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C-banding polymorphism and the analysis of nucleolar activity in *Dasypyrum villosum* (L.) Candargy, its added chromosomes to hexaploid wheat and the amphiploid *Triticum dicoccum* – *D. villosum*

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Summary. C-banding patterns and nucleolar activity were analyzed in *Dasypyrum villosum*, its added chromosomes to hexaploid wheat and the hexaploid amphiploid *Triticum dicoccum–D. villosum*. Two different populations of the allogamous species *D. villosum* (2n = 14, VV) from Greece and Italy were analyzed showing a similar polymorphism for C-banding pattern. Six of the seven addition lines were identified by their characteristic C-banding pattern. No polymorphism between both members of each added alien chromosome was found. Furthermore, nucleolar activity and competition were studied by using silver staining procedure. In *D. villosum* only one chromosome pair, A, was found to be responsible for organizing nucleoli. The results obtained in the amphiploid and in the addition lines demonstrate that nucleolar activity is restricted to SAT-chromosomes 1B and 6B of wheat, while those of *D. villosum* remain inactive.

Key words: C-banding pattern – Nucleolar activity – *Dasypyrum villosum* – Wheat 'Chinese Spring' – *D. villosum* addition lines – *Triticum dicoccum–D. villosum*

Introduction

Several species closely related to wheat are known to possess important agronomic characters that make them very interesting for wheat improvement. Many studies have been carried out in wild species related to wheat, however in *Dasypyrum villosum* (formerly *Haynaldia villosa*), an anual allogamous diploid species (2n = 14, VV) native to Mediterranean and Caucasus areas, which has been shown to carry genes for resistance to different diseases, very few studies have been realized.

Part of the evidence for the close relationship of the genus *Dasypyrum* with other genera of *Triticeae* is its crossability with species of those genera, for example, with diploid and tetraploid species of *Triticum* (Tschermak 1934; Von Berg 1935; Hyde 1953; Halloran 1966a and b; Blanco and Proceddu 1983; Blanco et al. 1983a, b; Jan et al. 1986), *Secale cereale* (Sando 1935; Kostoff and Arutinova 1937; Nakajima 1951; Stefani 1986), *Aegilops* (Sears 1941, 1953) and *Elytrigia* (White 1940).

For this reason *D. villosum* is very interesting for analyzing the evolutionary relationships of *Triticeae*. Hence we report here the C-banding patterns of *D. villosum* and its added chromosomes to hexaploid wheat and its amphiploid with tetraploid wheat.

Material and methods

The material analyzed consists of two populations of *Dasypyrum villosum* (L.) Candargy (2n = 14, VV) from Greece and Italy; six addition lines of *D. villosum* to hexaploid wheat cv. 'Chinese Spring' (2n = 44) 2H, 4H and 5H from Greece and 1H, 6H and 7H from a species grown in Italy, kindly provided by Dr. E. R. Sears, U.S.A. Six accesions of the amphiploid between *Triticum dicoccum* and *D. villosum* (*Haynaldotricum hungaricum*, 2n = 42, AABBVV) were kindly supplied by Dr. C. Lehmann, GDR.

Seeds were germinated on wet filter paper in Petri dishes at 20 °C. When primary roots were 1–2 cm long, they were excised and immersed in tap water at 4 °C for about 40 h to shorten the chromosomes. Root tips were subsequently fixed in acetic acid-ethanol (1:3). Anthers of CS–*D. villosum* addition line 5H were collected and fixed in acetic acid-ethanol (1:3).

C-banding and silver staining procedures were carried out according to Giraldez et al. (1979) and Lacadena et al. (1984a).

A comparative analysis of somatic metaphase cells by phase contrast, C-banding and Ag-NOR-staining was made. The nucleolar organizer chromosomes (SAT-chromosomes) were identified both with phase contrast and C-banding technique by comparing the same metaphase cell. Silver stained nucleolar organizer regions (Ag-NORs) were identified, being located at the secondary constrictions. Cytological identification of five of the six addition lines, namely 1H, 2H, 4H, 6H and 7H was made in somatic metaphase cells. Line 5H was characterised by analyzing PMCs at first meiotic metaphase in monosomic and disomic addition plants.

Statistical analysis of the distribution of nucleoli at interphase betwen different plants within each line was made by applying χ^2-contingency test. Since no significant differences for each material analyzed were detected, individual data were pooled. Relative chromosome lengths and arm ratios of *D. villosum* chromosomes were calculated in 30 complete undistorted metaphase cells.

The nomenclature of the addition lines were established by E. R. Sears, using morphological or biochemical markers (Driscoll 1983).

Results and discussion

C-banding and nucleolar activity of *D. villosum,* wheat cv. 'Chinese Spring'–*D. villosum* addition lines as well as the amphiploid *T. dicoccum–D. villosum* have been analyzed by means of phase contrast followed by C- or Ag-NOR-banding.

Description of C-banded chromosomes

1) *Dasypyrum villosum*

Two different populations of *D. villosum,* one from Greece and one from Italy were analyzed, each consisting of 15 and 18 plants, respectively. The chromosome complement of *D. villosum* consists of seven similar sized chromosome pairs with different arm ratios. Each chromosome pair could be identified by its characteristic C-banding pattern. Figure 1 shows a complete mitotic metaphase cell of *D. villosum* in phase contrast (a) and after using C-banding technique (b).

A polymorphism for C-heterochromatin between and within plants for each chromosome has been detected in both populations. The two accesions have shown similar C-banding polymorphism. A composite with the different chromosome types observed for each chromosome comprising the haploid complement is illustrated in Fig. 2.

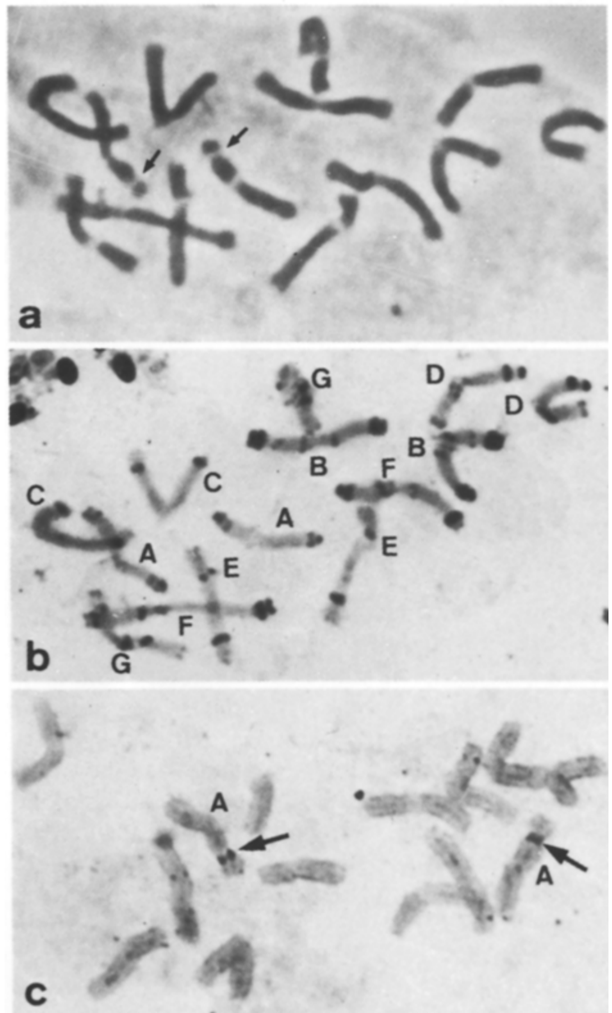

Fig. 1. Phase contrast (**a**), C-banding (**b**) and Ag-NOR-banding (**c**) of somatic metaphase cells of *Dasypyrum villosum.* Arrows indicate secondary constrictions and Ag-NOR bands

Chromosome A. It is the SAT-chromosome, submetacentric, showing an arm ratio of about 0.79. The secondary constriction is located in the subterminal region of the short arm. Telomeric and subtelomeric C-bands in both arms were always detected. The C-band found adjacent to the nucleolar organizer region varies from thick to virtually absent. A great range of variation has been observed for both bands in the long arm.

Cromosome B and F. These chromosomes are very similar in size, arm ratio and C-banding pattern and are sometimes difficult to distinguish. They are almost metacentric with an arm ratio of 0.90. In the majority of plants analyzed, prominent telomeric and subtelomeric C-bands were present in the short and long arm. Since both bands are closely located, in many cases they appear as a large terminal block. Major variation for both bands in the long arm was found, ranging from a very large block to a small but distinctive band for the subterminal band and to almost occasional absence of the telomeric band. Two pericentromeric C-bands were always detected in both arms, varying from thick to faint for chromosome F. In addition, a fine distal band in the long arm of chromosome B was also observed.

Chromosome C. This chromosome shows an arm ratio of 0.72 and is characterised by two thick distal bands in both arms, the one located in the short arm being nearly terminal.

Chromosome D. It is submetacentric, although the difference in arm length is more pronounced than in chromosome A, resulting in an arm ratio of 0.66. Clearly visible C-bands were present in telomeric regions of both arms. This chromosome is furthermore characterised by possessing an intensely stained subterminal block in the long arm. In addition small peri-

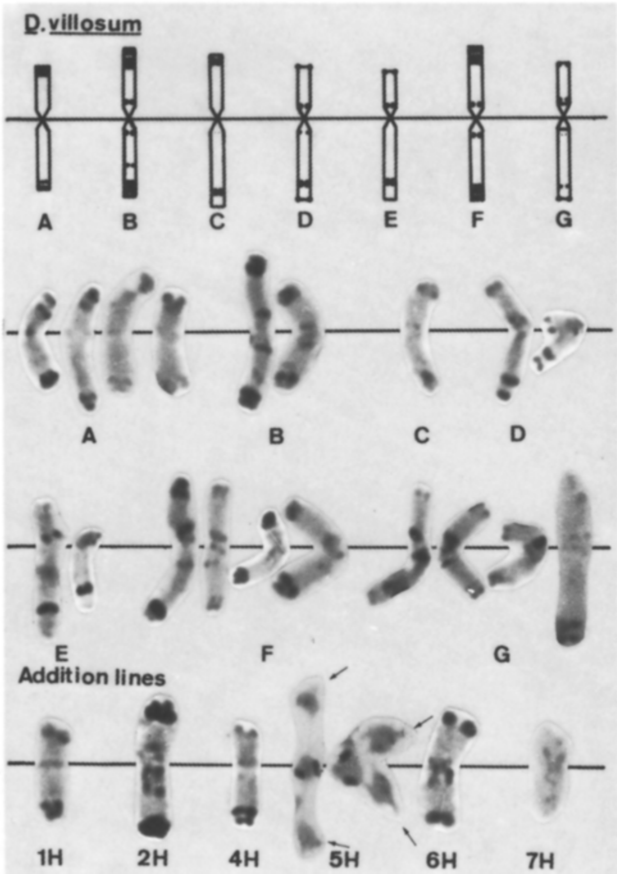

Fig. 2. Basic karyogram and C-banding polymorphism of *D. villosum* chromosomes in the diploid species and its addition lines with hexaploid wheat cv. 'Chinese Spring'. Arrows point to the centromeres

centromeric C-bands were also observed in both arms being larger in the short arm.

Chromosome E. This chromosome shows a similar arm ratio (0.61) as chromosome D and presents also a similar sized prominent block in the distal region of the long arm. On the contrary, virtually no telomeric bands were observed in both arms, although a very pronounced C-band close to the centromeres was always present in the short arm.

Chromosome G. It presents an arm ratio of 0.73, similar to those found in chromosome A and C. Small telomeric bands were present in both arms. Two bands closely located to each other and adjacent to the centromere were always present in the long and short arm, showing a high degree of polymorphism particular pronounced in the long arm. In addition a faint C-band was also observed in the distal region of the long arm.

All chromosomes show small centromeric bands of different intensity.

2) Wheat cv. 'Chinese Spring'–*D. villosum* addition lines

Six addition lines were analyzed and the alien chromosomes identified by their characteristic C-banding patterns. No differences in C-banding pattern could be observed either between the two homologues of each *D. villosum* added chromosome pair or between plants within each line analyzed. These results show that no polymorphism for C-heterochromatin of the added alien chromosomes exists.

Figure 3 shows somatic metaphase cells of the 'Chinese Spring'–*D. villosum* addition lines 1H (a) and 2H, 4H, 6H and 7H (e–h), respectively. A meiotic metaphase I cell of the addition line 5H is presented in Fig. 3 d. A representative chromosome of each of the 'Chinese Spring'–*D. villosum* addition line analyzed is given in Fig. 2.

Table 1. Silver-stained nucleolar organizer regions (Ag-NORs) and nucleoli observed in somatic metaphase and interphase cells in *Dasypyrum villosum,* the amphiploid *Triticum dicoccum–Dasypyrum villosum* (2n = 42) and in the addition lines *Triticum aestivum* cv. 'Chinese Spring'–*Dasypyrum villosum* (2n = 44)

Material	No. of plants	Metaphases	Ag-NORs	No. of nucleoli at interphase						Total cells
				1	2	3	4	5	6	
Dasypyrum villosum	39	318	2	6,587	1,793					8,650
Amphiploid										
T. dicoccum–D. villosum	60	142	4	6,606	10,616	7,335	1,187			25,744
Addition lines										
T. aestivum–D. villosum										
1H	34	235	4	3,171	4,095	3,086	406	11		10,769
2H	32	255	4	1,226	3,520	4,110	1,528	490	55	10,929
4H	30	233	4	1,080	3,370	3,708	1,164	567	39	9,928
5H	5	20	4	226	838	574	178			1,816
6H	32	307	4	1,798	4,872	2,860	1,077	18	6	10,631
7H	32	131	4	1,199	3,736	2,953	563	29	13	8,493

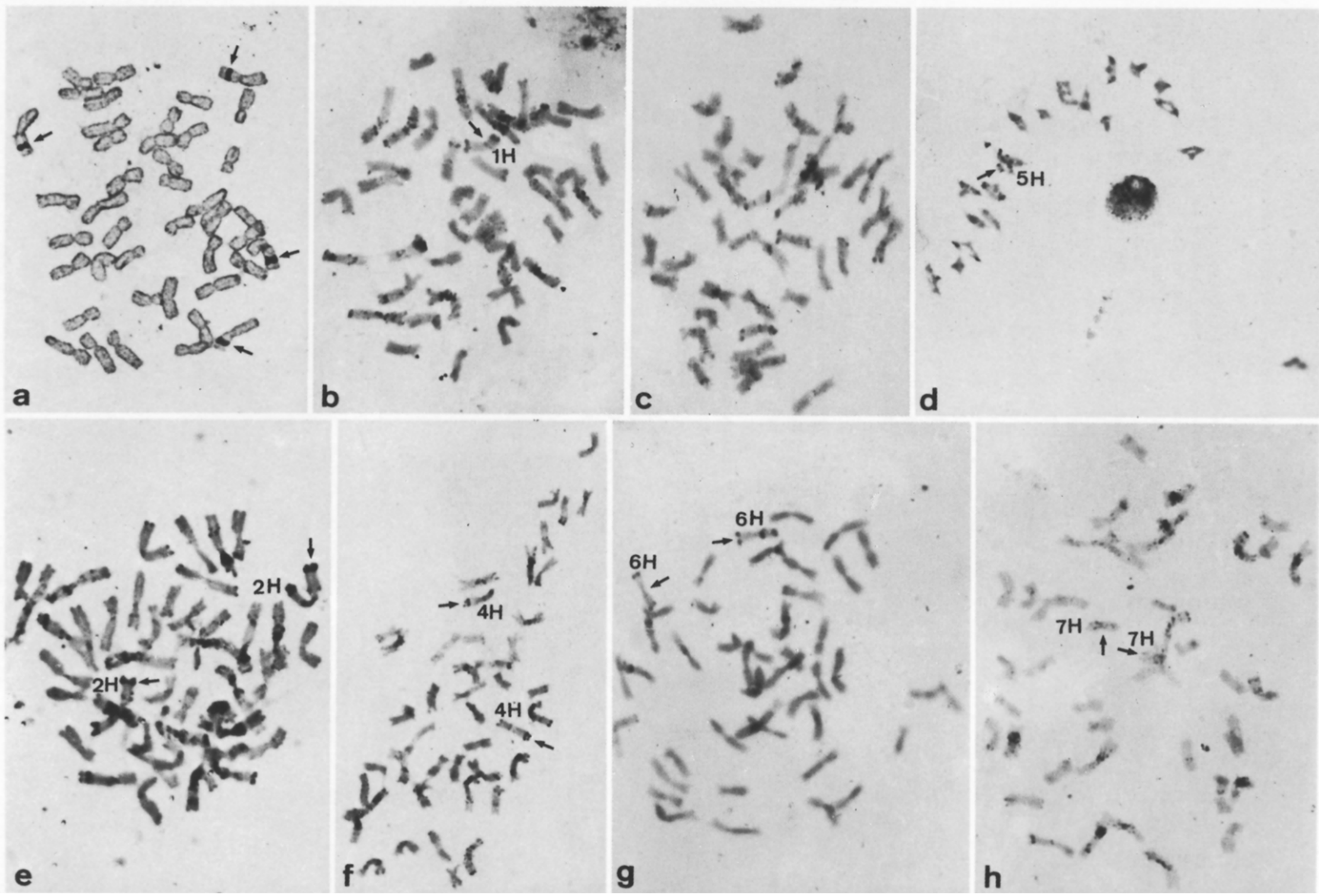

Fig. 3a–h. Somatic and meiotic metaphase cells of the different 'Chinese Spring'–*D. villosum* addition lines and the *T. dicoccum–D. villosum* amphiploid. **a, b** Ag-NOR and C-banding of the addition line 1H, respectively; **c** amphiploid; **d** C-banded PMC at metaphase I of the addition line 5H; **e–h** C-banded somatic metaphase cells of the addition lines 2H, 4H, 6H and 7H, respectively. Arrows indicate *D. villosum* chromosomes and Ag-NOR bands

The correspondence between the nomenclature of the chromosomes of the diploid species *Dasypyrum villosum* and those belonging to the addition lines was established according to their C-banding pattern being as follows:

1H = A, 2H = B, 4H = D, 5H = E, 6H = F, 7H = G.

Addition line 3H is missing in the set. Since six of the seven alien addition lines were identified, it is evident that the lacking chromosome 3H corresponds to chromosome C of *D. villosum.*

3) Amphiploid *Triticum dicoccum–D. villosum* (*Haynaldotricum hungericum*).

Figure 3c shows a C-banded somatic metaphase plate of the hexaploid amphiploid *Haynaldotricum hungaricum.* C-banding analysis was carried out in 5 plants per line. The six accessions analyzed contained the complete chromosome complement of *D. villosum.* A similar C-banding pattern was detected in all cases corresponding to the basic C-banding pattern of the wild species.

Analysis of nucleolar activity

Ag-NOR staining was applied in order to analyze the nucleolar activity in *D. villosum* and nucleolar competition in its addition lines with wheat cv. 'Chinese Spring' as well as in the amphiploid *Haynaldotricum hungaricum* (AABBVV). The results obtained are presented in Table 1.

In *D. villosum* one chromosome pair shows secondary constriction and positive Ag-NOR bands (Fig. 1a, c) respectively, which corresponds to the maximum number of nucleoli observed at interphase, which was two, indicating that only one chromosome pair possesses nucleolar activity. This chromosome was revealed by its C-banding pattern as chromosome A of *D. villosum* (Fig. 1b and 2).

In the amphiploid between *D. villosum* and *Triticum dicoccum hungaricum* and in the 'Chinese Spring'–*D. villosum* addition lines only four Ag-NOR bands were observed. The satellited chromosomes were identified as chromosome 1B and 6B, belonging to the B genome of wheat. The maximum number of nucleoli observed at interphase agrees with the number of Ag-NORs at metaphase. These results show that nucleolar activity is restricted to SAT-chromosomes of the B genome of wheat, while those of *D. villosum* remain inactive.

A similar amphiplasty of nucleolar activity has been observed in triticale, wheat-rye addition lines (Lacadena et al. 1984a; Cermeño et al. 1984, 1986) and in the amphiploid *Aegilops ventricosa–Secale cereale* (Orellana et al. 1983), where SAT-chromosome pair 1R of rye was suppressed by the presence of NOR-chromosomes of B genome of wheat and *Aegilops ventricosa,* respectively. However, contradictory results were obtained in *Elytrigia elongatum* and *A. ventricosa–*

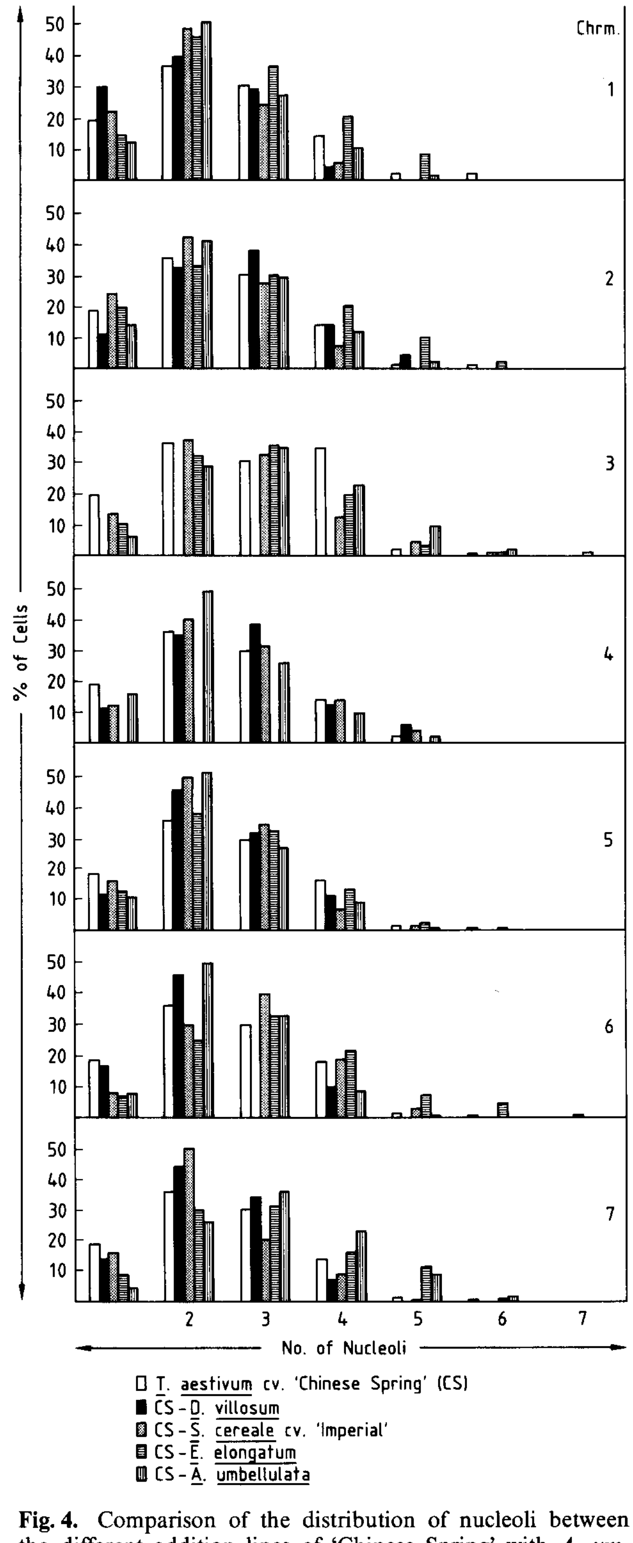

Fig. 4. Comparison of the distribution of nucleoli between the different addition lines of 'Chinese Spring' with *A. umbellulata**, *E. elongatum, S. cereale* and *D. villosum.* (Data were taken from Lacadena and Cermeño 1985)

Triticum amphiploids (Orellana et al. 1983; Lacadena et al. 1984b) in which codominance between SAT-chromosomes of the wild species and those belonging to cultivated wheat were found.

However, differences in the distribution of nucleoli were observed in other not SAT-addition lines analyzed, indicating an influence of the added *D. villosum* chromosomes over nucleolar activity of 1B, 6B and 5D of wheat, in respect to those found in *T. aestivum* cv. 'Chinese Spring'. A similar genetic control has been reported earlier by using different chromosome combinations of wheat (Viegas and Mello-Sampayo 1975).

A positive effect upon nucleolar activity was found in the addition lines 2H and 4H, showing a higher frequency of cells with 4, 5 and 6 nucleoli. On the contrary, a very low frequency of these classes were observed in 5H, 6H and 7H, in which cells with 5 or 6 nucleoli range from 0.0 to 0.3. Figure 4 shows a comparison of the behaviour of added alien chromosomes to 'Chinese Spring' upon the distribtuion pattern of nucleoli. A similar positive influence has been reported earlier (Cermeño et al. 1984; Lacadena et al. 1984b; Lacadena and Cermeño 1985), in addition lines of 'Chinese Spring' with *Secale cereale, Elytrigia elongatum* and *Aegilops umbellulata,* respectively.

In summary, this paper gives a detail description of C- and Ag-NOR banding patterns in *Dasypyrum villosum,* six of the seven addition lines 'Chinese Spring'–*D. villosum* and their amphiploid *T. dicoccum–D. villosum.* The C-banding patterns found were very distinctive, permitting to distinguish each chromosome of the complement of *D. villosum.* This fact offers the possibility of realizing further studies about the evolutionary relationships and homoeology of *D. villosum* chromosomes to those of wheat in a more precise way.

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