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Giemsa C-banding Pattern of Rye, *Secale cereale*

Lee, Woong-Jik, Jae-Wook Bang and Sang-Ryul Choi
(Department of Biology Education, Seoul National Universiy, Seoul)

Giemsa C-band에 의한 호밀의 核型分析

李雄稙 · 方在旭 · 崔上律
(서울大學校 生物敎育科)

ABSTRACT

Giemsa C-banding analysis with Korean rye strain revealed that individual chromosomes had characteristic banding patterns making it possible to distinguish the seven rye chromosomes one from another.

It seems to be evident that B-chromosomes in rye contain proportionally more heterochromatin than autosomes.

INTRODUCTION

On the basis of conventional cytological techniques, it was often difficult to make a precise classification of each chromosome in rye, *Secale cereale*, because some homologous have similar size and centromeric position, particulary in the case of excess contraction of chromosomes due to pretreatment.

C-banded techniques provided more reliable information for karyotype of rye (Gill and Kimber, 1974; Vosa, 1974; Verma and Rees, 1974). Banding techniques are also useful to study aneuploidy and chromosome rearrangements: Trisomic (Zeller, Kimber and Gill, 1977) translocation (de Vries and Sybenga, 1976; Lelley and Gustafson, 1979) and deletion (Singh and Röbbelen, 1976).

Giemsa banding technique is also applied to rye chromosome introduced in triticale (Darvey and Gustafson, 1975) and wheat-rye addition lines (Gustafson *et al.*, 1976; Gustafson and Krolow, 1978). By use of C-banding techniques heterochromatin polymorphism between different cultivars of rye was demonstrated by Weimarck (1975) and Lelly *et al* (1978). Bennett *et al.* (1977) studied DNA quantity in rye chromosomes as well as C-banding pattern of rye.

The present paper is concerned with the C-banded heterochromatin pattern in Korean rye with B-chromosomes.

MATERIALS AND METHODS

Rye (*Secale cereale*) strain maintained in our laboratory and a local rye variety collected from Ochun, Kyungi Province were used for the present investigation.

To collect root tips, rye plants were grown in small pots or seeds of rye were germinated in petri dishes with damp filter paper. Excised root tips were treated with 0.2% colchicine solution for a duration of 6 hours in the dark. Subsequently they were fixed in acetic acid glacial and stored in a refrigerator before use.

The stain procedure for C-banding was conducted

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according to the method described by Kimber and Gill(1975) except for the following modifications; 2 hours of enzyme softening and 10-12 minutes of Giemsa staining.

Lelly *et al.* (1978) used three band categories depending on the band size; primary, secondary and tertiary band. In the present work, thick thin and very thin bands were used. Thick, thin and very thin bands approximately corresponds to primary, secondary and tertiary bands which are classified by Lelly *et al* (1978).

For ordinary photomicrographs Kodak microfilm (ASA 32) was used together with Kodak 11 developer and high speed Ectachrome was used for color photomicrographs.

RESULTS

The good preparation of C-banding was obtained from strain 77-2-6 which was maintained in our laboratory and the analysis of the C-banding pattern was carried out with this strain.

Fig. 1 shows C-banding pattern of rye strain 77-2-6. As may be seen in the figure, this rye plant possesses 2 B-chromosomes in addition to 14 chromosomes of the normal complement. On the basis of C-banding the chromosomes in strain 77-2-6 are classified as follows:

Chromosome I: Nuclear chromosome

This chromosome has two thick bands; one terminal in the long arm and one terminal in the satelllite. A thin band which has variable intensity of stain is located adjacent to the nucleolar constriction in the short arm. A very thin facultative band is occassionally found in the middle of the long arm in some chromosomes.

Chromosome II: Median

Two thick terminal bands are located in each arm.

Chromosome III: Median

A terminal thick band is located in the short arm but the long arm has only a very thin band in terminal.

Chromosome IV: Sub-median

In addition to one thick terminal band in the short arm, this chromosome has a fairy thick or thin intercalary band in the long arm. A very thin band is located in telomere of the long arm.

Chromosome V: Sub-median

Thick terminal band is located in short arm. The long arm possesses two thick bands one sub-terminal and one intercalary position.

Chromosome VI: Sub-median

Thick terminal band is located in the short arm. The long arm shows the most complicated appearence in the banding pattern among seven homologous. Two thin sub-terminal bands are located in the long arm. Three very thin facultative bands are observed between the thin band and the centromere. Also a very thin band is located in the telomere of the long arm.

Chromosome VII: Median

This chromosome has a thick terminal band in both arms. A very thin band is located adjacent to the terminal band in the long arm. This sub-terminal band is very thin but appears consistently. Without this very thin band it is quite impossible to distinguish between chromosome Ⅱ and chromosome Ⅶ.

B Chromosome:

Thick band is located in terminal of the long arm. Two thin bands are located in sub-terminal and proximal position in the long arm.

The basic haploid karyotype of rye is illustrated in Fig. 2. The idiogram represents C-banded analysis obtained from rye strain 77-2-6.

As may be seen in Fig. 3. one preparation derived from Ochun local variety of rye shows intensive contraction of mitotic chromosome, which has reduction of chromosomes in half size. Other preparation of same strain showed normal size of chromosomes and such drastic contration of chromosomes were not observed. Whether this contraction of chro-

mosome was originated by pre-treatment of root tip or staining procedure of C-banding is not certain.

DISCUSSION

Banding patterns of chromosomes of rye (*Secale cereale*) have been mainly studied with Giemsa C-banding techniques whereas fluorescence banding methods with rye still remains in preliminary stage.

C-banding pattern of rye reported by various authors show slight differences. These variations represent polymorphism between various rye cultivars but somtimes it may be caused by different staining methods. Although banding patterns of chromosomes in the present work was not designated according to their homoeologous relationship with wheat, it resembles in some ways the results of Lelly *et al*(1978). In the present work, chromosome Ⅰ, Ⅳ and Ⅴ showed fairly good coincidence with chromosome 1R(b), 4R(b) and 5R(c) respectively designated by Lelly *et al.* (1978). Chromosome Ⅵ of the present work is quite similar with 6R(g) presented by Lelly *et al.*(1978) except the existence of a very thin terminal band in the long arm and three very thin bands, instead of two, between the sub-terminal thin band and the centromere.

In rye, three median chromosomes were often difficult to distinguish one from another due to similar chromosome morphology. In the present work it was quite possible to classify each chromosome due to the following charactics; chromosome Ⅱ with both terminal thick bands, Ⅲ with thick terminal band in only the short arm and Ⅶ with both terminal thick bands and a very thin sub-terminal band in the long arm.

Müntzing(1974) pointed out that B-chromosomes are frequently heterochromatic. Although precise calculations for heterochromatin was not conducted in the present work, it is quite evident that B-chromosome in rye contains proportionally more heterochromatin than autosome. Standard B-chromosome studied by de Vries and Sybenga(1976) revealed more a complicated banding pattern than the present work. In their result, the long arm contains a large terminal band together with three small bands, one constant and two facultative, and even in the short arm a small constant band exists. This banding pattern clearly showed the heterochromatic nature of B-chromosome in rye.

The origin of B-chromosomes has been discussed for so many years by many cytologists. Kranz(1968 and 1971) postulated that standard fragments of the B-chromosomes may be originated due to fragmentation of an autosome in the original genome of the genus *Secale*. Comparative banding study between B-chromosomes in rye and wild species of genus *Secale*, might be necessary and more improved techniques of banding that provide more intercalary bands which are useful for classification of individual chromosome is recommended.

摘 要

韓國産 호밀의 一系統의 核型을 Giemsa C-banding에 의하여 分析하였다. 各染色體는 特異한 band型을 나타내어 호밀의 7개의 染色體를 서로 識別할 수 있었다.

호밀의 B染色體는 A染色體(常染色體)보다 比例的으로 더 많은 heterochromatin을 含有하고 있다고 생각된다.

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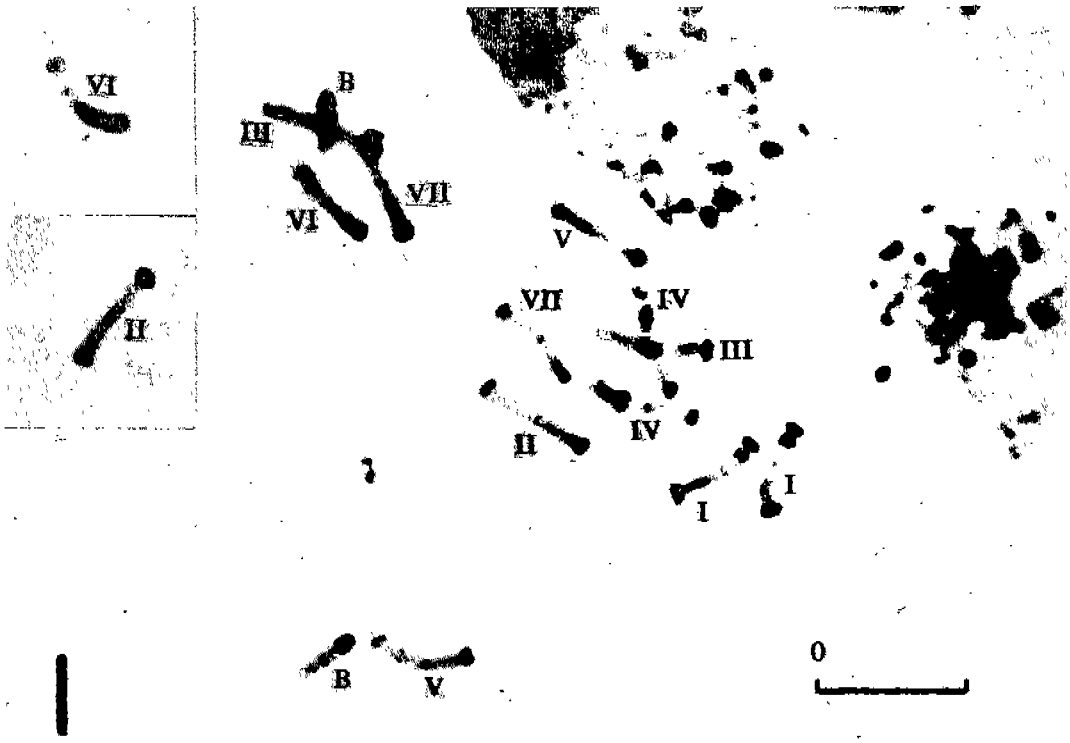

Fig. 1. Root tip mitosis of rye, *Secale cereale*, stained with C-banding technique. Chromosome complement is 2n=14+2B. Two chromosomes which were spread too widely are attached in the corner of photomicrograph.

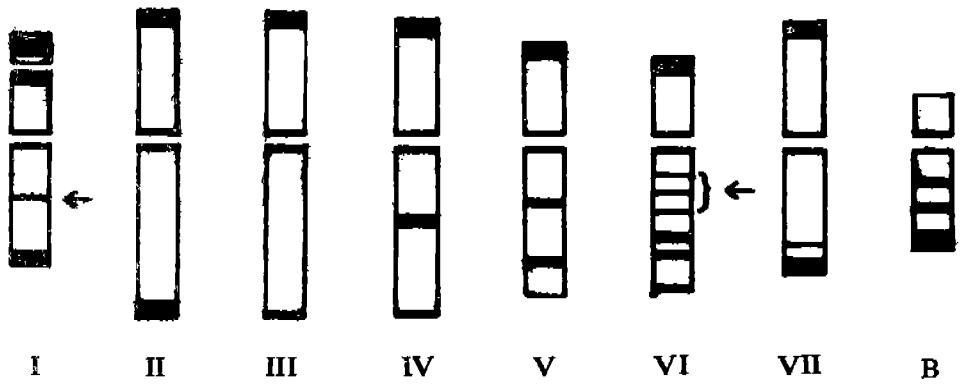

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Fig. 2. The idiogram showing C-banded pattern in rye, *Secale cereale*. Small occasional bands are arrowed.

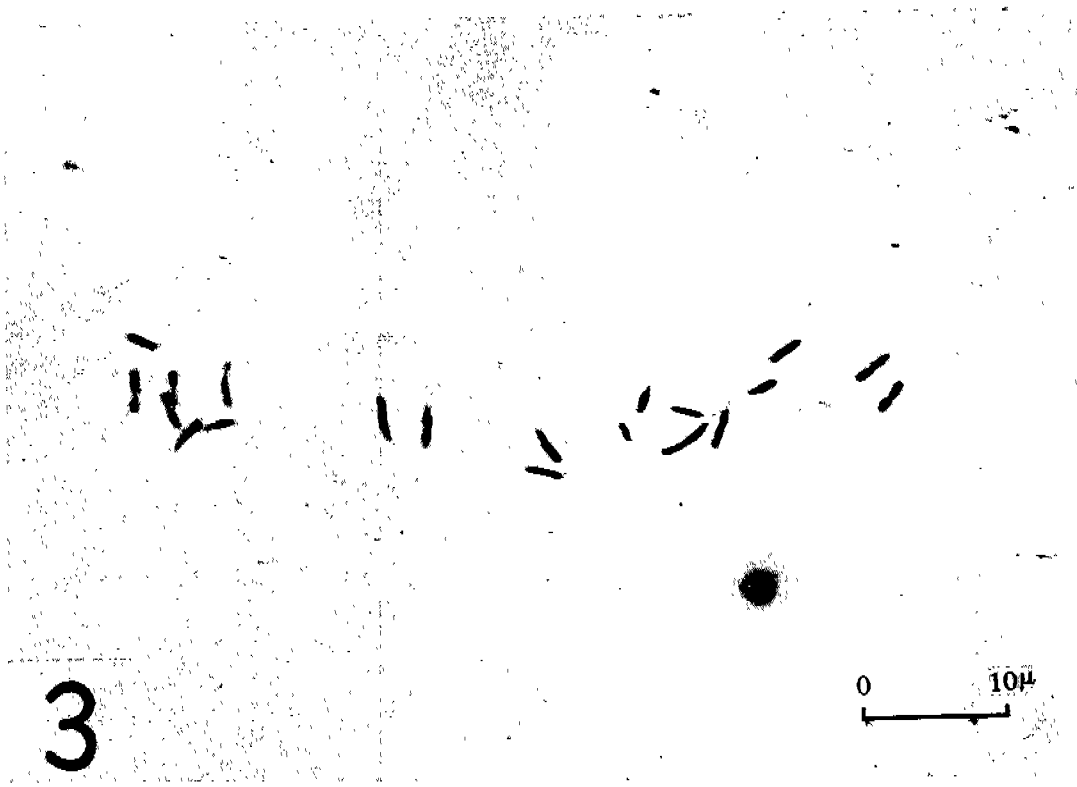

Fig. 3. Abnormally contracted chromosomes of rye due to C-banding treatment.