Meiotic chromosome of Pyricularia oryzae studied by fluorescence microscope using DAPI (4-6-diamidino-2-phenylindole) and G-banding method

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Abstract

Meiotic chromosome of Pyricularia oryzae were described using DAPI staining and G-banding method. With DAPI technique we have succeeded in observing chromosomes of this fungus at the sexual stage, but this technique failed to stain the sexual stage of this fungus. By using G-banding chromosome of Pyricularia oryzae showing the typical G-bands at prometaphase stage could be observed. The G-banding patterns between homologous chromosomes were apparently consistent, although the chromosome number 1 and 2 assumed as the shortest chromosome (1.8 um). The binding patterns of nos 3 and 4 (2.3 um) are quite similar to each other and also to chromosome nos 5 and 6 (2.2 um).

Keywords: Pyricularia oryzae - DAPI - G-banding

Introduction

It has been documented that Pyricularia oryzae has a large number of economically important crops; such as corn, millets, barley, rye, and wheat as its host. But none of them, except finger millet and wheat blast disease is consider to be a major disease. Many attempts have been made to estimate yield loss caused by this disease but exact figures are few. In some region damages were calculated to be more than 50% in epidemic areas (Urashima et al., 1993).

The discovery of the sexual cycle in Pyricularia oryzae (Kato and Yamaguchi, 1982) was the fundamental importance to conduct studies concerning nuclear division and chromosome behavior of the fungus. Leung and Williams (1986) and Nirmala (1995) reported, chromosome number of Pyricularia oryzae are determined to be six by using Giemsa staining procedure.

In continuing the attempt to make clear the meiotic chromosome of Pyricularia oryzae, we examined the meiotic chromosome by using DAPI (4-6-diamidino-2-phenylindole) and G-banding method. DAPI staining is one of the regional banding method which can be used to label subsets of chromatic regions in the chromosome complement. However only few studies have been performed on the DAPI staining during meiosis to date (Haaf et al., 1986).

Recently, Taga and Murata (1994) investi-
gated the cell division in germinating conidia and mitotic chromosomes of Botrytis cinerea, Alternaria alternata and Nectria haematococca by fluorescence staining using DAPI (4,6-diamidino-2-phenylindole). They reported this staining method (DAPI) was more useful to stain the chromosome than Giemsa staining as reported previously by Shirane et al., 1988 in germination conidia of Botrytis cinerea. They recognized the size of the chromosomes, the number of chromosome exhibited a threadlike structure by using fluorescence microscope and samples were stained by DAPI solution. It was demonstrated that G-bands were unequivocally present in some higher plants chromosomes (Kakeda et al., 1990). When these methods are used, it is still difficult to identify all chromosomes in plant species due to coarseness in pattern of bands. Kakeda et al. (1990) identified the wheat chromosome by G-banding and these chromosomes were recognized as a standard for most cytological studies. However they have not reported the use of DAPI procedure and G-band in the sexual stage of fungi. Therefore in this study we examined the use of DAPI and G-banding method in both asexual and sexual stages of Pyricularia oryzae with slight modification.

Materials and Methods

Preparation of conidia. Conidium isolate of Exsises cornaca strain of Pyricularia Z 2-1 (230) was grown on oat meal medium. The fungus was incubated for 4 days at 25°C and exposed 3 days near ultraviolet light, then sporulated in this condition for several days. Conidia were formed, collected, and washed with distilled water and concentrated into 1 x 10^6 spores/ml.

Observation of the chromosome. Conidia on slide glass were incubated 6-9 hours on Potato Dextrose broth (PDB), and the germinated conidia were washed by water, and were treated with methanol : acetic acid solution (methanol : acetic acid = 9:1, 17:3, 3:1 v/v) for 30 minutes each at room temperature and flame dried. They were immediately stained with DAPI.

Preparation of asexopore. Two isolates of Exsises cornaca strain of Pyricularia, G(10-1) (279) MAT 1-1 and Z(2-1) (230) MAT 1-2, were used in this study. Perithecia were produced 14 - 18 days after inoculation. Single perithecium was isolated from medium under stereo microscope, and then pretreated with colchicine for 2 hours. Perithecia were subsequently dipped in hypotonic solution (KCl) for 15 minutes at 37°C.

Staining preparation. Asci were fixed by methanol : acetic acid (3:1) 30 minutes and flame dried. Samples were stained by 1 um DAPI solution dissolved in NS buffer solution (20 mM Tris-HCl at 7.6, 0.25 M Sucrose; 1 mM EDTA; 1 mM MgCl2; 0.1 mM ZnSO4; 0.4 mM CaCl2; 0.5 % 2-mercapto ethanol). Samples were observed by a fluorescence microscope.

Slide preparation for G-band. The perithecia were treated with 0.05 % colchicine solution for 2 hours at 25°C. Perithecia were subsequently dipped in the Okinakis hypotonic solution (55mM KCl, 55 mM NaNO3, 55 mM CH3COONa, 10:2 for 30 minutes to 1 hour at 25°C. Perithecia were fixed in methanol:acetic acid = 3:1 centrifuged at 1500 rpm for 5 minutes and then placed in the fixation solution two times. Fixed perithecia were subsequently crushed on the slide glass and fixed overnight at 60°C. The sample were dipped into 0.25 % Trypsine solution for 10-20 seconds, and then washed by water and air dried. The samples were dipped in 70% alcohol for 1 minute. They were stained in 5% Giemsa solution for 10 minutes and after treatment, the slides briefly washed and air dried.

Results

After 9 hours incubating, the germinated conidia of P. oryzae fixed by methanol : acetic acid (9:1;17:3:3:1 v/v) stained by DAPI. Figure 1 shows the germinated conidia of P. oryzae fixed by methanol : acetic (3:1) and stained by DAPI. With 9 hour-old germinated conidia of P. oryzae some of the chromosomes have stained by DAPI solution. For sexual stage, DAPI solution could not work to stain the contents of ascus.

Figure 1. Germinating conidia of Pyricularia oryzae stained by DAPI observed under fluorescence microscope.

Figure 2. Chromosome identified by G-banding technique. They were obtained by trypsin and pretreatment by colchicine. Bar = 5 um.

Figure 3. G-banded chromosomes showing the karyotype. Bar = 5 um.

Chromosome of P. oryzae showing the typical G-band at prometaphase stage are presented in figure 2. Trypsine was used as an enzyme to cause the dispersion of the chromosome to enable better observation under the microscope. This treatment were carried out within one minute only in order to avoid chromosome becoming swollen or destroyed. After treatment with trypsin the chromosome could not be clearly observed, therefore Okinakis hypotonic solution was used after colchicine to enhance the clarity of the bands. For the fixation different ratio of methanol to acetic acid were tried but the best result was obtained when the ratio was 3:1. The pachytene chromosome related to G-band of metaphase chromosome in Pyricularia oryzae was not easily observable because separation of asci after crushing the perithecia was difficult.

Figure 3 shows the karyotype of a G-banded prometaphase plate, arranged tentatively in order of the chromosome number. The G-banding pattern between homologous chromosomes were apparently consistent, although the chromosome number 1 and 2 assume the shortest chromosome. The banding pattern of chromosome nos 3 and 4 are quite similar to each other and also to chromosome nos 5 and 6.
Discussion

In a study by Shirane et al., (1988), HCl-Giemsa technique could be used to observe the number of nuclei and chromosomes in germinating conidia of Botrytis cinerea. Moreover, Taga and Murata (1994), reported that by using DAPI technique chromosome number could be determined in Botrytis cinerea, Alternaria alternata, Nectria haematodes. In this work DAPI was successfully used to stain nucleus of conidia of Pyricularia oryzae (Figure 1) but did not work in sexual stage. From this study the reason for this failure could not be clarified. It can therefore be concluded that DAPI staining could become an important aid in studying chromosome of Pyricularia oryzae.

Using G-band staining method it is possible to obtain G-bands of chromosome in Pyricularia oryzae. For the enzymatic treatment using trypsin a concentration of only 0.25% was required to cause dispersal of the chromosome in Pyricularia oryzae. This was different from report in other plants where a concentration of 2% was used (Kakeda et al., 1990). One important problem associated with this method was that, the actual number of chromosomes in ascii may not be determined with certainty because some of them might be lost during the crushing process.

This study could not conclusively determine the karyotype of the chromosome in Pyricularia oryzae since some G-bands were not as clear. In Pyricularia oryzae chromosome size is 1.8-2.3 μm which about 1/4 of chromosome size 8.51 μm in wheat (Ezdo, 1986). Therefore the small size of chromosome presented a major difficulty encountered during observation of chromosome. The G-banding method of this fungi which have been developed here can be applied and practically used in other fungi.

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References


Introduction

Sugarcane is a large perennial grass. It belongs to the genus Saccharum, 2 complex genus which comprises six species. The genome organisation of these species are very complex. They are highly polyplody (Daniels and Roach, 1987). Collection and conservation of sugarcane germplasm are important for the most sugar research institute in the world (Roach, 1986; Berding and Roach, 1987). Indonesia has been known as one of the center of diversity of Saccharum germplasm. Since 1916 nine sugarcane collecting expeditions have been conducted, covering most areas of Indonesia. From North Sumatra to Irian Jaya (Berding and Koike, 1980; Lamadji, 1986; Sastrowijono et al., 1996; Tow et al., 1991). Hundreds of Saccharum germplasm accessions have been collected and conserved in the Indonesian Sugarcane Research Institute (ISRI).

Management of this large collection is complex and costly task. Furthermore, it is apparent that it often conserves more than one accession of the same genotype. It would, therefore, be advantageous to