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# A cytological study of promycelia and basidiospores and the chromosome number in Uromyces fabae

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**Abstract**
Freshly harvested teleutospores of *Uromyces fabae* germinate in 6–7 days of incubation at a temperature of 16 °C. Fully germinated teleutospores produce five celled promycelia. The upper four cells of a promycelium are functional with one nucleus in each cell whereas the lowermost cell remains anucleate. From each of the upper four cells of the promycelium arises a sterigma which develops a single basidiospore at its tip. The basidiospores are uninucleate initially but soon become binucleate by a mitotic division. Chromosomal counts in the promycelium as well as the basidiospore show four chromosomes, grouped in two pairs of different sizes.

## Introduction

Because of small chromosome size, cytological investigations of rust fungi have been very difficult. Wherever cytological studies were possible their preciseness was often shrouded in doubt and contradictory behaviour was often found by subsequent investigators. The case of *Puccinia sorghi* can be taken as an example wherein chromosome number was found to be four by Savile (1939), six by McGinnis (1956), and five by Pavgi *et al* (1960).

While studying the nuclear structure and behaviour in the uredinales, Savile (1939) came across four chromosomes in *Uromyces fabae*. To ascertain whether the obser – vations of Savile were correct, a detailed cytological study of promycelia and basidiospores of *U. fabae* was undertaken. The present communication describes the nuclear history in promycelium and basidiospore and chromosome number in *U. fabae*, the rust disease of *Lens culinaris* Medic.

## Materials and methods

Stem portions of infected lentil plants were collected from plants growing in a greenhouse and were washed first in running tap water for a few hours and then in double-distilled – sterilized water. The first treatment enabled the telial sori to be sufficiently softened whereas the second treatment eliminated spores of other fungi from the scope of the present studies. After this, the telial sori were carefully scraped and teased apart with a pair of teasing needles on a previously sterilized microslide. During teasing of the sori, small drops of sterilized water were added from time to time and the teleutospore suspension so obtained was periodically removed with a soft painting brush and applied to one side of a microslide. The teleutospores were

germinated following the technique described by Kapooria (1968). After 6–7 days, teleutospores started germinating. Slides showing various stages of germination were fixed in acetic acid-alcohol (1:3) for one hour and then flooded with 0.5% acetocarmine. Temporary preparations were made because such preparations were optically superior and were found more suitable for the study. The observations were recorded at magnification of × 1250.

## Results

Teleutospores developed on plants, if harvested and allowed to germinate, produced promycelia and basidiospores within 6–7 days at 16 °C. Attempts to germinate fresh teleutospores before November often failed whereas successful germination was always obtained when spores were collected after November. This indicates that teleutospores developed at higher temperatures, which usually prevailed in northern India before November, did not germinate, whereas those developed in colder periods, germinated profusely.

Near the apex of the single celled teleutospore, the inner wall protruded out through the outer wall and the originally papilla-like promycelium (Fig. 1 and 2) started developing until it became 60–65 μm in length. At about the time when the promycelium was very short still, the diploid nucleus moved from the cell of the teleutospore

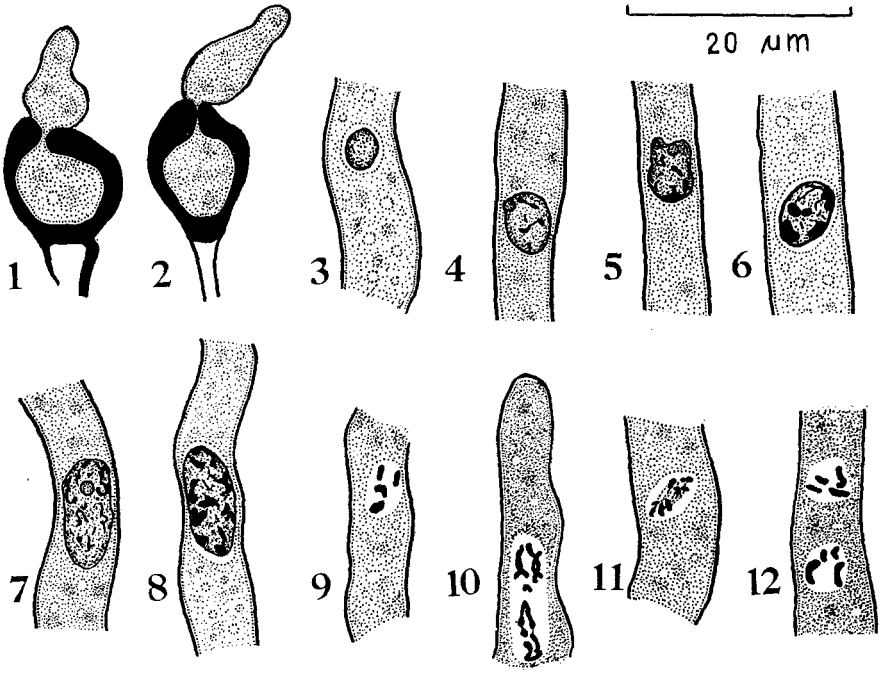


Fig. 1–12. *Uromyces fabae.* Various stages of nuclear division in promycelium. 1–2. Germinating teleutospores; 3–8. Prophase nuclei; 9. Metaphase; 10–12. Chromosomes in anaphase.
*Fig. 1–12. Uromyces fabae. Verschillende stadia in de kerndeling bij het promycelium. 1–2. Kiemende teleutosporen; 3–8. Profase; 9. Metafase; 10–12 Chromosomen in anafase.*

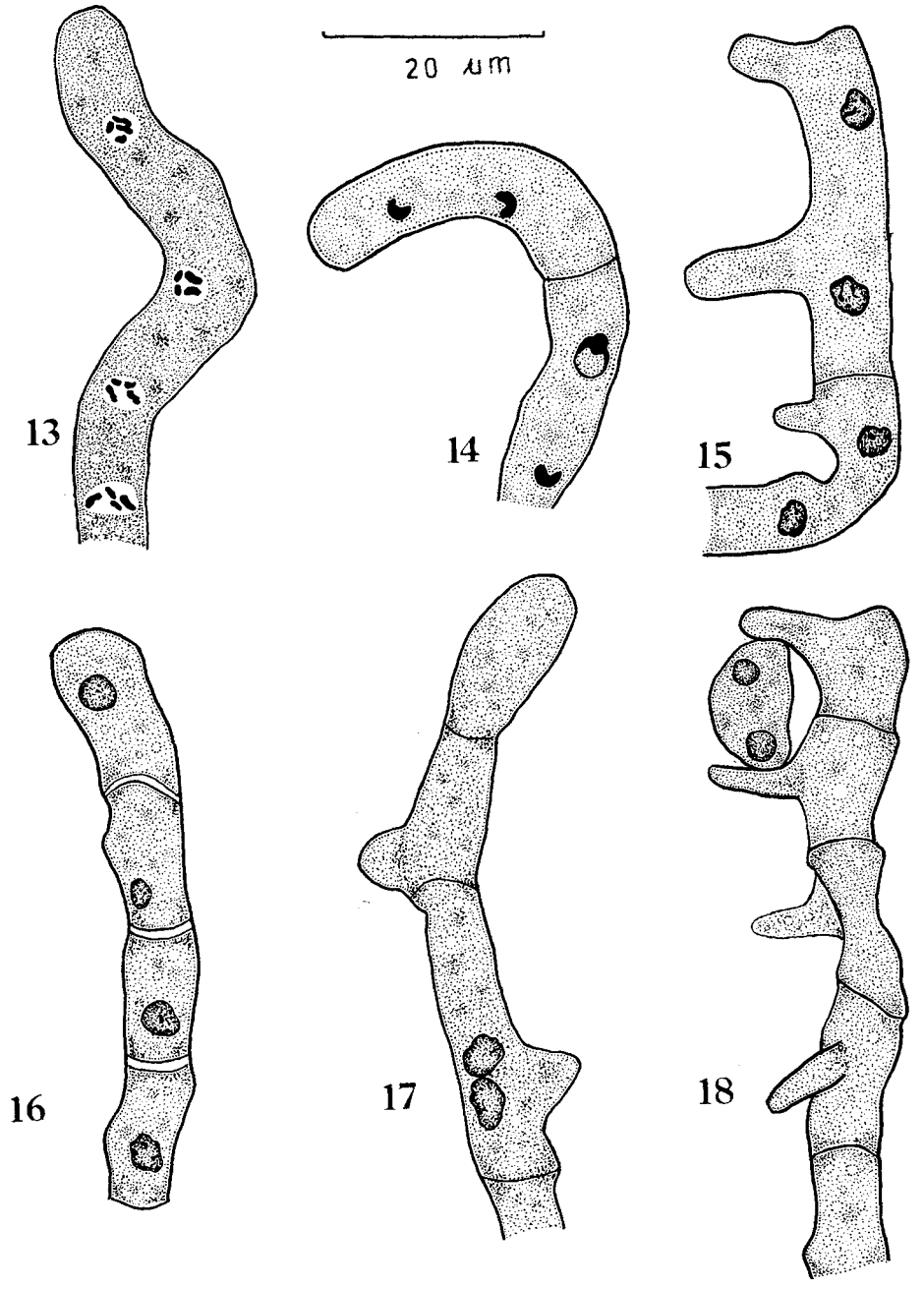


Fig. 13–18. Stages in the development of the promycelium. 13. Four-nucleate promycelium; 14–15. Four-nucleate promycelia with one septum in each; 16–17. Four- and three-septate promycelia, respectively. 18. A five-celled promycelium.

*Fig. 13–18. Stadia in de ontwikkeling van het promycelium. 13. Vierkernig promycelium; 14–15. Een septum gevormd. 16–17. Vier en drie septa aanwezig; 18. Vijfcellig promycelium.*

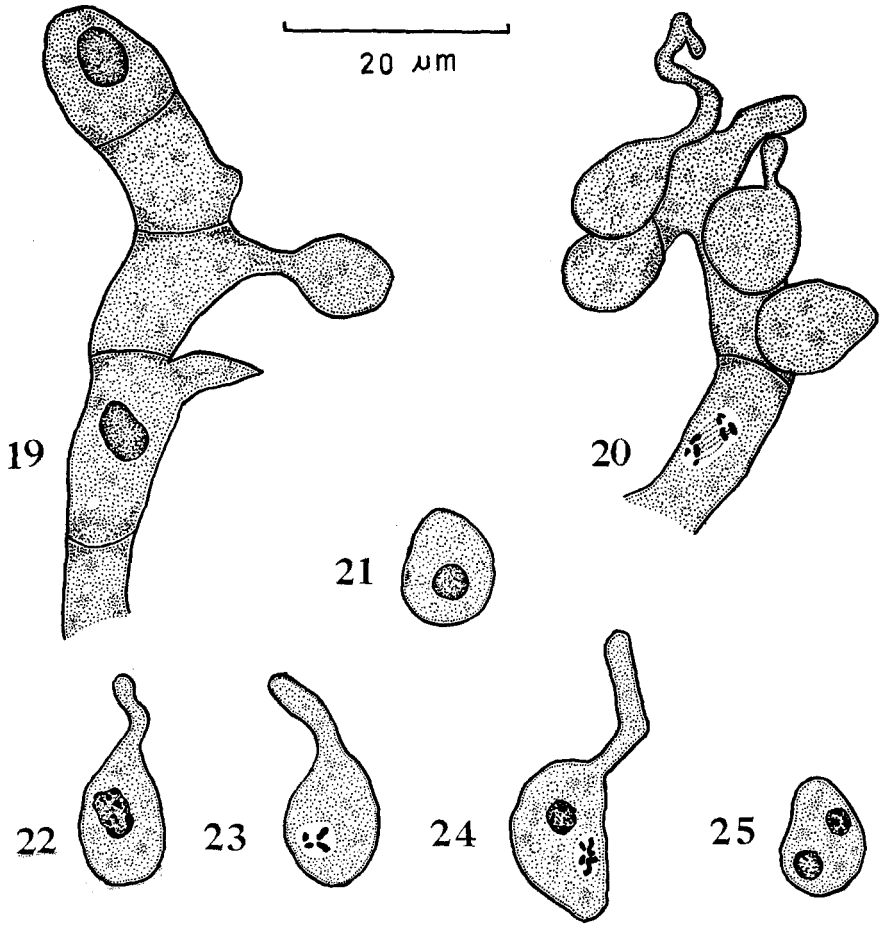


Fig. 19–25. Promycelia and basidiospores and their nuclear stages. 19. A five-celled promycelium with nuclei seen only in two cells and the initiation of sterigma and basidiospore formation in it; 20. A bent promycelium with attached basidiospores in germinating stages; 21. Basidiospore with nucleus; 22. Prophase nucleus; 23. Metaphase chromosomes; 24. A basidiospore with reconstituted and unreconstituted nuclei; 25. A binucleate basidiospore.

*Fig. 19–25. Kernstadia bij promycelia en basidiosporen. 19. Vijfcellig promycelium met kernen in 2 cellen. Begin van de vorming van sterigmata en basidiosporen; 20. Een gebogen promycelium met kiemende basidiosporen; 21. Basidiospore met kern; 22. Profase; 23. Metafase; 24 en 25. Tweekernige basidiosporen.*

and eventually became disposed about mid-way in the developing promycelium. The nucleus soon entered a period of considerable enlargement (Fig. 3–8). During these stages the chromosomes became fibrillar and thereafter shortened. The originally diploid nucleus underwent two successive divisions, one of which was a meiotic and the other mitotic (Fig. 9–15). The result was a four nucleate promycelium (Fig. 16). During the formation of septa in the promycelium, the four nuclei became so distributed in the four cells that a fifth cell at the base of the promycelium remained anucleate (Fig. 18 and 19). During nuclear division and septa formation in the pro-

mycelium, it was often noted that nuclei tended to appear crescent-shaped or ring-like (Fig. 14). Soon after the promycelium became four-celled, each of its cells developed a papillar sterigma (Fig. 15 and 17) which attained a length up to 10 μm. All four sterigmata arose from the same side of the promycelium or were alternate in arrangement (Fig. 15 and 17–19). On certain occasions it was observed that the promycelium curved backwards in such a manner that all sterigmata appeared to arise from the same level (Fig. 20). At the tip of each sterigma a basidiospore was borne which was oval in shape and 14.4 × 8.8 μm in size. Newly developed basidiospores were always uninucleate but soon became binucleate by a mitotic division (Fig. 21–25).
A study of the chromosomes at meta- and anaphases in the promycelium and metaphase in the basidiospore revealed four chromosomes arranged in two pairs of different sizes. Each of the chromosomes constituting the bigger pair measured 1.4 μm and that of the smaller pair 0.7 μm.

The germination of teleutospores and the formation of basidiospores showed a very regular and orderly sequence of events without any sign of abnormality. Nuclear divisions, both in promycelium and basidiospores, were also quite regular and normal.

## Discussion

Following teleutospore germination in *U. fabae*, the sequence of promycelium formation and its development and the nuclear events associated with it have been investigated. The study showed a normal type of promycelium as commonly found in the genera of rust fungi. In addition, however, a basal anucleate cell was always seen present in the promycelium. An anucleate basal cell was reported by Kapooria (1968) in *Puccinia penniseti* also although the developmental sequence in the two rust fungi was quite different.

Certain species of *Uromyces* have been the subject of earlier cytological investigations. Tischler (1916) studied representatives of the genera *Endophyllum*, *Cronartium*, *Melampsora*, *Thecopsora*, *Gymnosporangium*, *Uromyces*, *Puccinia*, *Phragmidium*, and *Coleosporium* and observed two chromosomes uniformly. However, Savile (1939) who was investigating nuclear structure and behaviour in species of the Uredinales, found four chromosomes in *U. fabae*, *U. hyperici*, *Puccinia sorghi*, *P. malvacearum* and *Melampsora bigelowii*. Although the cytological findings of Savile have been a matter of dispute for certain workers (Allen, 1933; McGinnis, 1956; and Pavgi *et al*, 1960) his reported case of four chromosomes in *U. fabae* appears confirmed in view of the observations reported here. Morphology of the chromosomal complement was studied in some detail and it was found that there were two pairs of chromosomes of different sizes.

## Samenvatting

*Een cytologisch onderzoek van de promycelia en basidiosporen, en het aantal chromosomen bij Uromyces fabae*

Teleutosporen van *Uromyces fabae* werden verzameld van linzeplanten. Incubatie van het verse materiaal leidde na 6–7 dagen tot kieming. Volledig gekiemde teleutosporen vormen 5-cellige promycelia. De bovenste 4 cellen van het promycelium hebben elk

een kern. Uit elk van deze 4 cellen ontstaat een sterigma met een basidiospore aan het uiteinde. De basidiosporen zijn aanvankelijk eenkernig maar worden spoedig tweekernig door een gewone kerndeling. De bovenste 4 cellen van het promycelium, en de basidiosporen hebben 4 chromosomen, in 2 paren van verschillende lengte. De onderste cel van het promycelium heeft geen kern.

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**Erratum**

In the article of A. van Dijkman and A. Kaars Sijpesteijn, A biochemical mechanism for the gene-for-gene resistance of tomato to *Cladosporium fulvum* (*Neth. J. Plant Pathol.* 77 (1971) 14-24), the drawings of Fig. 3 and 4, on page 20, should be interchanged. Thus the drawing placed as Fig. 4 belongs to the text of Fig. 3.