Vol. 16 No. 3–4 1988 Cereal Research Communications

A NEW BANDING TECHNIQUE FOR CHROMOSOMES OF WHEAT (*TRITICUM*) AND ITS RELATIVES*

X.M. Shang[1], R.C. Jackson[2] and H.T. Nguyen[1],
[1] Department of Plant and Soil Sciences, [2] Department of Biological Sciences,
Texas Tech University, Lubbock, Texas, 79409, U.S.A.

Summary

A new Giemsa banding technique has been developed for chromosomes of wheat and other cereals. After pretreatment with a dilute solution of 1-bromonaphthalene, fresh root tips are hydrolyzed in 5N HCl, squashed, and then air-dried. The air-dried slides are then treated with hot 1N HCl and 0.07N KOH at room temperature. The new procedure makes it easy to obtain well-separated and sharply-banded chromosomes, including centromeric banding, and the heterochromatin banding patterns are highly reproducible. We designate this as the HKG (HCl-KOH-Giemsa) banding method.

Index words: HKG banding, heterochromatin, chromosomes, wheat.

Introduction

Although there are many chromosome banding procedures that have been used in cytological and cytogenetical studies of wheats (Gill and Kimber,1974; Gerlach, 1977; Jewell, 1981; Hutchinson et al., 1982; Seal, 1982; Endo, 1986), these can be classified as BSG ($Ba(OH)_2$-2SSC-Giemsa) and HABG (Hot-Acid-Buffer-Giemsa) or N-banding, based on the reagents or key chemicals that have been applied to those procedures. However, all of these employ Giemsa staining. We face two difficult problems in applying the BSG banding procedures to the studies of the genetic diversity and heterochromatin differentiation of wild and cultivated diploid and polyploid wheats from different germplasm collections. One involves the low acid concentration (usually 0.1-0.2N HCl) that various procedures use to hydrolyze the root tips, but this acid concentration does not produce easily separated cells that can be squashed so as to give well-separated chromosomes. Another difficulty is that the $Ba(OH)_2$ easily reacts with CO_2 in air to form $BaCO_3$ particles that may prevent clear chromosome banding during the process.

* Contribution of the College of Agricultural Sciences, Texas Tech University. Journal No. T-4-220.

The N-banding technique has the advantages of simplicity, but it clearly shows the banding patterns of only 16 of the 21 pairs of hexaploid wheat chromosomes. The procedure usually does not band chromosomes 1A and 3 to 6D of hexaploid wheat and the A genome chromosomes of diploid *T. monococcum* (Gerlach, 1977; Endo and Gill, 1984).

The present communication describes a method using a high acid concentration (5N HCl) and a new base reagent (KOH) that allows one to make slides relatively easily and to show heterochromatin banding by the new Giemsa banding schedule.

Materials and Methods

Germination and pretreatment: Wheat seeds are germinated on the surface of 30 ml water in 125 ml flasks at room temperature as are all subsequent treatments. Root tips are collected from 12:00 to 1:30 p.m. and pretreated for 2.5 h. with an aqueous solution of 1-bromonaphthalene (.01 ml. stock solution in 10 ml of water; stock solution is 1 ml 1-bromonaphthalene in 100 ml absolute ethanol). The root tips then are washed twice in distilled water and hydrolyzed with 5N HCl for 20 minutes. This is followed by two washes in distilled water; the material is then strored in 45% acetic acid prior to squashing.

Slide preparation: Slides are cleaned in 95% ethanol and wiped dry. The meristematic part of the root, about 1 mm long, is cut from the root tip with a razor blade on a clean slide in a drop of 45% acetic acid. A coverglass is added and the meristematic tissue is spread by first tapping on the coverglass with a dissecting needle and then by squashing hard. Next, the slide is dipped in liquid nitrogen for 3 minutes, and then the coverglass is removed with a razor blade before the tissue thaws.

Banding: The slides are air-dried and stored for 3 to 7 days. Dried slides are then treated with 1 N HCl at 60C for 6 minutes and washed 4 times in distilled water for a total of 10 minutes at room temperature. Slides are then air-dried half a day, dipped into fresh 0.07 N KOH solution for 20-25 seconds followed by dipping into 1/15M Sorenson's phosphate buffer (pH 6.8) for 5-10 seconds with shaking, and then stained in 3% Gurr's improved Giemsa stain solution (3 ml stain in 100 ml 1/15 M Sorenson's phosphate buffer, pH 6.8) for 1-2 h., or until clear and sharp banding patterns appear. The slides can be kept in the stain solution 1 to 2 days without overstaining. The slides are then rinsed in

distilled water, air-dried, and mounted in a synthetic resin (Preserveaslide, Mathesin, Coleman and Bell Co.).

Results and Discussion

The banding patterns of wheat chromosomes visualized by the HKG procedure are shown in Figures 1-4. The chromosomes of the genomes of polyploid wheats can be easily identified based on chromosome size, arm ratio, and banding patterns.

The advantage of the HKG method is that the higher acid concentration (5 N HCl) can kill, fix, and hydrolyze root tips simultaneously. This is a 25-50 times greater acid concentration than used in the BSG banding techniques. It makes the meristematic tissue more easily squashed and yields well-spread chromosomes. The HKG method uses a low concentration of KOH, and this eliminates the potential particle contamination problem caused by the reaction of $Ba(OH)_2 + CO_2$. Furthermore, the HKG procedure shows the centric region and other banding patterns in the A genomes of diploid and polyploid wheats that are not seen with N-banding method (Fig.2). Another important consideration is that the heterochromatin banding patterns of wheat chromosomes are highly reproducible by the new method. Therefore, the HKG banding technique can be applied to the study of heterochromatin diversity and genome differentiation in many different germplasm collections. Heterochromatin banding patterns as genetic markers can be useful also in wheat breeding programs.

The terminology and classification of chromosome banding procedures have become complicated because there are a considerable number of banding methods and different kinds of heterochromatin reported (Schubert and Kieger, 1984; D'Amato, 1986). However, the same Giemsa stain solution has been used in many banding procedures, and the banding mechanisms are still not well understood. To avoid confusion, Schubert and Rieger (1984) proposed a neutral term, Giemsa band or Giemsa banding patterns, specified by positions such as centric, proximal, interstitial and telomeric to replace the terms of G-banding and C-banding. Obviously, the neutral term of Giemsa banding can also replace the terms N-bands or N-banding. However, to identify a specific Giemsa banding procedure, the best way to do so is to name the critical chemical or key steps and to add to the neutral term of Giemsa banding. For example, Iordansky et al. (1978) named their method BSG ($Ba(OH)_2$-2SSC-Giemsa) banding, and we have follwed this nomenclature by using the term HKG (HCl-KOH-Giemsa) banding for our method.

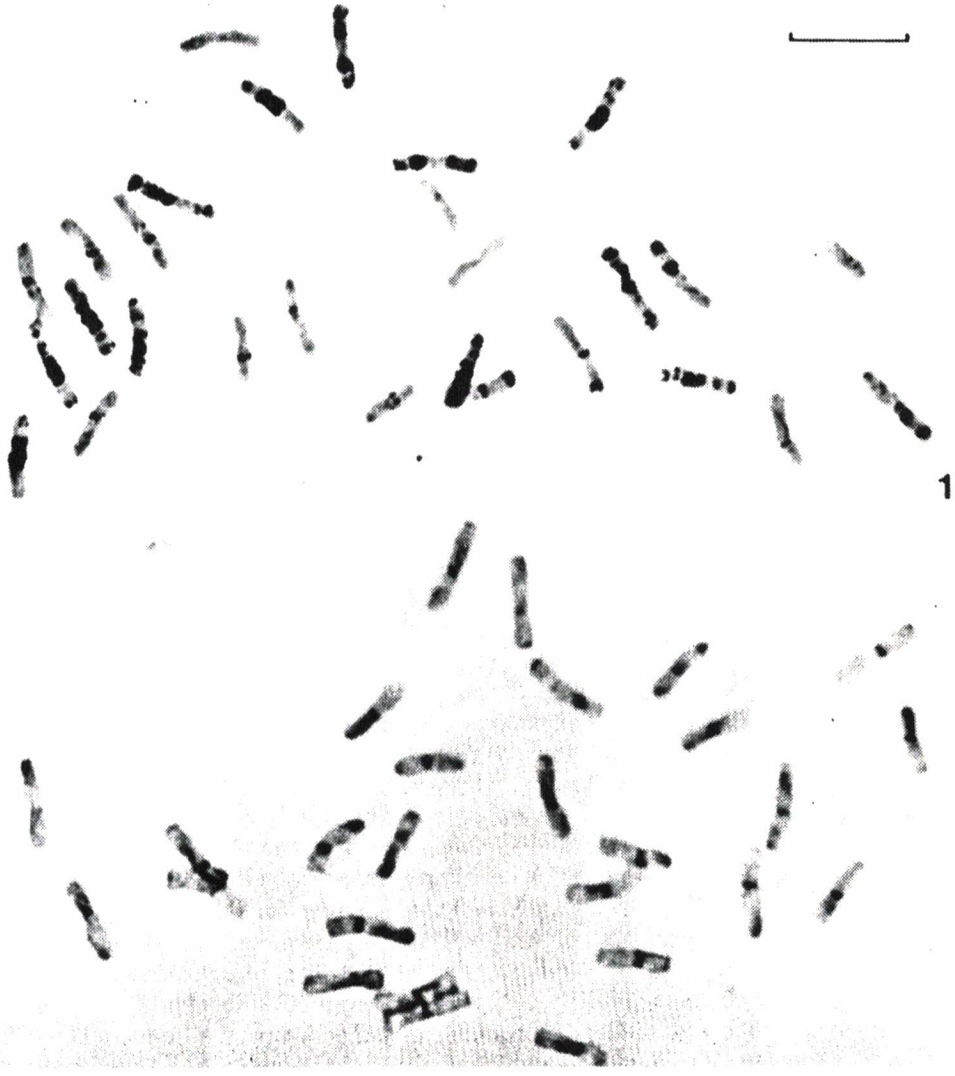

Fig. 1. HKG-banding patterns in the chromosomes of the wild tetraploid *T. dicoccoides* (PI488024). Fig. 2. HKG-banding patterns in chromosomes of an artificial tetraploid produced by chromosome doubling of the hybrid of *T. boeoticum* (G1004) and *T. urartu* (G1545); seeds obtained from the University of California., Riverside. Bar=10 *u*m.

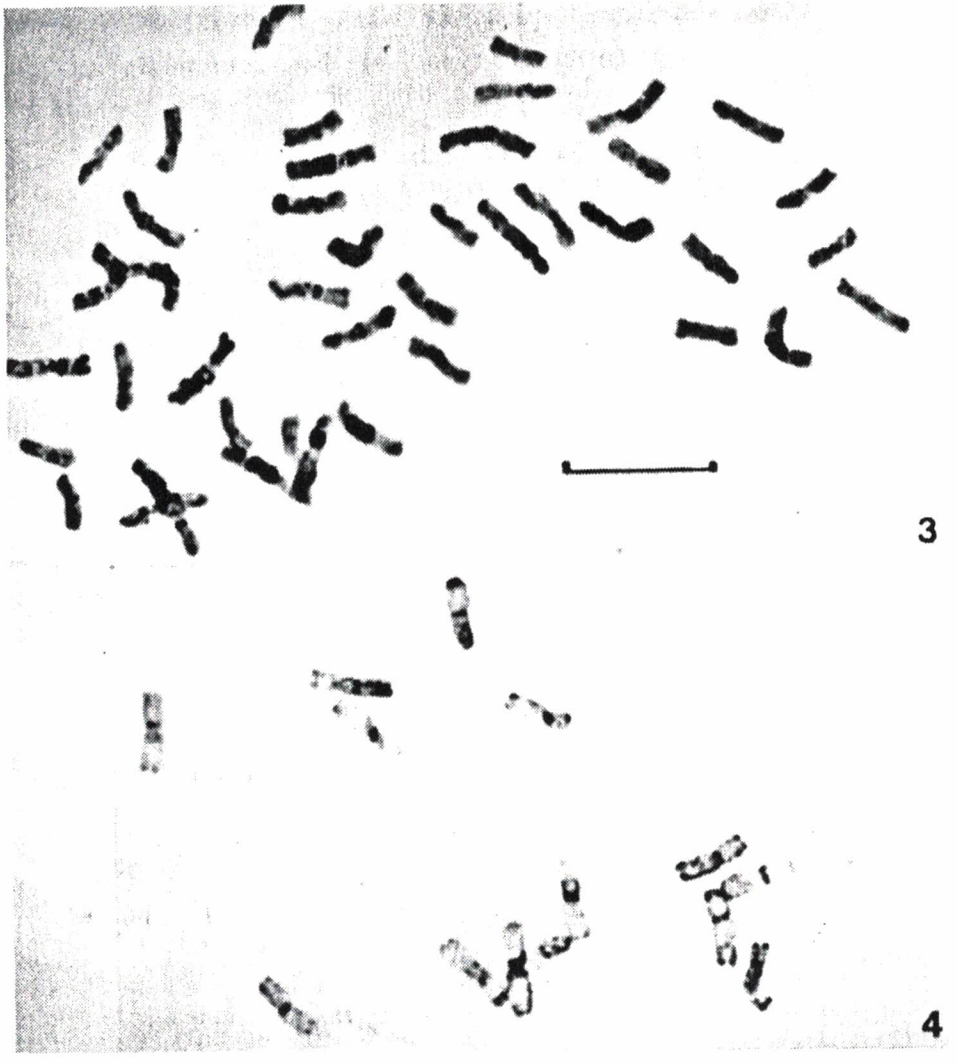

Fig. 3. HKG-banding patterns in the chromosomes of hexaploid bread wheat *T. aestivum* L. c v. TAM W-101. Fig. 4. HKG-banding patterns in the chromosomes of diploid *T. tauschii* (PI452130). Bar=10 *u*m.

References

D'Amato, F. 1986. Different types of heterochromatin in plant chromosomes investigated by Feulgen banding. Caryologia 39: 123-129.

Endo, T. R. 1986. Complete identification of common wheat chromosomes by means of the C-banding techniques. Jpn. J. Genet. 61: 89-93.

Endo, T. R. and Gill, B. S. 1984. Somatic karyotype, heterochromatin distribution, and nature of chromosome differentiation in common wheat, Triticum aestivum L. em. Thell. Chromosoma 89: 361-369.

Gerlach, W. L. 1977. N-banded karyotypes of wheat species. Chromosoma 49: 357-370.

Gill, B. S. and Kimber, G. 1974. A Giemsa C-banding technique for cereal chromosomes. Cereal Res. Commun. 2: 87-94.

Hutchinson, J., Miller, T.E., Jahier, T. and Shepherd, K. W. 1982. Comparison of the chromosomes of Triticum timopheevi with related wheats using the techniques of C-banding and in situ hybridization. Theor. Appl. Genet. 64: 31-40.

Jewell, D. C. 1981. Recognition of two types of positive staining chromosomal material by manipulation of critical steps in the N-banding technique. Stain Technol. 56: 227-234.

Iordansky, A. B., Zurabishvili, T. B., and Badaev, N. S. 1978. Lineardifferentiation of cereal chromosomes I. Common wheat and its supposed ancestors. Theor. Appl. Genet. 51: 145-152.

Schubert, I., and Rieger, R. 1984. G and/or C-bands in plant chromosomes? J. Cell Sci. 71: 113-120.

Seal, A. G. 1982. C-banded wheat chromosomes in wheat and triticale. Theor. Appl. Genet. 63: 39-47.

Received 18th April 1988