Neth. J. Pl. Path. 74 (1968) 2–7

Cytological studies of the germinating teliospores and basidiospores of Puccinia penniseti[1]

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Accepted 25 July, 1967

Abstract

The teliospores of *Puccinia penniseti* germinate within 48 hours if the optimum conditions of temperature and humidity are available to them. They have no resting period. On germination, they produce a three-celled promycelium in which the upper two cells contain nuclei and appear functional whereas the third, lowermost cell is without a nucleus. Each of the two cells normally gives rise to a quadrinucleate basidiospore, but occasionally uni- and binucleate basidiospores are produced. In these latter instances, the division of the nucleus in the basidiospore may continue until 8–16 nuclei have been produced. Presumably, all the nuclei but one degenerate before the basidiospore germinates to produce a monocaryon. A study of the nuclei in the promycelium as well as in the basidiospore shows that there are 5 chromosomes, 4 arranged in two pairs of different sizes, the fifth being the smallest and solitary.

Introduction

A knowledge of the chromosomal complements of the rust fungi has become indispensible ever since the existence of rust races has been recognized. Cytological studies with the host plants have mostly been easier because of the larger size of the chromosomes. In contrast, the extremely small size of the chromosomes in members of the Uredinales has posed some difficulty in their precise determination. However, earlier handicaps in the way of such studies have been largely overcome and many useful techniques have been described.

The cytological investigations of McGinnis (1953, 1954, 1956) revealed the haploid chromosome number in several species of *Puccinia*. Pavgi et al. (1960) and Payak (1962), also, described the haploid chromosome numbers of *P. sorghi* and *P. thwaitesii*, respectively. These reports provided a great impetus to cytopathologists and interest has been revived in the cytology of Uredinales.

In this communication the cytological events within the promycelium and basidiospore of *Puccinia penniseti* Zimm., the rust of *Pennisetum typhoides* Stapf et Hubb., have been studied. The haploid chromosome number in *P. penniseti* has also been investigated.

[1] This paper is based in part on a thesis presented in July, 1964, to the University of Agra, Agra, India, in partial fulfilment of the requiment for the Ph. D. degree.

Materials and methods

Leaves of pearl-millet, showing good telial infection, were collected from seedlings growing in a glasshouse and washed in running tap water for a few minutes. Later, they were rinsed 2–3 times in double-distilled water. Telial sori were picked up with a pair of 'OO' forceps and teased on a microscope slide with the help of steel needles. One to two drops of distilled water were placed on the teased material so as to make a suspension of the teliospores. Drops of this suspension were applied to one end of the slide and the teliospores incubated at 16–17 °C. Within 48 hours of incubation at the above temperature, fairly-good teliospore germination was achieved. Slides showing various stages of germination were fixed in aceto-alcohol (1:3) and Carnoy's fluid (6:3:1, 100% ethyl alcohol, glacial acetic acid and chloroform) for about an hour. Several pretreatments with 8-hydroxy-quinoline, aesculine and a mixture of the two in 1:1 ratio were also given in order to secure better shortening and separation of the chromosomes but with little difference in the results. The slides were flooded with 0.5% acetocarmine and temporary mounts prepared.

For a cytological study of basidiospores, the teliospores were germinated in situ. When they gave rise to basidiospores they were fixed and stained as described above. Cytological examinations were made at a magnification of × 970–1200.

Results

Good germination of teliospores is noted within 48 hours under suitable conditions of temperature and humidity. The initial indication of germination is the development of a short papilla-like outgrowth (Fig. 1) which finally attains a length of about 62 μ

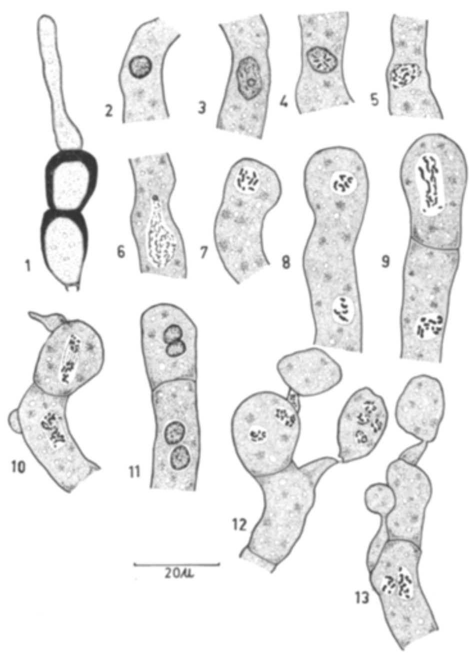

Fig. 1–13. *Puccinia penniseti.* Various stages in nuclear division in the promycelium.
1: germinating teliospore, 2–6: stages in prophase, 7–8: early and late anaphase, 9–10: second nuclear division, 11: second telophase, 12: promycelium showing basidiospore formation, 13: promycelium with a foot-cell and two functional cells.

Fig. 1–13. Diverse fasen van de kerndeling in het promycelium.
1. Kiemende teleutospore, 2–6: momenten in de profase, 7–8: vroege en late anafase, 9–10: tweede kerndeling, 11: tweede telofase, 12: promycelium met vorming van basidiosporen, 13: promycelium met basale cel en twee functionerende cellen.

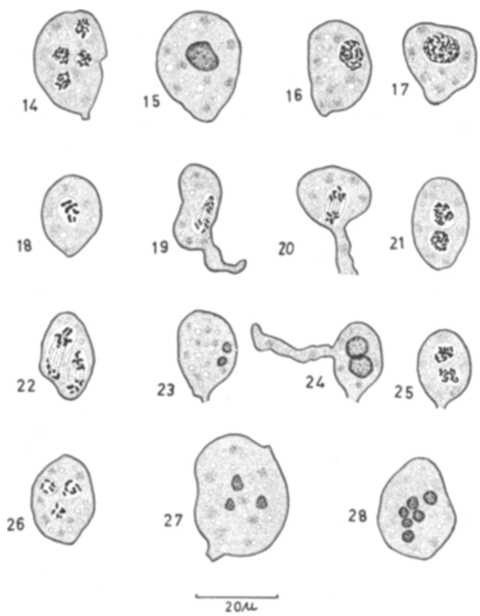

Fig. 14–28. Various stages in the formation of the basidiospore.
14: normal four-nucleate basidiospore, 15–17: prophase, 18: metaphase, showing five chromosomes, 19–21: first nuclear division in basidiospore, 22: second nuclear division in basidiospore, 23–28: two, three and six nucleate basidiospores. Fig. 15–28 are occasional stages in basidiospore development. The commonest occurrence is the formation of four-nucleate basidiospores, all the four nuclei being received directly from the corresponding cell of the promycelium.

Fig. 14–28. Diverse fasen in de vorming van de basidiosporen.
14: normale vierkernige basidiospore, 15–17: profase, 18: metafase met vijf chromosomen, 19–21: eerste kerndeling in de basidiospore, 22: tweede kerndeling in de basidiospore, 23–28: basidiosporen met respectievelijk twee, drie en zes kernen. 15–28: zijn minder gewone stadia in de ontwikkeling van de basidiosporen. Het meest voorkomend is de vorming van basidiosporen met vier kernen, die elk rechtstreeks van de betrokken cel van het promycelium afkomstig zijn.

(Fig. 12–13). This promycelium may arise from both the cells of the teliospore but usually it arises from the apical cell. The diploid nucleus of the teliospore now moves up into this tube and eventually lies in the centre of it being ready for the first nuclear division, which is presumably the reduction division. The nucleus undergoes a great increase in size (Fig. 2–5) and ultimately a chromatin reticulum with a nucleolus at

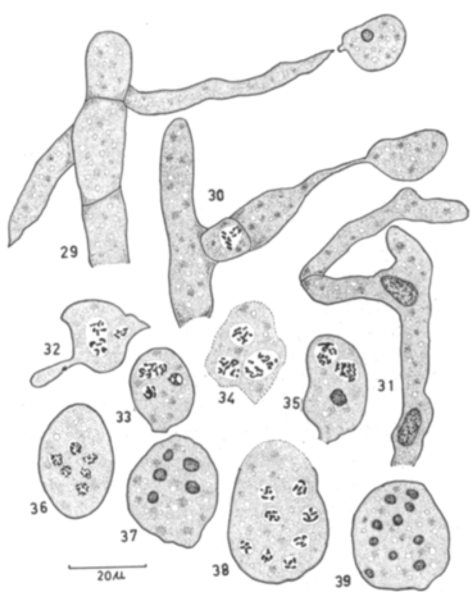

Fig. 29–39. Various abnormal promycelia and basidiospores.
29–31: some abnormal promycelia, 32–39: abnormal basidiospores with three, five, eight and ten nuclei.

Fig. 29–39. Diverse abnormale promycelia en basidiosporen.
29–31: enkele abnormale promycelia, 32–39: abnormale basidiosporen met drie, vijf, acht en tien kernen.

one end becomes prominent (Fig. 6). The chromosomes are very fibrillar at this stage but soon start condensing. No late prophase and metaphase could be seen in the promycelium during three years of this study, but early anaphase was noticed (Fig. 7), in which five chromosomes were seen in two groups. The two groups separate to enter into the late anaphase (Fig. 8). After the first nuclear division the nuclei may be reconstituted or they undergo the second division directly. When late anaphase is reached, a wall separates the two nuclei (Fig. 9). The promycelium is now three-celled, the upper two cells containing the nuclei and the lowermost being without a nucleus. Thus the lowermost cell of the promycelium may be considered as a foot cell. The nuclei of the functional cells of the promycelium undergo a third division and the result is the formation of four nuclei in each cell which pass directly to the developing basidiospores (Fig. 9–13). While the second nuclear division is taking place in the promycelium the side walls of this structure produce conical protuberances to form sterigmata (Fig. 10). Normally the sterigmata measure about 11 μ in length but when they have elongated abnormally, their shape and size are considerably changed (Fig. 29–30). Abnormally elongated sterigmata may measure up to 61 μ in length. Usually the sterigmata develop alternately but at times they may arise from the same side of the promycelium. Generally only two sterigmata are produced, each giving rise to a basidiospore. While the basidiospores are developing, a streaming movement of the chromosomal groups can be seen through the narrow passages of the sterigmata (Fig. 13).

The mature basidiospore is an oval body, 16 × 12 μ in size. However, when nuclear divisions in the basidiospore are irregular, their shape and size are considerably changed (Fig. 32–39). Generally a basidiospore receives four nuclei from the corresponding cell of the promycelium (Fig. 14), but at times a single nucleus may be seen in the basidiospore, apparently because there was only a single nuclear division in the promycelium. In such cases the nucleus continues its division in the basidiospore until there are four or more nuclei in it (Fig. 15–28). Mostly the basidiospore is four-nucleate, but sometimes 5–10 nuclei may be produced (Fig. 36–39).

Chromosome counts at the anaphase in the promycelium and at the meta- and anaphase in the basidiospore indicate that there are five haploid chromosomes in *Puccinia penniseti*. Morphologically, the chromosomes differ in their shape and size. There are two pairs, one bigger (2.94 μ in length) and one smaller (1.76 μ in length); the fifth, solitary, chromosome (1.18 μ in length), is the smallest of all.

Sometimes the promycelia exhibit abnormal structures due to excess amounts of water on the slides as a result of condensation (Fig. 29–31).

Discussion

The germination of the teliospores of *Puccinia penniseti* seems to be fairly consistent if optimum conditions of temperature and humidity are available to them. On germination they produce a three-celled promycelium with only two basidiospores. Since *P. penniseti* is a macrocyclic rust, having as the telial host pearl-millet *(Pennisetum typhoides)*, and as the aecial host brinjal (*Solanum melongena* L.), the formation of a three-celled promycelium in this organisms conflicts with the earlier report of Jackson (1935) who suggested that the production of two-celled promycelia is characteristic of microcyclic rusts and four-celled ones of macrocyclic rusts. Kulkarni (1958) reported that in the promycelium of *P. penniseti* three divisions take place instead of two, a common behaviour in other genera of Uredinales. The observations in the present study indicate that in certain cases all three divisions may be completed in the promycelium, or only one or two divisions may occur there, the remainder being completed in the basidiospores. Lindfors (1924) reported the production of two-celled promycelia in *Puccinia arenariae* and also the formation of binucleate basidiospores which on germination produce a binucleate mycelium. From the report of Lindfors it is not evident whether the promycelium is two-celled or three-celled, as has been observed in the case of *P. penniseti*. However, the production of quadrinucleate basidiospores in *P. penniseti* can be taken as a point where the behaviour in the two rusts differ. Nevertheless, at times binucleate basidiospores can also be found in *P. penniseti*. Binucleate basidiospores have been found in *Coleosporium sonchi-arvensis* (Holden and Harper, 1902), *Puccinia sorghi* (Pavgi et al., 1960) and *P. thwaitesii* (Payak, 1962). A quadrinucleate basidiospore was seen only once by Savile (1939) in *Uromyces lespedezae-procumbentis*.

Dandeno (1907), while working on *P. malvacearum*, noted a swelling at the base of the germinating teliospores and considered it "peculiar to the mallow rust". Such inflations were also reported to occur in *P. malvacearum* parasitic on *Althaea rosea* and *Malva rotundifolia* by Taubenhaus (1911). He found that this swelling was certainly not a uniform and constant characteristic of the germinating teliospores of this species and that it occurred only in autumn. He suggested that the spores germinate with greater vigour during autumn and that therefore the promycelium swells up rapidly at first and elongates later. Such inflations were also seen in the present study during the period from October to February. Since the moisture film is not uniformly distributed on the slides, the promycelia may attenuate for lack of moisture and hence their bases appear in the form of swellings.

As mentioned above, there are five chromosomes in *P. penniseti*, four arranged in two pairs of dissimilar sizes, the fifth, the smallest chromosome, being solitary. Five chromosomes have also been reported for *P. sorghy* by Pavgi et al. (1960) and these have a similar morphology to those of *P. penniseti*. McGinnis (1956) worked out the chromosome number in relation to sexual behaviour in a few species of *Puccinia* and concluded that homothallic species have four chromosomes whereas heterothallic ones have only three. Thus the presence of five chromosomes in *P. sorghi* (Pavgi et al., 1960) conflicts with this generalisation of McGinnis, which is further contradicted by the presence of five chromosomes in *P. penniseti* (the material of the present study), which is homothallic (Dalela and Sinha, 1962). The idea of there being a fixed chromosome number in relation to sexual behaviour has also been refuted by Payak (1962).

Acknowledgments

I express my gratitude to Dr. S. Sinha, Professor and Head of the Department of Botany, Agra College, Agra, India, for his keen interest in the problem and for his constant guidance throughout the course of the study. I also record my indebtedness to Dr. M. N. Gupta, Associate Professor in Botany, Agra College, Agra, India, for his help in the cytological techniques and in the making of the camera-lucida-drawings.

Samenvatting

Cytologische studie van de kieming der teleutosporen en basidiosporen van Puccinia penniseti

De teleutosporen van *Puccinia penniseti*, de veroorzaker van de roest van *Pennisetum typhoides*, kiemen bij optimale temperatuur en vochtigheid binnen 48 uur. Ze vertonen geen kiemrust. Bij kieming produceren deze teleutosporen een driecellig promycelium. De terminale cel en de middencel bezitten elk een kern, de basale cel is kernloos. Het is normaal dat de beide eerstgenoemde cellen elk een vierkernige basidiospore vormen, doch soms wordt ook een één- of tweekernige basidiospore gevormd; in het laatste geval kan de kerndeling in de basidiospore doorgaan tot 8 of 16 kernen ontstaan zijn. Vermoedelijk degenereren alle kernen op één na voordat de basidiospore kiemt en een mycelium met éénkernige cellen vormt.

Uit bestudering van de kernen in het promycelium en van die van de basidiosporen bleek dat er vijf chromosomen zijn, waarvan vier in twee paren van verschillende afmetingen en één, het kleinste, afzonderlijk.

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