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Cytogenetic investigations in rye, wheat and triticale

3. C-banding of tetra- and hexaploid wheat by Giemsa- and/or Leishman staining

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Summary. Chromosomes of tetra- and hexaploid wheat have been individually characterized by Giemsa and/or Leishman C-banding techniques. Appropriate methodological modifications resulted in almost identical staining of chromosomes of tetraploid wheat with Giemsa and Leishman solutions. Additionally comparison of Giemsa banded chromosomes of the A- and B-genome of *Triticum turgidum* 34 and *Triticum aestivum* cv 'Jubilar' reveals similar or corresponding patterns in all homologous chromosomes with the exception of chromosome 7B. Apart from this intervarietal variation in certain homologous chromosomes of both wheat cultivars, intravarietal polymorphism is verified.

Key words: Wheat – Giemsa/Leishman C-banding – Polymorphism

Introduction

A number of cereals have been subjected to chromosome banding techniques, *Secale cereale* L. (Schweizer 1973; Bennett et al. 1977; Giraldez et al. 1979) and *Hordeum vulgare* L. (Linde-Laursen 1975; Kimber et al. 1976) being the most extensively studied.

There are fewer reports on differential staining of wheat chromosomes in *Triticum* species (Gill and Kimber 1974; Iordanski et al. 1978a, c; Bebeli and Kaltsikes 1985) or in triticale (Gustafson and Krolow 1978; Lukaszewski and Gustafson 1983).

Usually in cytogenetic research Giemsa stain is used to identify cereal chromosomes (Jones 1978; Seal and Bennett 1981) while Leishman solution or other Romanowski stains are applied less frequently (Darvey and Gustafson 1975; Noda 1981; Seal 1982; Endo 1986). In the majority of wheat chromosomes the Leishman and the Wright solutions produced a far more distinctive banding pattern than Giemsa, which stains chromosomes 1B and 6B differently.

In this paper, banding patterns of tetra- and hexaploid wheat chromosomes stained with Giemsa and/or Leishman are compared to ascertain differences not only between, but also within the same karyotypes. Additionally, the suitability of Giemsa and Leishman stain for the employed C-banding technique is discussed.

Materials and methods

The following wheat genotypes were analyzed: *T. turgidum* 34 (tetraploid) and *T. aestivum* cv 'Jubilar' (hexaploid). The material was kindly supplied by Dr. G. Oettler, Landessaatzuchtanstalt Stuttgart-Hohenheim.

Wheat chromosomes were C-banded using the technique described by Martin and Hesemann (1987) with the following modifications: roots were hydrolyzed in 0.1 N HCl at 57 °C for 4 min and transferred into pectinase solution (Serva) for further maceration: 5% solution of pectinase Rohament P5 from *Aspergillus niger* (0.2–0.3 units/mg) for 1 h, or 5% solution of pectinase Rohament P5 from *Aspergillus niger* (0.53 units/mg) for 2 h, or 10% solution of pectinase from *Aspergillus niger* (0.3 units/mg) for 3–3.5 h.

A 2.7% barium hydroxide solution was used to denature DNA and proteins in Giemsa C-banding; slides were immersed in Giemsa solution for 7 h. For Leishman staining, slides were incubated in 3.5% barium hydroxide solution for 11 min at 47 °C, placed in Leishman stain (Merck), and diluted 1 : 4 with Sörensen phosphate buffer, pH 6.7, for 3–4 h.

Karyotypes were constructed from complete chromosomes which showed the maximum possible banding patterns in at least 15 different metaphase plates. The size of each band was estimated and drawn in a chart indicating total length and arm ration of the respective chromosome. The genomic and homoeologous relationships of chromosomes were identified according to the classification of Gill and Kimber (1974).

Table 1. Symbols for Giemsa and Leishman C-bands

Type	Position	Size			
		Small	Medium	Large	Very large
Constant	Centromeric				
	Interstitial Telomeric				
	NOR-associated				
Inconstant	Interstitial Telomeric				

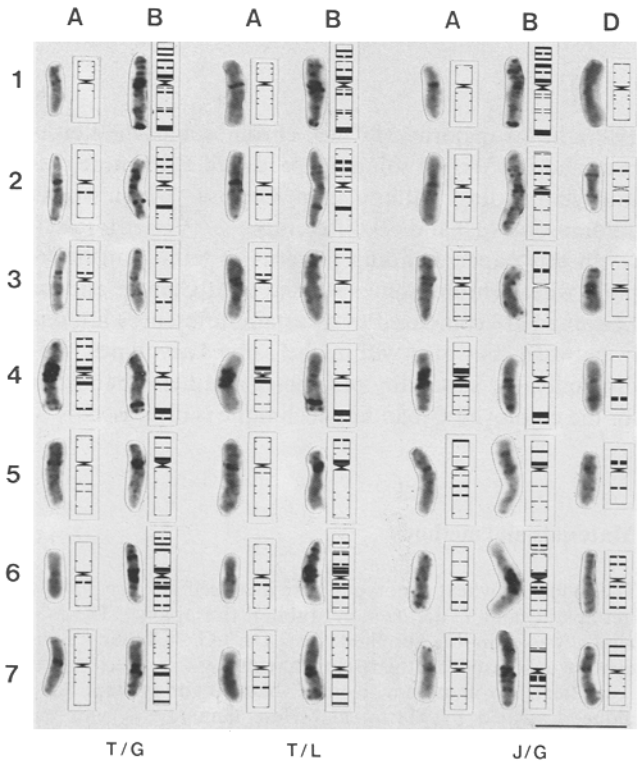

Fig. 1. Giemsa (G) and Leishman (L) C-banding patterns of chromosomes of *T. turgidum* 34 (T) and *T. aestivum* cv. 'Jubilar' (J). Bar represents 10 μm

Differentially lcoated C-bands were divided into two types, depending on their frequency of appearance: (1) constant bands, seen in all examined specimens of a specific wheat chromosome, or (2) inconstant bands, detected in only 50%–70% of examined chromosomes of the same type.

In karyograms, C-bands are symbolized as shown in Table 1; the solid lines represent intensively stained bands which can be seen only occasionally as two adjacent, separate dots. The broken lines represent laterally adjacent stained dots with variable staining intensities, which are only occasionally fused into one dot or band. The expression of bands and dots depends on the degree of condensation of the respective chromosomal regions, the amount of detectable constitutive heterochromatin, and the method employed.

Results

The C-banding patterns of the chromosomes of *T. turgidum* 34 and *T. aestivum* cv 'Jubilar' are shown in Fig. 1.

Tetraploid wheat

In homologous chromosomes differentially stained with Giemsa or Leishman a correlation could be established between the employed stain and both size and appearance of the corresponding heterochromatic segments.

A-genome: In Giemsa marked chromosomes medium-sized constant bands predominated; the use of Leishman solution resulted in a higher percentage of inconstant bands.

B-genome: Giemsa C-bands were mostly medium-sized while Leishman revealed smaller and larger bands. The number of inconstant bands was not a result of the dye employed.

Hexaploid wheat

In the majority of Giemsa stained chromosomes of *T. aestivum* cv 'Jubilar' and *T. turgidum* 34 the distribution of C-bands in homologues was similar (see Fig. 1). The chromosomes 3A, 6A, 7A and 3B of both forms showed a slightly different banding pattern. The homologous chromosomes 7B were, however, unequally stained.

In A-, B- and D-genome of hexaploid wheat there were more constant bands than inconstant bands, while the latter were more frequent in Giemsa stained chromosomes of tetraploid wheat.

In general, chromosomes of the B-genome are most heterochromatic and bear the highest number of C-bands. An exceptional is chromosome 4B, which is reallocated to the A-genome by different authors (Dvořák 1983; Lapitan et al. 1984; Bolsheva et al. 1986). Chromosomes of the D-genome are less heterochromatic than those of the A-genome.

Certain chromosomes of the B-genome of *T. turgidum* 34 and *T. aestivum* cv 'Jubilar' revealed polymorphisms in their banding patterns. The amount of detectable constitutive heterochromatin varied not only between different cells, but also within the same cell. So far, this type of polymorphism has been preferentially studied in rye chromosomes (Weimarck 1975; Lelley et al. 1978; Hesemann et al. 1987), while C-band polymorphism in wheat chromosomes has been only sporadically analyzed (Bolsheva et al. 1986). Individual

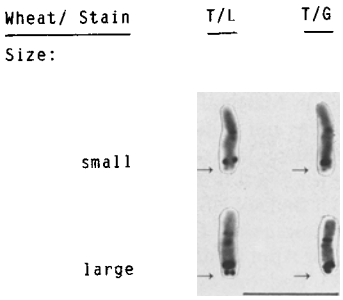

Fig. 2. Different size of the terminal band in the long arm of chromosome 4B. Bar represents 10 μm

Table 2. Different size of the terminal band in the long arm of chromosome 1B. G: Giemsa; L: Leishman; J: *Triticum aestivum* cv 'Jubilar'; T: *Triticum turgidum* 34

Wheat/stain			T/L	T/G	J/G
Chromosomes investigated			71	53	78
Size	Small	(%)	34	28	32
	Medium	(%)	53	54	50
	Large	(%)	13	18	18

wheat chromosomes displayed considerable variation in the amount of heterochromatin, more often in terminal than in interstitial bands. Polymorphism cannot be ascertained in the majority of wheat chromosomes, as wheat chromosomes bear smaller amounts of detectable constitutive heterochromatin than rye chromosomes which are rarely localized at telomeres (Bennett et al. 1977). In this paper, only conspicuous variations in size of telomeric bands of chromosome 1B of tetra- and hexaploid wheat and chromosome 4B of tetraploid wheat are presented (see Table 2 and Fig. 2).

Discussion

Comparison of the C-banding patterns of wheat chromosomes presented in this paper with the ones established by other authors has been difficult because of the different material and techniques involved in the studies.

Comparison of the C-banding patterns of *T. turgidum* cv '20' detected by Gustafson and Krolow (1978) with Giemsa or Leishman stained chromosomes of *T. turgidum* 34 showed that most homologues were similar. However, the classification of Gustafson and Krolow (1978) is only in accord with Gill and Kimber (1974), Lukaszewski and Gustafson (1983), and our results based on the assumption that the designation of chromosome 2A, 3A, 6A, 7A and 7B of *T. turgidum* cv '20' is changed to 7A, 4A, 4B, 3A and 6A respectively. The distribution of constitutive heterochromatin in chromosome 4A and 4B of *T. turgidum* cv '20' did not correspond with any chromosome of *T. turgidum* 34, while the remaining seven chromosomes were adequately banded and classified.

Chromosomes of tetra- and hexaploid wheat and triticale subjected to Leishman staining (Seal 1982) showed C-banding patterns closely resembling their N-banding patterns. Consequently the total number of distinctive bands was smaller. However, Leishman C-banding by Lukaszewski and Gustafson (1983) resulted in a higher number of diagnostic bands, and therefore facilitated the identification of all wheat chromosomes of *T. aestivum* cv 'Chinese Spring'.

In the present study, Giemsa staining of most chromosomes of *T. aestivum* cv 'Jubilar' produced a similar banding pattern to their homologues of *T. aestivum* cv 'Chinese Spring' by Lukaszewski and Gustafson (1983). Due to material rather than to methodological differences, the position of C-bands diverges significantly in the chromosomes 2B, 3B, 2D and 3D. Contrary to Seal and Bennett (1982), banding patterns of wheat chromosomes revealed by Giemsa- and Leishman staining were almost identical, which may be attributed to the modified techniques employed. Staining intensity and size of individual bands differed significantly in only a few chromosomes of *T. turgidum* 34 – e.g. Leishman solution produced smaller and fainter bands at the centromere of chromosome 4B and nucleolar organizer region of chromosome 6B, whereas the short arm of chromosome 2B showed a more distinctive pattern than the it's Giemsa-stained homologue. The terminal band in the long arm of chromosome 4B is usually small after Giemsa or Leishman staining; large bands in the respective region occur more after application of Leishman solution. Further investigations will clarify whether slightly methodological changes caused these variations, or if the chemical compounds of the two dyes react differently upon identical DNA-sequences.

Small structural alterations were detected in both populations of autogamous and allogamous plants (Weimarck 1975). In our material, polymorphism of C-bands occurred more frequently in different chromosomes of tetraploid than of hexaploid wheat. On the other hand, Bolsheva et al. (1986) observed more polymorphic chromosomes in hexaploid than in tetraploid wheat.

Gustafson et al. (1983) reported that structural variations in rye chromosomes can arise spontaneously in somatic cells by a process involving chromosome breakage and deletion, or redistribution of telomeric segments. Our results imply that in wheat chromosomes, even within cells of the same plant, deletions are predominant, yet they are not common. Both this intravarietal polymorphism and the intervarietal variants,

for instance those of chromosome 7B, probably originate in the diverging evolution and different agricultural utilization of the respective wheat strains. Therefore the qualification of different *Triticum* species for effective triticale breeding may also be variable.

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