Rice Meiotic Chromosome Spread Preparation of Pollen Mother Cells

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[Abstract] In this protocol, we describe a simple and efficient method for meiotic chromosome spread preparation in rice pollen mother cells. Meiotic chromosome preparation by spreading itself is an important technique for plant cytogenetics (Higgins *et al.*, 2004; Chelysheva *et al.*, 2012; Wang *et al.*, 2009); furthermore, it is a crucial step for applying other cytogenetic methods including Fluorescence *in situ* hybridization (FISH) and immunostaining.

Materials and Reagents

1. Young panicles containing meiocytes of rice (Oryza sativa ssp japonica cv. Zhonghua 11)
2. 70%, 90% and 100% ethanol (Analytical Reagents)
3. 60% (v/v) acetic acid (Analytical Reagents)
4. Carmine (Sigma-Aldrich, catalog number: C1022-25G)
5. Liquid nitrogen
6. Vectashield Mounting Medium with DAPI (Vector Laboratories, catalog number: H-1200)
7. Carnoy's fixative (see Recipes)
8. Aceto-carmine (see Recipes)

Equipment

1. Stereo microscope
2. Fluorescence microscope (Zeiss, model: AX10)
3. Microscopic slides and cover slips
4. Dissection needles and fine forceps
5. Heating block
6. CCD camera (Hamamatsu Photonics K.K., model: ORCA-R2 C10600)

Procedure

1. Fix young panicles by immersing whole young panicles (from 4 cm to 15 cm in length) at

proper developmental stages into about 100 ml freshly prepared Carnoy's solution at room temperature for 2-4 h and store the fixative materials in Carnoy's solution at 4 °C.

2. Dissect about 15 florets under stereo microscope and remove all parts except anthers on a microscopic slide.
3. Put the anthers into 20 µl Aceto-carmine stain on a slide, and keep cutting the anthers with a small scalpel until no obvious large debris were observed.
4. Add 30 µl 60% (v/v) acetic acid to chopped anther on the slide and put the slide on a heating block at 50 °C for 2 min.
5. Cover the slide with a piece of glass cover slip and put the slide and cover slip under a piece of filter paper, then press the cover slip down tightly with thumb for 10 sec to squash the meiocytes. Keep the slide at -20 °C for at least 30 min (keep the glass slides from cracking when it was placed into liquid nitrogen next step).
6. Put the squashed slide into liquid nitrogen for 5 min and remove the cover slip with a scalpel.
7. Dehydrate the slides for 2 min in 40 ml of 70% ethanol, 90% ethanol and 100% ethanol, sequentially.
8. Add 15 µl VECTASHIELD Mounting Medium with DAPI on the air-dried slide, and seal the slide with a glass cover slip.
9. Observe the chromosome spreads under fluorescence microscope and capture the images with CCD camera (Figure 1).

Representative data

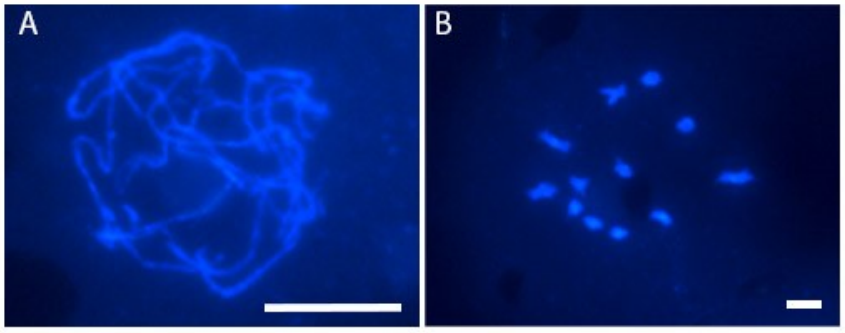

Figure 1. Male meiotic chromosome spread by DAPI staining at pachytene (A) and diakinesis (B) in wild type. Bar= 5 µm

Recipes

1. Carnoy's fixative

Ethanol: Glacial acetic acid 3: 1 (v/v)

Freshly prepared

2. Aceto-carmine

   0.5 g carmine was dissolved in 100 ml boiling 45% acetic acid

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