Bothalia, 10, 2: 329—334

Somatic Nuclear Division in Stemphylium botryosum

by

K. T. van Warmelo*

ABSTRACT

Nuclear division in an isolate of *Stemphylium botryosum* obtained from lucerne was investigated using the HCl-Giemsa technique. Vegetative mycelium was predominantly monokaryotic. Chromosome counts at metaphase gave a tentative haploid number of six. Six chromosomes could again be distinguished at late anaphase. Migration of nuclei between hyphae was observed. The conclusion is drawn that somatic divisions in this fungus are strictly mitotic.

INTRODUCTION

This investigation on the somatic nuclear division in *Stemphylium botryosum* was undertaken to investigate the mechanism and regularity of genome replications at the divisions. This would give an indication of the possible degree of aneuploidy and the stability of specific genomes which could be useful in any future investigation of the pathogenicity of this fungus.

The genus *Stemphylium* is considered to be closely related to the genus *Alternaria* and a comparison of their respective chromosome numbers and sizes could perhaps demonstrate the closeness of this relationship.

Hartmann (1964) investigated nuclear divisions in *Alternaria tenuis* and found that these followed a typical mitotic sequence. The haploid chromosome number was determined as five.

Despite many early investigations on somatic divisions in fungi (Olive, 1953; Hrushovetz, 1956) it is only recently that close attention has been given to, and success attained with, the fine structure of dividing somatic fungal nuclei.

Several possible mechanisms of division have been put forward by workers on many different fungi. These have included amitosis (Bakerspigel, 1961, 1962; Robinow, 1957a, 1957b; Saksena, 1961), atypical mitosis by means of a nuclear filament (Dowding & Weijer, 1961; Dowding, 1966; Weijer & Weisberg, 1966) and a variant of mitosis (Aist & Wilson, 1967) the interpretation of which has since been modified (Aist & Wilson, 1968).

Typical mitosis has, in contrast, been found in different genera by different workers (Somers, Wagner & Hsu, 1960; Hall, 1963; Ward & Ciurysek, 1961, 1962; Hartmann, 1964; Rogers, 1965; Hosford & Gries, 1966; Shatla & Sinclair, 1966; Knox-Davies, 1966, 1967).

* Plant Protection Research Institute, Private Bag 134, Pretoria. Present address: Dept. of Botany, Rand Afrikaans University, P.O. Box 524, Johannesburg.

Ward & Ciurysek (1962) summarised and discussed the various mechanisms of somatic division put forward by many different workers, both from the logical and from the factual point of view and concluded that ". . . the evidence . . . constitute strong grounds, therefore, for the conclusion that the somatic nuclei of fungi divide in the same manner as those of higher organisms."

Materials and Methods

The strain of *Stemphylium botryosum* used in this investigation was isolated from lucerne. The fungus was maintained on Difco malt agar. Best growth was obtained at 25°C while fairly satisfactory growth was seen at 16-17°C. Unfortunately, however, a noticeable decrease in sporulation vigour at each successive sub-culture occurred until all cultures were virtually sterile. Even transfers using conidia as inoculum failed to regenerate the original capacity for sporulation.

The nuclei were stained with the HCl-Giemsa stain used on a large number of fungi by many different workers (Hrushovetz, 1956; Ward & Ciurysek, 1961, 1962; Rogers, 1965; van Warmelo, 1966; Knox-Davies, 1966, 1967). Best results were obtained by taking blocks of agar and mycelium cut from a culture through the various solutions instead of using macerated air-dried mycelium (Ward & Ciurysek, 1962) or mycelium on Cellophane (Roane, 1952).

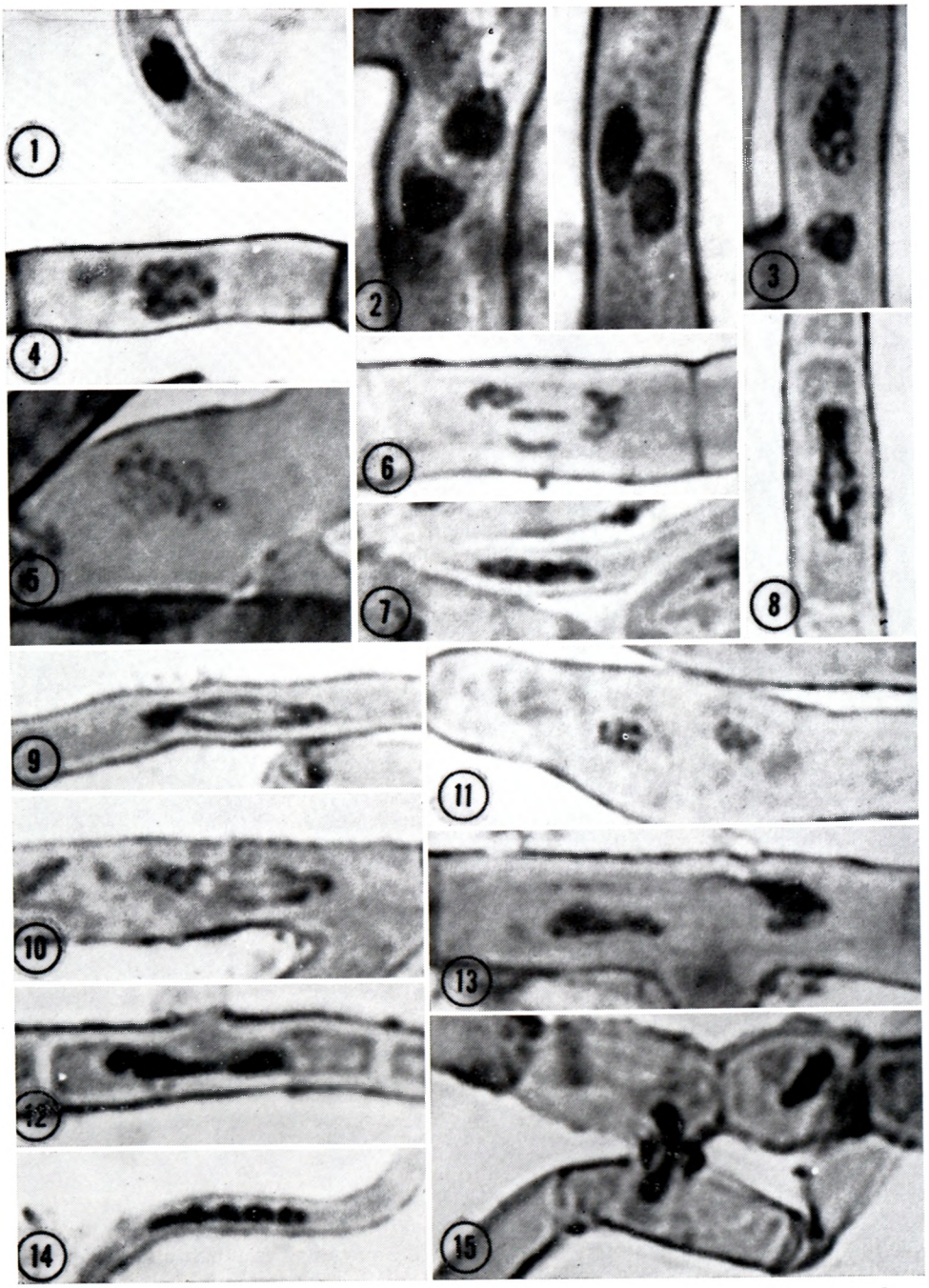
In the following descriptions of the nuclei, the nomenclature for the nuclear status of cells will be according to Jinks & Simchen (1966).

Results

Interphase

Vegetative mycelium with the nuclei in interphase was seen to be predominantly monokaryotic (Fig. 1). The nuclei were large, ellipsoid, usually centrally placed in the cells and showing little or no structural differentiation. These nuclei stained very well. Not infrequently, however, dikaryotic mycelial cells could be found interspersed between the monokaryotic cells (Fig. 2). These nuclei were similar in size, shape, degree of visible differentiation and staining intensity to the nuclei illustrated in Fig. 1. The dikaryotic condition was often associated with branched or anastomosed cells. Frequently, however, two nuclei could be found in cells where reasons for their presence were not immediately apparent.

Plate 1.— All figures are at a magnification of 3500x.
Fig. 1. Monokaryotic mycelium with nucleus in interphase.
Fig. 2. Dikaryotic mycelium with nuclei in interphase.
Fig. 3. Very early prophase nucleus showing structural differentiation.
Fig. 4. Prophase nucleus showing condensation of chromosomal material and nucleolus.
Fig. 5. Prophase nucleus showing weakly staining chromosomes.
Fig. 6. Metaphase chromosomes.
Fig. 7. Early anaphase.
Fig. 8. Anaphase with chromosomal material arranged on the outside of the spindle.
Fig. 9. Anaphase at further stage than Fig. 8.
Fig. 10. Late anaphase showing six chromosomes in left-hand nucleus.
Fig. 11. Early telophase showing six chromosomes in left-hand nucleus.
Fig. 12. Condensed late telophase nuclei.
Fig. 13. Post-division interphase nuclei.
Fig. 14. Beaded nucleus in narrow mycelium.
Fig. 15. Nucleus migrating between hyphae.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Prophase

At very early prophase the nucleus became structurally differentiated (Fig. 3), and a network of bands began to appear. The nucleus was, apparently, still surrounded by the nuclear membrane at this stage. Occasionally the nucleolus could be distinguished. At later prophase, nuclei showed a marked condensation of chromosomal material (Fig. 4). The nuclear membrane appeared to be still intact at this stage. Towards the end of prophase the chromosomes were still not highly condensed and could be seen as thin, rather weakly staining strands (Fig. 5). The nucleolus was often no longer visible and the nuclear membrane had apparently disappeared.

Metaphase

At metaphase discrete chromosomes could be seen (Fif. 6). The chromosomes were much contracted compared with prophase and stained fairly intensely. A count at this stage gave a tentative haploid chromosome number of six.

Anaphase

At anaphase the chromosomes were highly condensed and considerably smaller than at metaphase. In Fig. 7 the chromosome clumps have just begun to move apart on a structure which is regarded as a spindle. Continued movement of the chromosomes on the spindle (Fig. 8) produced a rhomboidal shape with the most densely staining material arranged around the outside of the spindle. Movement of the chromosomes continued until there was marked aggregation towards the poles with the spindle showing a large clear central area (Fig. 9). Remnants of the spindle could still be seen at late anaphase (Fig. 10). Movement of the chromosomes towards the poles appeared to be unsynchronised as at the left-hand pole the chromosomes were grouped fairly closely together and could be counted, whereas the chromosomes towards the right-hand pole were still moving. A count at the left-hand pole again gave a chromosome number of six.

Telophase

At early telophase the spindle was no longer visible (Fig. 11). The chromosomes were closely grouped but still distinct. Nucleoli were not seen. A count of the chromosomes in the left-hand daughter nucleus once more gave a total of six.

At late telophase (Fig. 12) the nuclei were highly condensed, small and often of irregular shape. No structural differentiation could be observed.

Post-division interphase

Daughter nuclei going into interphase (Fig. 13) enlarged, became somewhat diffuse, while irregular in shape, and stained as intensely as interphase nuclei before division.

General

In addition to the wide mycelium in Figures 1 and 2, a narrow mycelium was also observed, mainly at the surface of the culture medium. This narrow mycelium (Fig. 14) was also monokaryotic but the nuclei were much elongated and sometimes attentuated. Not much structural differentiation could be observed but the nuclei appeared moniliform. This shape of the nucleus is regarded as being due to the small hyphal diameter.

Although migration of nuclei from one cell to another along a hypha was not observed, migration of nuclei through anastomosing hyphae, which were frequently formed, was seen (Fig. 15). It is, therefore, highly likely that migration of nuclei along individual hyphae can occur as well.

Occasionally filamentous nuclei were observed. These showed varying numbers of granular thickenings and were very similar to the thread-like nuclei described by Dowding (1966).

Discussion

The staining time was considerably longer than that recommended by Ward & Ciurysek (1962) but was found to be the minimum time acceptable. After being stained for three hours mycelial nuclei were barely visible and the material was left to stain overnight. The fact that individual chromosomes could be distinguished at several stages is adequate proof that the staining time was not too long.

It is interesting to note the close similarity between the chromosome number of *Alternaria tenuis* (5) reported by Hartmann (1964) and the chromosome number (6) reported here for *Stemphylium botryosum*. In the absence of more detailed chromosomal data, however, no further inferences as to the relationship between these genera can be drawn.

The regularity of the divisions appeared to be high, which led to a low incidence of aneuploidy. This means that the stability of a specific genome will be high, subject of course to heterokaryotic selection.

Although occasional structures similar to the filament described by Dowding (1966) were seen they were not considered to be of any great importance, mainly because of their scarcity. In the face of an overwhelming number of figures suggestive of true mitosis, the "filaments" were considered as artifacts or transient chromosomal arrangements.

Ward & Ciurysek (1962) formulated the criteria for mitosis, i.e. demonstrable chromosomes, their alignment on a metaphase plate and the separation of chromatids to daughter nuclei. It is considered that, in the investigation reported here, these requirements were met, viz. chromosomes were demonstrated at several stages of division, the alignment on a metaphase plate was perhaps not shown but was at least suggested and movement of chromatids on a spindle was seen. It is, therefore, believed that the somatic divisions in *Stemphylium botryosum* can be accepted to be strictly mitotic.

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