A Technique for Staining Rice Chromosomes

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Studies of rice chromosomes are handicapped by the small size of the chromosomes and the lack of a satisfactory technique for revealing the details of chromosome structure. No analysis of the mitotic complement has yet been satisfactory. Only a few workers (Nandi 1936, Pathak 1940, Hu 1958, 1960, 1964, Ishii and Mitsukuri 1960, Sen 1963) have attempted to analyze the mitotic metaphase complement. Neither have haploids yielded an appreciable advance toward identification of the chromosomes, although Hu (1958, 1960) obtained well stretched preparations through cold treatment. Disagreement is considerable even as to the centromeric position of the different chromosomes.

Attention therefore, has switched to pachytene analysis (Shastry and Misra 1961, Shastry et al. 1960, Shastry 1964), but success was only moderate. Identification of each chromosome by its morphological features is still a problem. It is difficult to stain pachytene chromosomes so that details are in critical contrast. The bright-field light microscope does not accentuate the differences in chromosome segments and chromomeres to the same extent as does phase-contrast. Overstaining of chromosomes with phase-microscopy is frequently a problem, obliterating all the details. The techniques described below overcome such difficulties by combining critical staining with phase-contrast microscopy.

Method for root tip metaphases

1) Pretreat growing roots from germinating rice seeds when they are about 2 cm long with a saturated aqueous solution of α-bromonaphthalene for 1–1½ hours at room temperature.

2) Cut out the roots and fix in 3:1 ethanol acetic acid to which ferric chloride crystals (about 0.5 gm/500 ml fixative) have been dissolved to give it a fairly deep straw color. Fix for 3 to 15 days. Roots can be left in fixative for more than 2 months without any difficulty in obtaining good preparations. This long fixation permits good adsorption of iron onto the chromosomes. Deeply stained, sharply contrasted chromosomes are obtained without adding more iron to the stain.

3) Cut root tip (0.6–1 mm) from the fixed roots and place in two small drops of diluted acetocarmine (0.25%). Maceration of the tip is optional.

4) Place coverslip, and heat over a spirit flame without boiling until the material turns almost black. No hydrolysis with HCl is necessary.

5) Squash with blunt needle-point. One moderately firm tap on the tip should
6) Once the cells are separated, apply firm pressure on the coverslip under a blotter to spread out the cells.

7) Examine under microscope (phase 2, objectives $16\times$ or $40\times$). Each root tip usually yields 10–15 well-spread metaphase plates.

Method for meiotic prophase (pachytene, diplotene)

1) Fix immature panicles in the same fixative as for root tip (1:3 acetic alcohol + FeCl₃) between 10:30 A.M. and 1 P.M. The spikelets need not be cut open. Usually the best stage for collection for meiotic studies is when the junctura of the flag leaf is about 1 cm out of the boot. Fix for at least 3 days to as long as 20 days. The whole panicles can be left in the fixative for 2 months or more at room temperature without any appreciable difficulty. Metaphase chromosomes tend to become difficult to spread after prolonged fixation.

2) Identify stage of meiosis by examining an anther from a spikelet squashed in a drop of 1% acetocarmine stain.

3) Place anther in desired stage of division in a drop of 1% acetocarmine. Break up to release the pollen mother cells. Remove debris. Another drop of acetocarmine may be added before placing the coverslip.

4) Heat over a spirit flame for at least 30 seconds (intermittently) almost to the point of boiling. Two small drops of acetocarmine may be placed on the sides of the coverslip to prevent drying out.

5) Squash under one or two thicknesses of blotting paper. The pressure to be applied depends on the stage and length of fixation.

6) Slides can be made permanent with 'Euparal' mounting medium following dehydration in absolute alcohol.

Note: In general, for condensed chromosomes, use light stain (0.25–0.5%). A higher strength of carmine will overstain such chromosomes for phase-microscopy.

Results and discussion

Mitotic chromosomes are stained deep black against a slightly stained cytoplasmic background. In pachytene, all the chromomeres stain darkly, giving very clear-cut details against a uniform slightly stained background. A pretreatment with α-bromonaphthalene, not used by other workers, accentuated the primary and secondary constrictions.

Meiotic divisions in any one spikelet are very nearly synchronized, with a few cells in an earlier or later stage than the predominant one, so that one anther is enough to identify the meiotic stage of development. Spikelet development in rice proceeds from the tip to the base of the panicle. Late pachytene chromosomes offer well-spread preparations, but for pairing studies, some confusion over homology can arise occasionally since the bivalents may have begun separating at various points.

Diakinesis, metaphase I, anaphase I, metaphase II, and anaphase II stages (in general all condensed chromosomes) need weak stains (0.25–0.5%), whereas pachytene needs 1% or more for study under phase-contrast. Stains of 1.5% or more tend to overstain the pachytene chromosomes.

Abstract

A technique is described for staining both mitotic and meiotic chromosomes of rice. Important features are: comparatively long fixation; elimination of hydrolysis
for root tip chromosomes; and phase-contrast microscopy. The chromosomes stain deeply, the amount being varied to suit any particular purpose by using different strengths of the stain. The technique brings out the morphological features of the chromosomes in their greatest detail.

References