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The DNA Content of the Individual Chromosomes of Rye

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Summary. The relative DNA content of individual chromosomes of *Secale cereale* L. was determined in 25 cells by microdensitometry of Feulgen stained preparations. The correlation value between relative DNA content and relative chromosome length was $r = 0.61$ for all 328 chromosomes measured. However, the correlation coefficients calculated for individual cells as well as for mean values always approached 1. Taking into account the structure of rye chromosomes, this indicates that microdensitometric results may not be accurate when large quantities of heterochromatic DNA sequences are present in analyzed material.

Key words: Chromosomes – DNA – Heterochromatin – Microdensitometry – Rye

Introduction

The progress in triticale (× *Triticosecale* Wittmack) cytogenetics that has been achieved within the last decade has resulted in growing interest in rye (*Secale cereale* L.) chromosomes. The rye genome has been blamed for most of the triticale's disadvantages, i.e., poor meiotic stability and shrivelled grain (for review of triticale cytogenetics see Gustafson 1976; 1982). The rye genome has 34% more DNA than the largest of the wheat genomes (B-genome) and has large blocks of heterochromatin at the telomeres. It has prolonged meiotic and mitotic cycles as compared to the wheat component of triticale and contributes to univalency and formation of aberrant endosperm nuclei. Gustafson and Bennett (1976) postulated that natural selection acts in hexaploid triticale in favor of genotypes where the difference in the DNA content and chromosome structure between wheat and rye has been reduced either by substitution of the longest (and presumably with highest DNA content) rye chromosomes by their shorter wheat homoeologues or by deletions of the telomeric heterochromatin. It has also been established that the elimination frequency of rye chromosomes from ABR wheat-rye F_1 hybrids is strongly correlated to the chromosome size (Lukaszewski et al. 1980).

It did not seem probable that chromosome size alone could play a role in the elimination frequency, therefore it was decided to measure the DNA content of the individual chromosomes of the rye which was used in the elimination study. The choice of the material was therefore predetermined even though and inbred line rather than a population would have been more suitable. Some data on the DNA content of the chromosomes of an inbred rye have been presented by Hennen and Casperson (1973).

Of several methods available the ultraspectrophotometry of Feulgen stained preparations was chosen because of the simplicity of measurements and interpretation. However, this method requires a good supply of complete plates with excellent chromosome spreads and these proved to be more difficult to find than was originally believed.

Materials and Methods

Seeds of a commercial rye cultivar 'Dankowskie Zlote' were germinated in the dark on moist filter paper in Petri dishes. After pretreatment in a 1:1 mixture of 0.01 M colchicine and 0.001 M 8-hydroxyquinoline at 18 °C for 3.5 h, the seedlings were washed in ice-water and the root tips were fixed in 3:1 absolute ethanol: glacial acetic acid and refrigerated. They were hydrolyzed in 1N HCl at 60 °C for 10 min, stained in Schiff's reagent, washed three times in SO_2 water, treated with pectinase and squashed in a drop of 45% acetic acid on special purity slides. Cover slips were sealed with gelatin.

Selected metaphase plates containing no more than two overlapped chromosomes were photographed and scanned in visible light at 545 nm using an OPTON UV-5 ultramicrodesitometer. A cross section of the light beam was 0.5 μ and the distance between two successive scans was 0.5 μ, yielding about 90–110 readings per chromosome and 1300–1600 readings per cell. Each cell was scanned three times

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at different angles and the mean value of the three readings was regarded as a total extinction value for the complement. Each of the chromosomes was identified on the photographs on the basis of arm ratio and relative length according to Darvey and Gustafson (1975), and their mean extinction values from the corresponding computer print-outs were expressed as a percentage of the total extinction value for the complement. Thus, a relative DNA content value for each chromosome was obtained. To calculate the real value of the DNA content the standard amount of 33.14 pg DNA per 4C nuclei for *S. cereale* cv. 'Petkus Spring' was adopted after Bennett et al. (1977).

In total, 25 metaphase plates from 25 different plants were analyzed on which 328 chromosomes were identified and individually measured. The remaining 22 chromosomes were overlapped so that they could not be individually measured.

Results and Discussion

The total extinction values from complete plates ranged from 39.1 to 52.3, a result of differences in staining intensity between different plates (the extinction value represents the percentage of the light absorbed by the Feulgen stain). The study, however, dealt with relative values for individual chromosomes so that data from different plates could be easily compared.

The relative extinction values for individual chromosomes ranged from 12.42 to 16.48. The mean values of the relative DNA content, calculated DNA content in pg, arm ratios and relative chromosome length values are given in Table 1. The variation in the DNA content was higher than that of chromosome size, both within chromosome pairs on one plate as well as between chromosomes from different plates. This could be due both to a lower accuracy of DNA measurements and to a variation of the DNA content between different plants. Chromosome polymorphism has already been reported in rye populations (Weimarck 1975; Giraldez et al. 1979). Variations in the chromosome size were higher than those presented earlier (Pieritz 1970; Hennen and Casperson 1973), which may indicate a larger variation in this population. On the other hand, the variation of the relative DNA content of the individual chromosomes was essentially the same as that presented by Hennen and Casperson (1973) for an inbred line, despite the difference in sample size.

The correlation coefficient for the relative chromosome length vs. relative DNA content for all 328 chromosomes was 0.61 and was lower than that obtained for wheat chromosomes measured in anaphase I ($r=0.82$, Nishikawa 1970) or calculated for wheat, $r=0.74$, by Gustafson and Bennett (1976). The low correlation coefficient indicated variation of the measured characters between different plates.

The calculated value was difficult to compare to those given by others because the method of calculation was not given by Nishikawa (1970), while the value given by Gustafson and Bennett (1976) was calculated for mean values of DNA content and chromosome size. The correlation coefficient calculated for the mean values given in Table 1 was $r=0.998$ and it always approached 1 (0.998 and up) when calculated for every cell. The same high correlation can be calculated for the mean values given by Hennen and Casperson (1973) for an inbred rye ($r=0.97$) or for values given by Radley (1966) for *Protomnodon bicolor* ($r=0.996$). This indicates that the chromosome size is a linear function of its DNA content, which is probably true for chromosomes where DNA is evenly distributed along their length. However, this is not the case in rye because four chromosomes of rye have large blocks of heterochromatin at the telomeres of both arms while the remaining three chromosomes have heterochromatic blocks at the telomeres of short arms. In addition, blocks of interstitial heterochromatin exist on every chromosome (Lima-de-Faria 1952; Darvey and Gustafson 1975). The density of heterochromatin is higher than that of euchromatin. Exact values are not known but estimations range from 1.5 for *Secale* sp. (Bennett et al. 1977) to 7.62 for *Lolium* sp. (Thomas 1981). The estimation for *Secale* seems to be very conservative and was based on Jones and Rees' (1968) finding that the DNA density in B chromosomes of rye in 1.5 times higher than that in A chromosomes. However, B chromosomes of rye are not totally heterochromatic. Verma and Rees (1974) and Kranz and Goethe (1976) showed that the size of heterochromatic C-bands is about the same on both B chromosomes and A chromosomes. The B chromosomes are about ½ shorter and so the proportion of heterochromatin is about two times higher. From this it may be concluded that a very rough estimation of the density of

Table 1. Relative and absolute[a] DNA content, relative length and arm ratio of the chromosomes of *Secale cereale* cv. 'Dankowskie Zlote'

Chromosome	Number of chromosomes measured	Arm ratio	Relative length %	Relative DNA content %	DNA content[a] pg
1R	50	1.36±0.08	13.32±0.17	13.16±0.40	4.36±0.13
2R	46	1.22±0.07	15.48±0.23	15.52±0.59	5.14±0.20
3R	44	1.07±0.03	14.00±0.16	13.83±0.33	4.58±0.10
4R	47	1.42±0.11	14.26±0.17	14.36±0.29	4.76±0.09
5R	48	1.91±0.16	13.52±0.11	13.61±0.24	4.51±0.08
6R	48	1.72±0.13	14.54±0.24	14.66±0.57	4.86±0.18
7R	45	1.13±0.06	14.88±0.25	14.88±0.31	4.93±0.09

[a] Standard content adopted was 33.14 pg DNA per 4c nuclei, after Bennett et al. (1977)

DNA in heterochromatin might be about 4 times higher than in euchromatin. On the other hand, however, Rees and Hutchinson (1973) and Carlson (1978) suggested that the higher density of B chromosomes may result from allocycly and be quite independent of heterochromatin.

If the amount of the DNA per length unit in heterochromatin is much higher than in euchromatin it should be expected that the chromosomes with two telomeric bands contain more DNA than size alone indicates and more than the chromosomes with only one band. The amount of heterochromatin in the individual chromosomes of *S. cereale* ranges from 7.83 to 16.95% (Bennett et al. 1977): therefore, chromosome 1R, which is the shortest in the karyotype but which has 14.9% of its length occupied by C-bands, will contain more DNA than the second shortest chromosome 5R, which has only 9.06% of the heterochromatin. The same should be true for chromosome 3R and 4R. Moreover, the density of heterochromatic blocks may vary between different chromosomes. It was shown that the composition of bands of different chromosomes vary as to the number of different repeats present (Jones 1980). Therefore, the DNA content of the individual chromosomes would depend on its length (providing the DNA is evenly distributed in euchromatin), and on the amount and the composition of heterochromatin. Thus the correlation values between chromosome size and the DNA content should be lower or even insignificant. Yet, this is not the case in this and other studies.

Hennen and Casperson (1973) presented graphs of the DNA distribution along chromosomes of rye. They showed higher concentrations at some telomeres but the height of the peaks was much lower than should be expected assuming that DNA concentration in heterochromatin is much higher than in euchromatin. On one hand it might be due to the material studied; inbred lines of rye often show much smaller C-bands than populations (Giraldez et al. 1979; Lukaszewski, unpubl. data) but it may also indicate that the ultramicrodensitometry is not sensitive enough to allow for distinction of the differences in the DNA concentration with good accuracy. One of the assumptions in this technique is that the intensity of Feulgen staining is proportional to the amount of the DNA present in the chromosome. However, C-bands are not visible on Feulgen stained preparation even in *Lolium* sp., where the heterochromatin was found to be 7.62 times more dense than euchromatin (Thomas 1981). On the other hand, the microdensitometers record the mean value of light absorption in light beam. With the relatively small size of bands as compared to the cross section of the light beam, it tends to "flatten" the peaks of higher DNA concentrations.

Therefore, it seems that the ultramicrodensitometry is not a reliable method of measuring DNA content in objects where its concentrations changes rapidly between different points, as it does in the chromosomes of rye. Biochemical methods are probably capable of yielding more accurate estimations especially now, when lines isogenic for presence/absence of certain heterochromatic C-bands are available in Triticale (Merker 1976; Roupakias and Kaltsikes 1977; Gustafson and Bennett 1982).

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