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NUCLEAR BEHAVIOR DURING TELIOSPOSE GERMINATION IN *USTILAGO TRITICI* AND *U. NUDA*

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Abstract

When germinated upon water, the mature teliospores of *Ustilago tritici* and *U. nuda* gave rise to a 4-celled monokaryotic promycelium. Nuclei moved from adjacent promycelial cells into a conjugation tube. The conjugation tube elongated into a branching monokaryotic or dikaryotic mycelium. The haploid number of chromosomes appeared to be 4. The growth of these two smut fungi on potato dextrose agar was not identical.

Introduction

Since the cytological study of *Ustilago longissima* (Schlecht.) Meyen by Schmitz (1879) many investigators have made significant contributions to the cytology of smut fungi. Nuclear behavior in *U. tritici* (Pers.) Rostr., and *U. nuda* (Jens.) Rostr., was investigated by Lutman (1910), Paravicini (1917), Hutting (1931), Wang (1934), Lang de la Camp (1936), Thren (1940) and Popp (1955). Popp (1955) showed that when the spores germinated on potato dextrose agar (PDA) or water agar (WA), promycelia formed which directly gave rise to branching hyphae. On PDA the nuclear behaviour and branching of *U. nuda* were distinctly different from that of *U. tritici*, whereas on water agar there was little difference between the species.

The present study was undertaken to evaluate the nuclear behaviour in *U. tritici* and *U. nuda* during germination in water.

Materials and Methods

Teliospores of *U. tritici* and *U. nuda* were germinated in sterile tap water in Petri dishes at 28°C. The spores were examined in different stages of germination during a period of 36 hr. The germinating spores were stained with cotton blue in lactophenol to observe structural details. For studies of nuclear behaviour, germinated spores were affixed to a microscope slide using Haupt's adhesive, and fixed in freshly prepared Lu's fixative (n-butylalcohol; glacial acetic acid: 10% aqueous chromic acid; 3:2:1, v/v) for 12 hr (Lu, 1962).

The fixed material was rinsed in distilled water and hydrolysed in 10% HCl at 36°C for 30 minutes, rinsed thoroughly in distilled water and rinsed in 0.2 M phosphate buffer at pH 7.2. A stock solution of Giemsa stain was prepared by dissolving 0.5 gm of Giemsa powder in 33 ml of glycerine at 55°C for 1-2 hr. After cooling, 33 ml of methanol was added. The stock was freshly diluted with 15 vols distilled water just before use. The material was stained for 10 min and the process of staining was observed under a microscope at 100X. The stain was drained off the slide and a few drops of 2% sodium hydroxide were added to the material. After the nuclei turned dark pink (about 1 min) the slide was rinsed in distilled water.

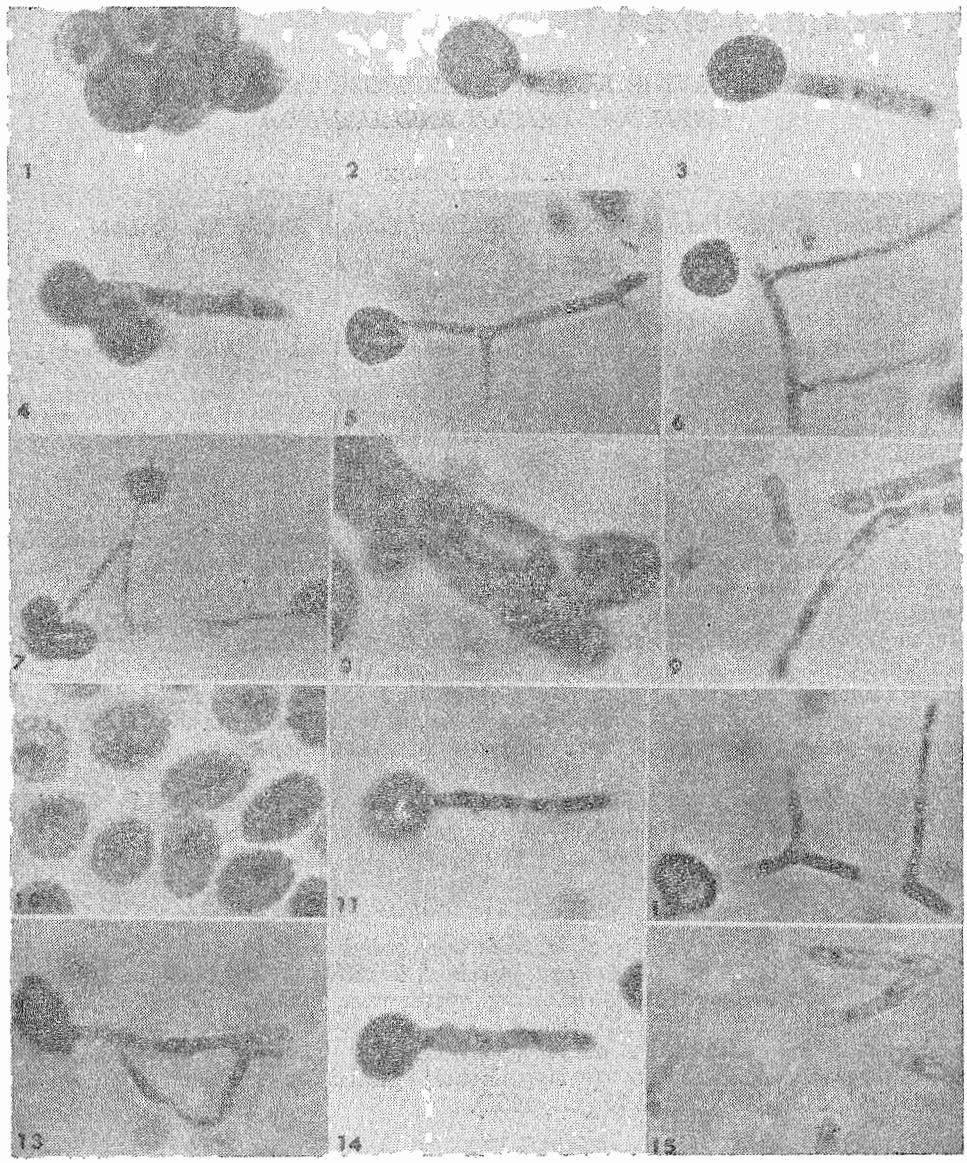

Fig. 1-9. *Ustilago tritici:* 1) Mature teliospores with single diploid nuclei; 2) Germinating teliospores with nucleus migrating from the spore into the promycelium; 3) Germinating teliospore with three haploid nuclei in the promycelium and one haploid nucleus in the spore; 4) Promycelium with four haploid nuclei and the initiation of a conjugation tube; 5) Promycelium with four nuclei and two young conjugation tubes; 6) Nuclei migrating into the conjugation tubes; 7) Nuclei in conjugation tubes; 8) Condensed chromosomes in nuclei; 9) Uninucleate cells from PDA culture.

Fig. 10-15. *Ustilago nuda:* 10) Mature teliospores with single diploid nuclei; 11) Four-celled promycelium with a haploid nucleus in each cell and a conjugation tube initial; 12) Promycelium with two conjugation tubes and two nuclei in one tube; 13) Fusion of two promycelial branches; 14) Condensed chromosomes in nuclei; 15) Uninucleate cells from PDA culture.

(Magnification Fig. 1-6, 11-14: X 1900; Fig. 7, 10: X 900; Fig. 8: X 2500; Fig. 9,15: X 1500)

To observe nuclei in resting spores, the spores were soaked in sterile tap water for 2 hr and fixed for 12 hr in Lu's fixative. The fixative was removed by filtering through a 0.8u Millipore filter. The spore walls were bleached in 3% hydrogen peroxide for 30 min and then rinsed 3-4 times with distilled water by filtering as above.

Forty-day old cultures of *U. tritici* and *U. nuda* grown on PDA were fixed, hydrolysed and stained as above. The stained material kept well for a couple of weeks and therafter faded.

Results

The teliospores of *U. tritici* usually contained a single nucleus (Fig. 1). This was judged to be dipoloid on the basis of its larger size than the nuclei in germinated spores. Prior to the production of the promycelium the nucleus inside the teliospores enlarged and moved towards the periphery. The wall ruptured at the weaker side, which was usually light colored, and a papillum broke through the wall giving rise to the young promycelium. The single nucleus moved partly into the promycelium (Fig. 2). By 12 hr, nuclear divsion had occurred and three well defined nuclei were clearly visible in the promycelium while the fourth nucleus remained within the spore (Fig. 3). As the promycelium grew further the nucleus in the spore moved into the promycelium and a conjugation tube started developing at the point of a septum (Fig. 4). Two conjugation tubes often formed (Fig. 5). After 24 hr the promycelium started branching at the septa and nuclei from adjacent cells migrated into the newly formed conjugation tubes. The conjugation tubes elongated into branches. The nuclei moved further into the conjugation tubes and division of the nuclei continued (Fig. 6). After 36 hr the branches from the promycelium continued to elongate and divided by means of septa into daughter cells which were monocaryotic (Fig. 7). The promycelium remained connected to the spore by older portions from which the protoplasm became evacuated.

The nuclear behaviour in *U. nuda* was found to be somewhat similar to that observed in *U. tritici*. The teliospores had a single diploid nucleus (Fig. 10) and produced a 4-celled promycelium in water (Fig. 11). As the promycelium grew further, conjugation tubes started developing opposite septa (Fig. 11). The nuclei migrated into the conjugation tube and division of the nuclei continued. Older cells of the promycelium became empty in the wake of evacuating protoplasm. The nuclei came together in pairs, giving rise to binucleate cells and establishing the dicaryotic condition (Fig. 12). Fusion of promycelial branches with other cells of the promycelium was often seen in *U. nuda* (Fig. 13).

Four dark colored bodies embedded in the nuclear membrane in the promycelial tips of *U. tritici* and *U. nuda* were discernible (Fig. 8, 14). These were thought to be chromosomes. However, since the nuclear envelope may remain intact during somatic nuclear division in fungi (Moore, 1965), it is possible that these chromatic structures are chromosome complements of daughter nuclei rather than single chromosomes.

U. tritici and *U. nuda* were found to be slow growing on PDA. In cultures of *U. tritici* profuse budding above the surface was more common. *U. nuda* budded within the medium. In 40-day old cultures the sporulating hyphae of *U. tritici* and

U. nuda were relatively large in diameter and subdivided into many small vacuolate cells with thick walls which were usually brown. These cells were uninucleate (Fig. 9, 15).

Discussion

The HCl-Giemsa technique used in the present study gave good contrast and demonstrated the nucleus and condensed chromatin in the stained material.

The teliospores of *U tritici* and *U. nuda* germinated readily in water at 28°C giving rise to promycelia which branched and ultimately developed into a network of mycelia. No spordia were produced in water. Meiosis may occur in the germinating spore or in the young promycelium in agreement with the interpretation of Wang (1934). The promycelia of both the smuts become subdivided in 2 and then into 4 cells with one nucleus in each cell which on the basis of behavior and from the standpoint of amount of chromosomal material is considered to be haploid. The haploid number of chromosomes appeared to be 4. These findings were in contrast to the observations of Wang (1934) who reported that the haploid number in *U. nuda* was n = 2.

The nuclear behavior of *U. tritici* and *U. nuda* were similar to each other when grown in water, except for the tendency of promycelia of *U. tritici* to be monocaryotic. Popp (1955) observed the germination of these smuts on water agar. He noted that the germination of spores of *U. tritici* was similar to *U. nuda*, but that promycelial branches of *U. tritici* often remained monocaryotic whereas those of *U. nuda* were dicaryotic. The nuclear behavior and conjugation tube formation observed here for spores germinated in water at 28°C were similar to those reported by Popp for germination on water agar, but unlike those reported on PDA.

The growth characters in PDA cultures were not identical. This is in conformity with the findings of Thren (1940) who observed that the smuts on wheat and barley were not identical in their mode of growth.

In the present study promycelia stained with HCl Giemsa showed metachromatic granules which showed Brownian movement in the vacuoles near the growing tip. Similar bodies were reported in *Tilletia caries* (D.C.) Tul. by Yen (1937).

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