

Chapter 1

C-Banding of Plant Chromosomes

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Abstract

C-banding is used to differentially stain metaphase chromosomes in organisms having appreciable amounts of constitutive heterochromatin. Its primary benefits are that it is an inexpensive and a relatively fast method of identifying individual chromosomes and morphological or karyotypic variation, including large chromosomal rearrangements and aneuploidies. We currently employ this technique with considerable effect in genome analysis of oat (*Avena sativa*) and related grass species, though it has been most extensively used for chromosome analysis of wheat (*Triticum aestivum*) and its relatives of the Triticeae.

Key words C-banding, Oats, *Avena*, Triticeae pooideae, Karyotyping

1 Introduction

The C-banding technique has been used extensively since the 1970s for cytogenetic studies of plant morphology, most notably in the Triticeae (Fig. 1), and its use has persisted through the era of molecular cytogenetics due to its relatively low cost and rapidity (for comprehensive review, *see* ref. 1). The first reports were published in 1973 in *Allium* [2–4], *Trillium*, *Vicia*, *Fritillaria*, and *Scilla* [5], and in the Triticeae for rye [6]. For example, C-banding is a more practical method than *in situ* hybridization for identifying gross morphological or karyotypic variation (Fig. 2) in large numbers of samples. It is also a much more accessible technique for scientists in the developing world, since C-banding includes no requirement for fluorescent microscopy, DNA labeling and hybridization reagents, or even computer-based imaging systems.

The basic C-banding method involves a series of chemical treatment steps of metaphase chromosome preparations affixed to standard microscope slides. Typically, metaphase chromosomes are accumulated through pretreatment with mitotic spindle inhibitors such as colchicine, colcemid (demecolcine), amiprophos-methyl (APM), 8-hydroxyquinoline, monobromonaphthalene, trifluralin

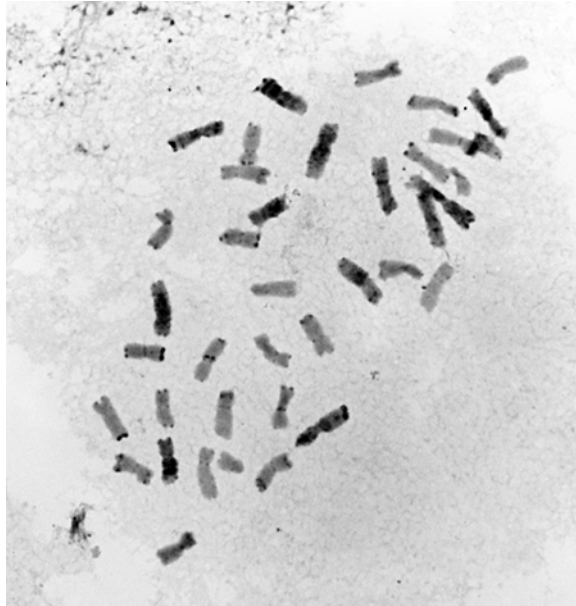


Fig. 1 C-banded somatic metaphase chromosomes of *Avena sativa*

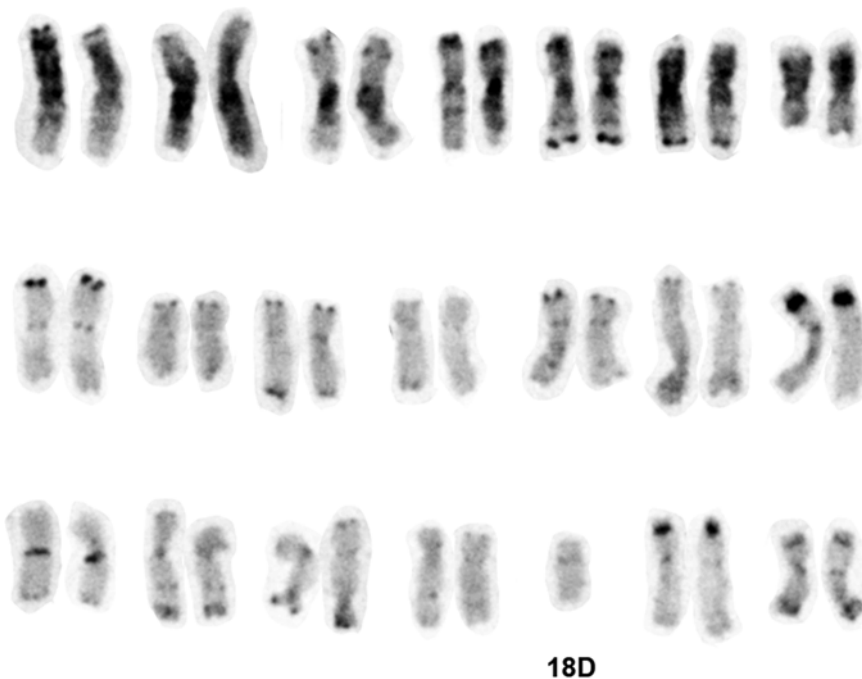


Fig. 2 C-banding karyotype of an *Avena sativa* plant monosomic for chromosome 18D (labeled)

(Treflan), ice water, or nitrous oxide gas. The chemical treatment steps of the prepared slides consist of an initial depurination wash in hot, dilute HCl, a wash in a concentrated alkaline solution at room temperature to denature the chromatin, a controlled renaturation wash in saline sodium citrate (SSC), and then staining in a phosphate-buffered eosin methylene blue-based stain.

The basic staining procedure was a modification from Giraldez et al. [7] and was first applied to *Avena* chromosomes in 1993 [8]. The inclusion of nitrous oxide gas treatment to arrest metaphase root-tip chromosomes follows the procedure of Kato [9].

2 Materials

Solutions do not need to be prepared with ultrapure water, as long as they are cycled through within a few months and are stored in the refrigerator.

2.1 Solutions

1. Farmer's Solution: 3:1 95 % ethanol: glacial acetic acid.
2. 45 % (v/v) acetic acid.
3. 100 % ethanol.
4. 20× SSC stock: 3 M NaCl, 0.3 M Na-citrate pH 7.0, adjusting pH with HCl and 5 M EDTA, if necessary.
5. 2× SSC is freshly prepared from a 20× SSC.
6. Giemsa stain solution: 1/15 M phosphate buffer [1:3 (KH₂PO₄:Na₂HPO₄)]. Geisma can be stored in tightly sealed bottles at room temperature (*see Note 1*).
7. Barium hydroxide: barium hydroxide octahydrate can be stored in tightly sealed bottles at room temperature. Solution is made by dissolving 300 g of Ba(OH)₂·8H₂O in 500 mL of distilled water. As long as crystals remain at the bottom of the flask, distilled water can be added to increase the volume of the saturated solution.
8. 0.2 M HCl: made freshly each time from a 1 M HCl stock.
9. Xylene.

2.2 Materials

1. Nitric oxide gas (N₂O).
2. Toluene-based mounting medium (e.g., Cytoseal).
3. Cover slip (#1 thickness).
4. Slide jars or trays for slide treatments.
5. Light microscope with phase contrast capability.

3 Methods

3.1 Root-Tip Metaphase Chromosome Pretreatment

1. Germinate seeds, embryo side up, on filter paper in petri plates at 19–21 °C in distilled water.
2. Excise root tips when 1–2 cm long, and treat with 130–150 psi N₂O gas for 1.5–3 h.
3. Fix in Farmer's Solution overnight at room temperature.

3.2 Chromosome Preparations

1. Make a preparation of mitotic chromosomes in 45% acetic acid. You can tap the cover slip over the root tip with a pencil tip while holding one edge of the cover slip stable using two fingers to break up the meristem. Another way to do it is to microdissect the root tip and squeeze or scrape out the meristematic cells and then discard the remainder of the root tip. If the mitotic index is high, either method will work fine.

IMPORTANT: Do not use acetocarmine! Chromosome preparations can only be inspected using phase contrast microscopy at this step.

2. Heat the bottom of the slide gently using the open flame of an alcohol burner until it is hot to the touch. If it boils, remake the preparation.
3. Freeze slide on dry ice 5 min, pry off the cover slip using the edge of a razor blade, dehydrate in 100% ethanol for at least 2 h, and then air-dry.

3.3 C-Banding Protocol

1. Treat slides with 0.2 M HCl, 60 °C for 2.5 min *precisely* (*see Note 2*), rinse, let slides drain *but not* totally dry.
2. Soak slides in saturated BaOH₂ at room temperature for approximately 7 min, and replace barium hydroxide completely with gently running warm (not hot) tap water (*see Note 3*). Then remove the slides and let them drain *but not* totally dry.
3. Soak slides in 2× SSC at 60 °C, for approx. 40 min, then shake off solution gently.
4. Stain slides in Giemsa stain solution (*see Note 1*). To check staining progress after 10 min, carefully remove slides to avoid iridescent filmy precipitate on the stain surface and rinse in two changes of tap water and then drain excess water. Slides can be inspected under a cover slip while wet and then float cover slip off in water (*see Note 4*).
5. Soak slides for a few minutes in xylene; mount using a small drop of mounting medium under a cover slip (#1 thickness) (*see Note 5*).
6. Observed slides using light microscope. Dark C-bands correspond to heterochromatic regions of the chromosomes. In plants, these bands are typically found at the centromeric and telomeric regions of the chromosome.

4 Notes

1. The Giemsa stain source is critical; I have used prepared liquid Giemsa from Sigma-Aldrich (#GS500) for many years, and the quality has been consistently excellent up to approximately 6 months past the expiration date on the bottle. In the past, I

have also used Leishman and Wright stains, but would not recommend them now.

2. IMPORTANT: Timing is critical! If not treated for a sufficient length of time, the staining will be overly blue; too long of a treatment results in pale pink staining.
3. For BaOH₂ treatment, break surface film on the BaOH₂ solution with the edge of the slide to avoid depositing a layer of the precipitate film over the area containing the chromosome preparation! Slides should not be lifted directly out of the solution; instead, displace the BaOH₂ solution with a slow stream of warm tap water for 1–2 min to avoid deposition of barium carbonate precipitate onto the slide surface. Timing in BaOH₂ solution is not critical, ±1 min.
4. If chromosomes are too purple, increase amount of time in the HCl step. If chromosomes are too pink, decrease time in HCl.
5. Mounting medium should be clear and colorless so that it will not crack or discolor with age (e.g., Cytoseal). Do not use Euparal media.

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