spores germinated individually suggesting their physiological coherence in the teliospore head. Thus the cytological observations do not justify the erection of the genus *Kernkampella*.

Summary

Nuclear behaviour in the basidium and basidiospores of *Ravenelia breyniae* Syd. is described. Haploid chromosome complement ($n=6$) is determined from the metaphase I and II of the basidial cytology. Numerous aberrations are encountered during teliospore germination and nuclear behaviour in basidium and basidiospores. Possible consequences of these aberrant types are discussed in view of the host-parasite relationship.

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