MICROSPECTROPHOTOMETRIC ANALYSIS OF THE NUCLEAR DNA CONTENT IN *Tilletia indica* Mitra*

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ANALISIS MICROSPECTROFOTOMETRICOS DEL CONTENIDO DE ADN EN NUCLEOS DE *Tilletia indica* Mitra

SUMMARY

To determine changes in ploidy during the life cycle of *Tilletia indica*, various stages of the organism were stained with two fluorochromes. Results of cytofluorometric studies showed that an orderly transition of diplo-, haplo- and dikaryophase occurs in the life cycle of T. *indica*. The relative DNA content in probasidial cells was consistently greater than that of nuclei in promycelia, primary and secondary sporidia, and somatic cells. This indicates that karyogamy in teliospores is followed by meiosis and mitosis during and after teliospore germination.

RESUMEN

Para determinar los cambios en ploidía durante el ciclo de vida de *Tilletia indica*, varios estados del organismo se tiñeron con dos fluorocromos. Resultados de estudios citofluorométricos demostraron que una ordenada transición de diplo- a haplo-, y dicariofases ocurren en el ciclo de vida de T. *indica*. El contenido relativo de ADN en células probasidiales fué consistentemente mayor que el de núcleos en el promicelio, esporidios primarios y secundarios, y células somáticas. Esto indica que la cariogamia en las teliosporas es seguida por la meiosis y mitosis durante y después de la germinación de las teliosporas.

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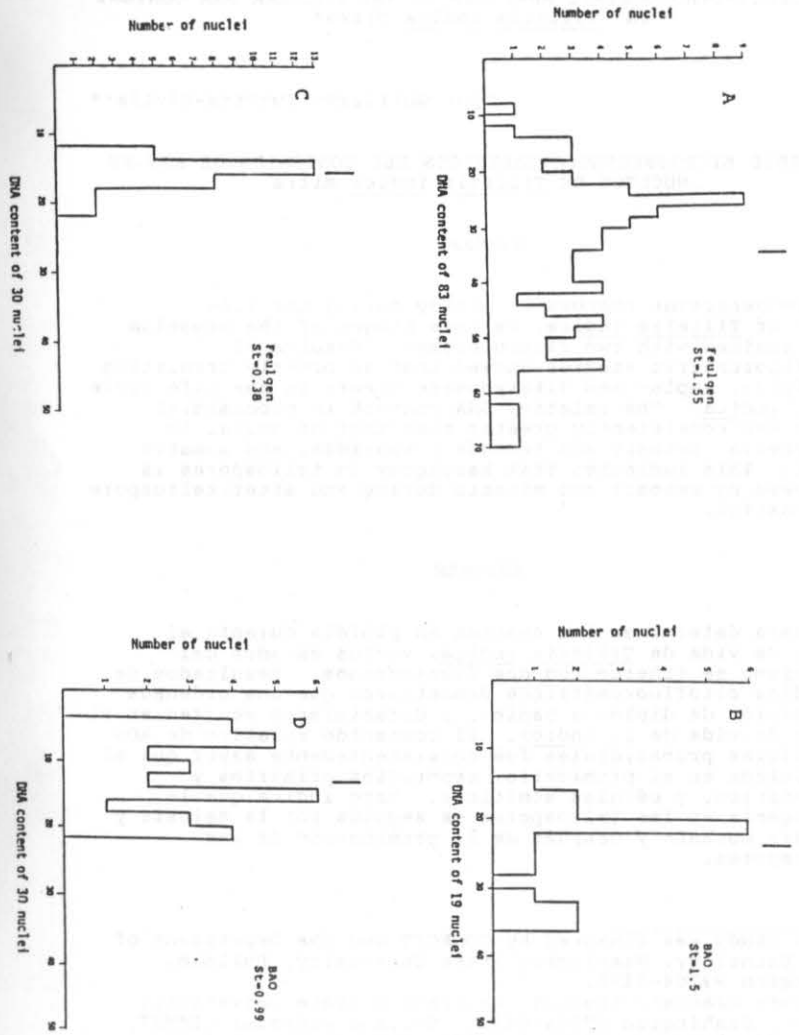

Fig. 1. Frequency distribution of DNA content (in arbitrary units) in promycelia (A, B) and in primary sporidia (C, D) of Tilletia indica stained with Feulgen and BAO. Values are indicated by a vertical line. St= standard error.

INTRODUCTION

To date, chromosomes have yet to be convincingly demonstrated in any species of smut fungi. It has been difficult to see chromosomes, largely because of their minuteness. Therefore, workers in recent years have used microspectrophotometric techniques to study ploidy levels at different stages of fungal life cycles, specially in certain Oomycetes (Rogers, 1973).

Because of its sensitivity, quantitative fluorescence microscopy is a very valuable technique in cytochemistry, and has been widely used in plant and animal cell research (Ruch, 1970). For example, cytofluorometric techniques have been widely used to study cytology of Myxomycetes (Therrien, 1966; Mohberg and Rusch, 1971; Yemma and Therrien, 1972; Mohberg et al., 1973; Therrien and Yemma, 1974; Collins and Therrien, 1976); Oomycetes (Bryant and Howard, 1969; Mortimer and Shaw, 1975); Ascomycetes (Bell and Therrien, 1977); and Basidiomycetes (Williams and Mendgen, 1975; Peabody and Motta, 1978). These techniques have provided important data, which have helped to identify changes in ploidy during fungal life cycles.

As far as it is known, cytofluorometric studies have not previously been used in cytological research related to Ustilaginales. The goal of this study was to demonstrate the changes in ploidy during the life cycle of Tilletia indica and establish a correlation with the nuclear cycle reported by Fuentes-Dávila and Durán (1986).

MATERIALS AND METHODS

Preparation of Different Stages of the Fungus

For cytophotometric studies, probasidial cells, primary and secondary sporidia, and somatic cells were obtained by the methods described by Fuentes-Dávila and Durán (1986). Basidia, primary and secondary sporidia, were grown on 0.8% water agar and sporogenous mycelia were obtained from incipiently infected wheat ovaries (artificially inoculated) on potato sucrose with 0.8% agar. Blocks of media were inverted onto microscope slides and fixed in 50% ethyl alcohol:formaldehyde:glacial acetic acid (18:1:1, vvv). The material was fixed 4-6 hours, drained, flooded with chrome alum (0.01g chromium potassium sulphate, 0.1g gelatin, and 100ml glass distilled water) and dried for 12-16 hours on a slide warmer at 40-45 °C. The dried preparations were then hydrated for 30-45 minutes at 37 °C in water, during this time the agar was carefully removed, and the specimens hydrolyzed in 3.5N HCl at 37 °C for 20 minutes (Fand, 1970). After hydrolysis, the material was rinsed in deionized water, stained with Schiff's base reagent [hereafter referred to as Feulgen

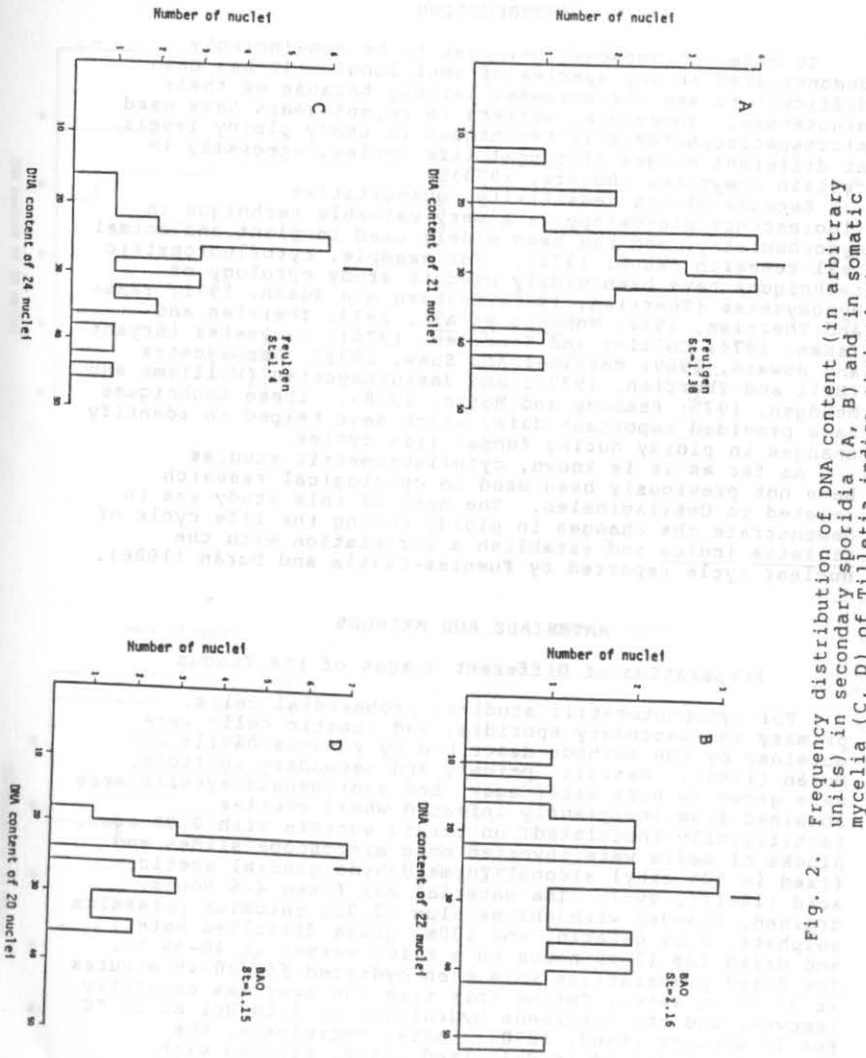

Fig. 2. Frequency distribution of DNA content (in arbitrary units) in secondary sporidia (A, B) and in somatic mycelia (C, D) of Tilletia indica stained with Feulgen and BAO. Values are indicated by a vertical line. St= standard error.

(Bryant and Howard, 1969)] for 1 hour, rinsed with three changes of a fresh mixture of sulfite water [deionized water:10% potassium metabisulfate:1N HCl (90:5:5, vvv)], rinsed in deionized water, dehydrated in an alcohol:xylene series, and mounted in nonfluorescent Entellan resin. This material was examined with a Leitz Dialux 20 EB fluorescence microscope with an N2 Leitz filter block, and a bandpass filter in the 530-560nm range. In other series of experiments the material was also stained with 2,5-bis[4'-aminophenyl(1')]-1,3,4-oxadiazole, hereafter referred to as BAO. The staining procedure was that described by Hull et al. (1982) differing only in that fixation was accomplished in Farmer's fixative [absolute alcohol:glacial acetic acid (3:1, vv)]. The preparations were rinsed in 1N HCl at 4 °C after hydrolysis, and then stained 3 hours in fresh staining solution. This material was examined with an A filter block with a bandpass filter in the 340-380nm range. Fluorescent intensities of individual nuclei were read with an MPV Leitz console which was calibrated to 100% excitation using a GG-17 uranyl glass standard. Since absolute DNA was not measured, intensity readings were given as relative or arbitrary units.

RESULTS

Consistent results were obtained with both fluorochromes. The frequency distributions of DNA content of nuclei (in relative units) during different stages of the life cycle are shown in 12 histograms (Figs. 1-3). Mean DNA content of nuclei in promycelia stained with Feulgen and BAO was 34.86 and 23.91 units, respectively [(Fig. 1A and B), hereafter mentioned in the sequence indicated to avoid repetition]. Nuclei of primary sporidia showed a mean DNA content of 15.97 and 12.63 units (Fig. 1C and D). For secondary sporidia mean values of 28.20 and 28.24 units were found (Fig. 2A and B) which is approximately twice the DNA content of primary sporidia. The mean DNA content of somatic mycelia was 28.23 and 25.75 units (Fig. 2C and D) which is similar to values of secondary sporidia. In sporogenous mycelia in which the conjugately associated nuclei were individually measured, the mean values were 30.33 and 27.14 units (Fig. 3A and B). In binucleate probasidia 33.90 and 34.28 mean values were found (Fig. 3C and D). Values of sporogenous mycelia and probasidia are similar to the mean values of secondary sporidia and somatic mycelia. It is noteworthy that large numbers of conjugately associated nuclei were consistently seen in sporogenous mycelia. The large numbers of nuclei demonstrated, were both consistent and impressive; they thus served as a basis for comparing relative mean DNA values with those of nuclei in promycelia, primary and

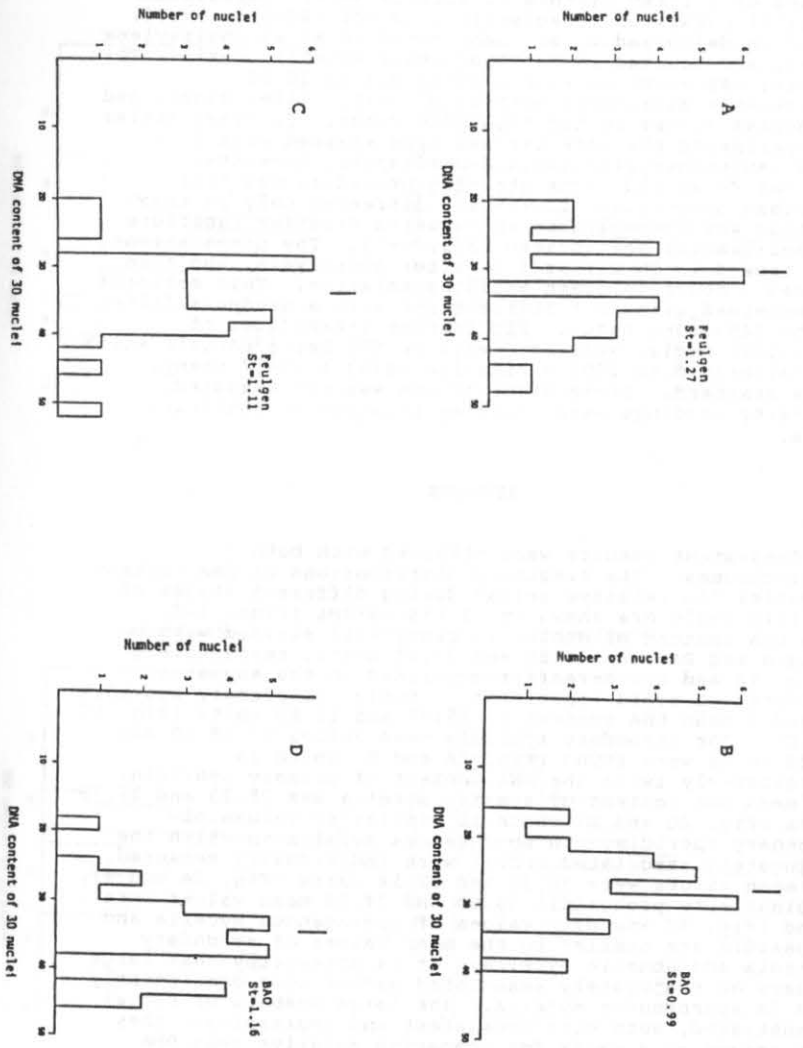

Fig. 3. Frequency distribution of DNA content (in arbitrary units) in sporogenous mycelia (A, B) and in probasidia (C, D) of Tilletia indica stained with Feulgen and BAO. Values are indicated by a vertical line. St= standard error.

secondary sporidia, somatic mycelia, probasidia with two nuclei and those with one.

DISCUSSION

Quantitative changes in ploidy (albeit relative) consistently detected by means of cytofluorometry, corroborated the nuclear cycle of T. indica, which had been demonstrated by Fuentes-Dávila and Durán (1986) using HCl-Giemsa and Hematoxylin.

The designations 1C for replicated haploid nuclei, 2C for replicating haploid nuclei or replicated diploid nuclei, and 4C for replicating diploid nuclei have been used to describe the nuclear DNA content during different metabolic states of nuclei. These designations were described by Swift (1950) and Howard and Pelc (1953), and have been subsequently used in cytofluorometric studies by other workers, and will be used in this discussion.

The transition from 1C to 4C during the nuclear cycle of the fungus, clearly showed that meiosis took place during spore germination and that karyogamy occurred in probasidia. Several workers experimenting with fungi of several classes have alluded to the 1C values of nuclei formed immediately after meiosis. Apparently, in 1C nuclei, the chromatin is highly condensed and hence tends to fluoresce less intensively than chromatin in nuclei of similar ploidy in which the chromatin is replicating and more diffuse. Evidences of this phenomenon have been reported in Saprolegnia terrestris Cookson ex Seymour (Bryant and Howard, 1969) and in Phytophthora drechsleri Tucker (Mortimer and Shaw , 1975). Mounting evidence suggests mounting that haploid nuclei in the life cycle of Saprolegniales and Peronosporales may be limited to products of meiosis in male and female gametangia. Moreover, fluorometric comparisons of haploid nuclei of Saprolegniales and Peronosporales both showed 1C values. All of the above mentioned workers reported 4C nuclei in gametangia prior to meiosis. Besides, Therrien (1966) reported that spores of Didymium iridis (Ditm.) Fr. and Didymium nigripes (Link) Fr., which characteristically form after meiosis, are haploid with 1C nuclei. Peabody and Motta (1978) have shown the same phenomenon in basidiospores of Armillaria mellea (Vahl ex Fr.) Kummer.

Secondary sporidia of T. indica consistently showed twice the DNA content of primary sporidia, and were assumed to be in a replicating haploid state (2C); however, others were in the replicated haploid state (1C) and a few in the replicating (4C) state. In the latter case, the 4C state may have resulted from the diffuse condition of chromatin, or possibly from products of fusion between sporidia, although it must be emphasized that fusions in culture were never seen, or if they were, they could not be

phenotypically distinguished.

Nuclei of somatic mycelia were consistently in a replicating haploid state (2C) which seemingly is in agreement with the characteristically rapid manner in which the fungus grew in culture. The mean fluorescence values were similar to those of secondary sporidia. Nuclei of sporogenous mycelia also were frequently in a replicating 2C state. The 2C values obtained for conjugately associated nuclei were expected on the whole, since sporogenous mycelia also grew very rapidly.

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