Theor Appl Genet (1984) 68: 75–80

Analysis of nucleolar activity in *Agropyron elongatum*, its amphiploid with *Triticum aestivum* and the chromosome addition lines

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Received September 29, 1983
Communicated by R. Riley

Summary. The nucleolar organizer activity of the *Agropyron elongatum,* its amphiploid with hexaploid wheat (*Triticum aestivum*) and the chromosome addition lines is analyzed by the silver-staining procedure. Four Ag-NORs are observed in *A. elongatum* corresponding to the chromosomes 6E and 7E. In the amphiploid *T. aestivum – A. elongatum,* eight Ag-NORs are observed which corresponds the wheat chromosomes 1B and 6B and to the *elongatum* chromosomes 6E and 7E. Thus, there is codominance in the nucleolar organizer activity of the chromosomes of the two species. However, a partial amphiplasty is detected since less than 8 Ag-NORs (7 up to 4) are observed in some metaphase cells; the chromosomes 6E and 7E are occasionally suppressed by wheat chromosomes. This conclusion is confirmed by the behaviour of the addition lines since only in those corresponding to the chromosomes 6E and 7E are the *elongatum* chromosomes nucleolar active although occasionally they can be suppressed by wheat chromosomes.

Key words: Amphiplasty – Ag-NOR – Addition lines – Nucleolar organizer – *Agropyron elongatum – Triticum aestivum*

Introduction

Nucleolar organizer regions (NORs) are the sites of rRNA genes. Highly reproducible silver-staining methods have been recently developed for the differential staining of NORs (Ag-NORs) in plant chromosomes (Hizume et al. 1980; Sato et al. 1980; Lacadena et al. 1984). The silver procedure can be used to visualize gene functionality at the rDNA sites with conventional light microscopy since the Ag-staining reaction of NORs at metaphase cells is an indication of genetic activity during the preceding interphase.

We have recently initiated the analysis of the nuclear competition in several cereal and related species (Lacadena et al. 1984; Cermeño et al. 1984; Orellana et al. 1984; Santos et al. 1984). In this paper we extend these studies to *Agropyron elongatum,* its amphiploid with common wheat and the chromosome addition lines in order to analyze the behaviour of the *elongatum* NORs in the presence of the wheat chromosomes.

Materials and methods

Four plants of *Agropyron elongatum* (2n = 14) were supplied by Dr. D. R. Dewey, Crops Research Laboratory, Utah State University, Logan.

Four plants of the amphiploid *Triticum aesticum* cv. 'Chinese Spring'–*A. elongatum* were supplied by Dr. G. E. Hart, Plant Science Department, Texas D and M University, Texas.

Six out of the seven possible chromosome addition lines *T. aestivum* cv. 'Chinese Spring'–*A. elongatum* for chromosomes 1E, 2E, 3E, 5E, 6E and 7E were supplied by Dr. G. E. Hart.

Seeds were germinated on wet filter paper in Petri dishes at 20 °C. When primary roots were 1 cm long they were excised and immersed in tap water at 0 °C for 36–48 h to shorten the chromosomes. The tips were fixed in acetic alcohol 1:3.

A comparative analysis of metaphase somatic cells by phase contrast, C-banding (according to Giraldez et al. 1979) and Ag-staining (according to Lacadena et al. 1984) was made.

Results

Silver-stained nucleolar organizer regions (Ag-NORs) are located in close correspondence with the sec-

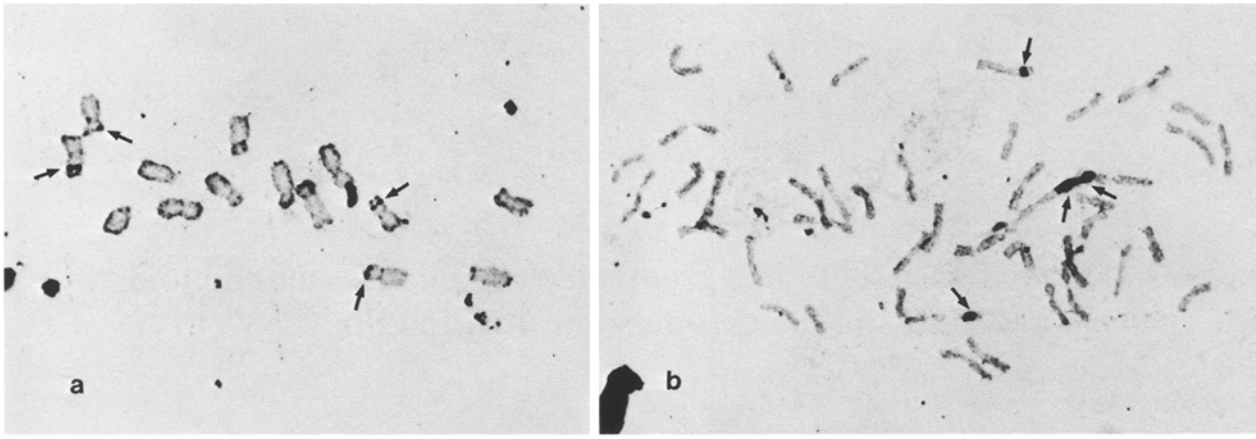

Fig. 1. Ag-stained somatic metaphase cells of *Agropyron elongatum* (**a**) and *Triticum aestivum* cv. 'Chinese Spring' (**b**). *Arrows* indicate Ag-NORs

Table 1. Ag-NORs and nucleoli observed, respectively, in somatic metaphase and interphase cells in *Agropyron elongatum*, its amphiploid with *Triticum aestivum* and the chromosome addition lines

Material	No. of plants	Meta-phases	Ag-NORs			No. of nucleoli at interphase[a]										
			1B+ 6B	6E	7E	1	2	3	4	5	6	7	8	9	10	Total
A. elongatum	4	7	–	2	2	277	172	40	11	–	–	–	–	–	–	500
		3	4	–	–											
		2	4	–	1											
		2	4	1	–											
Amphiploid		4	4	1	1											
T. aestivum cv. 'Chinese Spring'– *A. elongatum*	4	3	4	–	2	21	67	104	76	56	42	16	13	4	1	400
		2	4	2	–											
		3	4	2	1											
		1	4	1	2											
		10	4	2	2											
Addition lines																
T. aestivum cv. 'Chinese Spring' *A. elongatum* (CSE)																
CSE-1E	5	12	4	–	–	56	180	144	80	29	1	–	–	–	–	400
CSE-2E	5	26	4	–	–	60	208	186	124	37	15	–	–	–	–	630
CSE-3E	3	12	4	–	–	30	95	105	58	10	2	–	–	–	–	300
CSE-5E	4	11	4	–	–	64	189	165	66	15	4	–	–	–	–	503
		5	4	–	–											
CSE-6E	3	4	4	1	–	21	74	98	66	23	15	3	–	–	–	300
		2	4	2	–											
CSE-7E	3	4	4	–	–	27	93	97	50	36	4	4	–	–	–	311

[a] Contingency tests of the distributions of the number of nucleoli observed in the different individual of each plant type were not significant and, consequently, the data were pooled

ondary constrictions observed by phase contrast (Lacadena et al. 1984; Cermeño et al. 1984; Orellana et al. 1984; Santos et al. 1984).

From the comparison of the same metaphase cell by phase contrast and C-banding, it was demonstrated that the chromosomes which appear as satellized by phase contrast can be identified as the nucleolus organizer chromosomes by their C-banding patterns. Therefore by phase contrast, C-Banding and Ag-staining techniques one can identify which chromosomes have NORs genetically active during the preceding interphase.

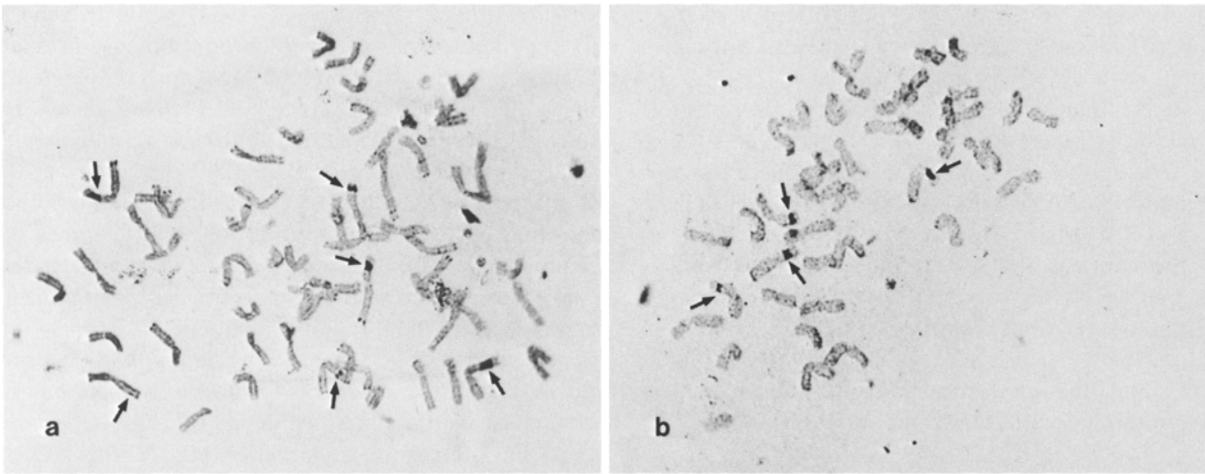

Fig. 2. Ag-stained somatic metaphase cells of 'Chinese Spring'–*A. ellongatum* amphiploid (**a**) and 'Chinese Spring'–*A. elongatum* 6E addition line (**b**). *Arrows* indicate Ag-NORs

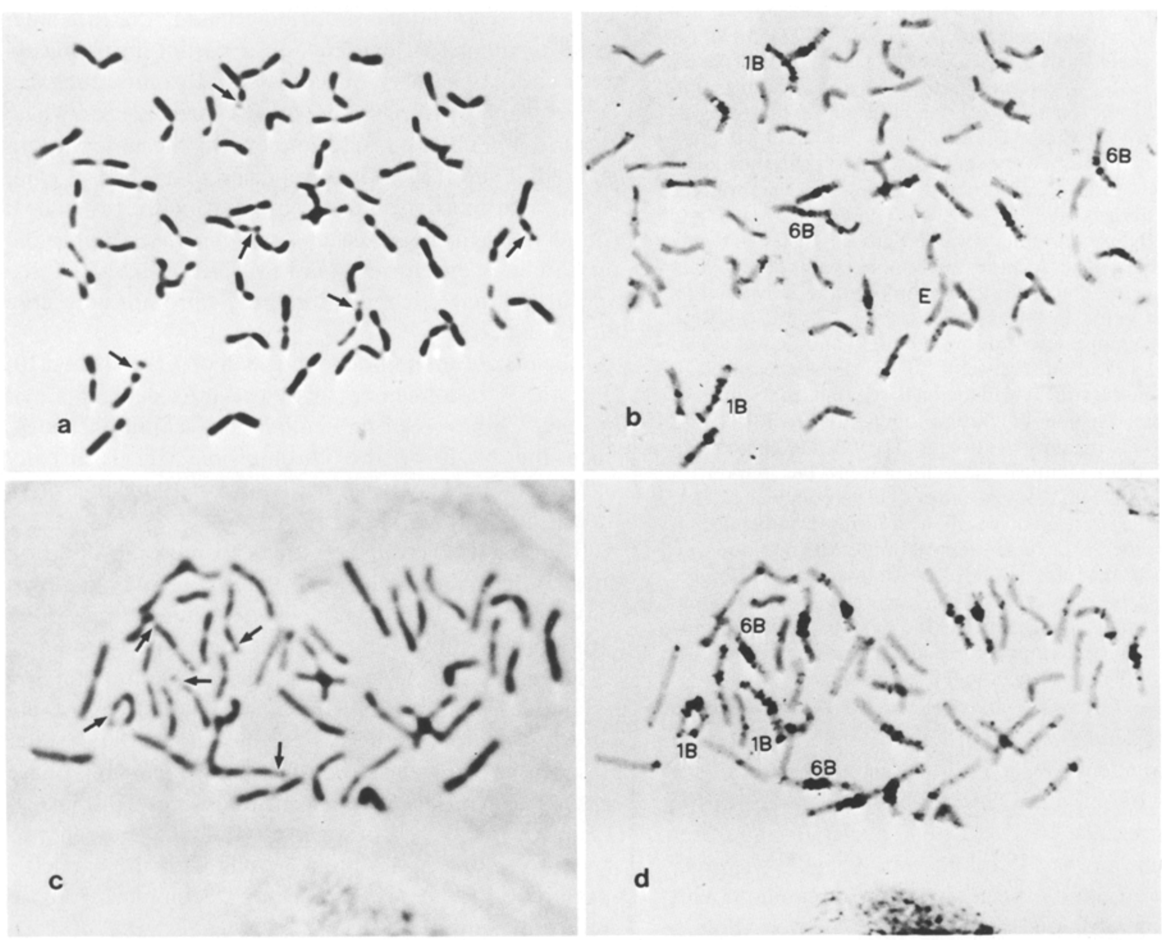

Fig. 3a–d. Phase contrast and C-banded metaphase cells of 'Chinese Spring'–*A. elongatum* amphiploid and 6E chromosome addition line. **a** and **b** 'Chinese Spring'–*A. elongatum* amphiploid, phase contrast and C-banding of the same cell. **c** and **d** 'Chinese Spring'–*A. elongatum* 6E addition line, phase contrast and C-banding of the same cell. *Arrows* indicate secondary constrictions

Four Ag-NORs have been observed both in *A. elongatum* (Fig. 1a) and *T. aestivum* cv. 'Chinese Spring' (Fig. 1b).

In somatic metaphase cells of the amphiploid *T. aestivum* cv. 'Chinese Spring'–*A. elongatum* and the 'Chinese Spring' 6E chromosome addition line, a variable number of Ag-NORs (8 up to 4 and 6 up to 4, respectively) have been found (Fig. 2). In all cases, when phase contrast and C-banding techniques were applied, the Ag-NORs corresponding to 1B and 6B wheat chromosomes were always detected (Fig. 3).

The numbers of Ag-NORs detected at mitotic metaphase and the distribution of the number of nucleoli at interphase are shown in Table 1.

Discussion

The karyotype of *Agropyron elongatum* has been described as being formed by seven pairs of meta- or submetacentric chromosomes with two pairs of satellited chromosomes (Matsumura and Sakamoto 1955; Cauderon 1958; Evans 1962; Schulz-Schaeffer and Jurasits 1962; Heneen and Runemark 1972; Dvořák and Knott 1974). The chromosome which possesses a large terminal satellite on the short arm was formerly designated as V by Evans (1962) and VII by Dvořák and Knott (1974). More recently, however, it has been identified as the chromosome 6E on the basis of its homoeology with the group 6 of wheat (Dvořák 1980). The other satellited chromosome possesses a very small satellite on the short arm, thus the NOR is located close to the telomere. This chromosome was formerly designated as IV by Evans (1962) and Dvořák and Knott (1974). In further studies it was designated as 3E by Dvořák (1980) on the basis of homoeology analysis. However, several lines of evidence suggest that the original chromosome 3E was structurally altered prior to the isolation of the disomic addition lines used in cytogenetic studies (Dvořák and Knott 1974; Dvořák 1975, 1980). Chromosome 3E has been observed to pair with 7E in certain hybrids, which suggests that chromosome 3E has a portion of chromosome 7E (Dvořák 1980). Hart (in personal communication to Dvořák 1980) detected the presence of an identical locus for an enzyme marker on chromosomes 3E and 7E. All this evidence indicates that chromosome 3E was involved in a reciprocal translocation with chromosome 7E prior to the isolation of the addition line we have used in this investigation. In summary, we will make reference to chromosome 7E in this text as the chromosome carrying the small satellite since the results obtained (Table 1) show that it bears the nucleolar organizer region as a consequence of the above mentioned interchange.

Four Ag-NORs are observed in all the metaphase cells of *Apropyron elongatum* (Fig. 1a). The maximum number of nucleoli observed at interphase agrees with the number of active NORs (Ag-NORs) present in the cells. The presence of two active nucleolar organizer chromosomes in the haploid set of *A. elongatum,* which is a diploid species, is in agreement with previous data obtained in such other related diploid species as *Triticum monococcum, T. thaudar, T. aegilopoides, T. urartu* and *T. sinskajae* (Gerlach et al. 1980), *Aegilops speltoides* (Hutchinson and Miller 1982), *Hordeum vulgare* (Santos et al. 1984) while in others only one nucleolar organizer chromosome is present (or active) in the haploid chromosome complement. This is the case, for instance, of *Aegilops squarrosa* (Hutchinson and Miller 1982) and rye, *Secale cereale* (Miller et al. 1980; Appels et al. 1980; Lacadena et al. 1984). From the evolutionary point of view, it would be interesting to analyze the reason why the genomes of diploid species likely arising from a common ancestral genome have different numbers of nucleolus organizer chromosomes.

From the results observed in the amphiploid *T. aestivum* cv. 'Chinese Spring'–*Agropyron elongatum* it becomes clear that eight (four pairs) Ag-NORs are very often present in metaphase cells. These NORs correspond to the chromosomes 1B and 6B of wheat and the chromosomes 6E and 7E of *A. elongatum.* However, in some cells only 7, 6, 5 or even 4 Ag-NORs are detected (the 1B and 6B Ag-NORs were always present) (Figs. 2a and 3a, b). This can be due to two reasons; on one hand, to the difficulty of distinguishing clearly the very small terminal Ag-NOR of chromosome 7E from telomeric artefacts and, on the other hand, to the actual existence of a partial amphiplasty: the nucleolar activity of *elongatum* chromosomes can be occasionally suppressed by the presence of wheat chromosomes. This would agree with the observations made by Evans (1962) who reported that when *A. elongatum* chromosomes are combined with the wheat complement in the *T. durum–A. elongatum* amphiploid the satellites on chromosome IV (7E) and V (5E) disappear so that even the satellited chromosomes lose their identity.

The maximum number of nucleoli observed (9 and 10) (Fig. 4a) is in agreement with previous data obtained in the 'Chinese Spring' cultivar of common wheat since the NOR of the chromosome 5D is usually active although it is not easily detected by the silver procedure (Cermeño et al. 1984).

The results obtained in the chromosome addition lines confirm the nucleolar organizer activity of chromosomes 6E and 7E of *A. elongatum* (in addition to the 1B and 6B) in the presence of the wheat chromosomes.

However, it is worth mentioning that in the corresponding addition line the nucleolar activity of the *elongatum* chromosome 6E can be suppressed by the presence of the wheat complement since metaphase cells with 4 (those of wheat chromosomes 1B and 6B) (Figs. 2b and 3c, d) and 5 Ag-NORs have been observed (Table 1) in addition to cells with the expected 6 Ag-NORs. These results are in agreement with those of Dvořák and Knott (1974) who, analyzing by conventional techniques the disomic addition line VII (6E), reported that somatic metaphase cells with more than four satellited chromosomes were very rarely ob-

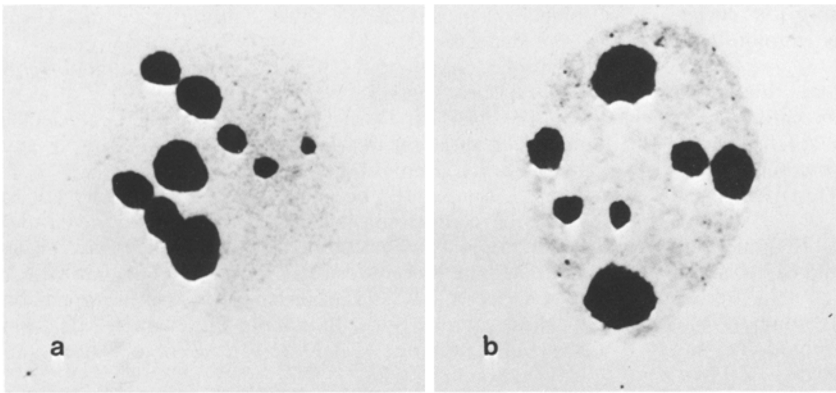

Fig. 4a, b. Ag-stained interphase cells. **a** interphase nucleus with 9 nucleoli corresponding to 'Chinese Spring'–*A. elongatum* amphiploid. **b** interphase nucleus with 7 nucleoli corresponding to 7E chromosome addition line

Table 2. Summary of nucleolar competition (amphiplasty) in different interspecific combinations analyzed by silver-staining procedure

Interspecific combination	Amphiplasty	Remarks	Authority
Wheat-rye	Yes	1R suppressed	Lacadena et al. (1984) Cermeño et al. (1984)
Wheat-barley	No	6B Ag-NOR size diminished by presence of 6H	Santos et al. (1984)
Barley-rye	Yes	1R suppressed	Ramsay and Dyer (1983)
Wheat-*Ae. ventricosa*	No		Orellana et al. (1984)
Wheat-*A. elongatum*	Yes, but partial	6E and 7E occasionally supressed	this work

served; however, in the PMCs the secondary constrictions in this chromosome were occasionally seen. It is also worth mentioning that in the disomic substitution 6B/6E, only one satellite pair (probably 1B) was observed in the root tip cells (Dvořák 1980).

On the other hand, the four Ag-NORs reported for the addition line 7E (Table 1), refer to those of 1B and 6B wheat chromosomes since, as indicated above, the very small and terminal satellite of chromosome 7E makes it difficult to distinguish the corresponding Ag-NOR from possible telomeric artefacts. However, the maximum number of 7 nucleoli observed at interphase is solid evidence for the nucleolar activity of the *elongatum* chromosome since the maximum number of nucleoli observed in the 'Chinese Spring' cultivar of wheat is six (Cermeño et al. 1984) (Fig. 4b).

In summary, one can conclude that a partial amphiplasty occurs in chromosomes 6E and 7E of *Agropyron elongatum* in the presence of wheat chromosomes since its nucleolar organizer activity, manifestated as Ag-NORs, is occasionally suppressed in both amphiploid and chromosome addition lines.

From the comparison of the results with those previously obtained in different such interspecific combinations as wheat-rye (Lacadena et al. 1984; Cermeño et al. 1984), wheat-barley (Santos et al. 1984), barley-rye (Ramsey and Dyer 1983) and wheat-*Aegilops ventricosa* (Orellana et al. 1984) (Table 2), a gradual seriation of "NOR strength" (nucleolar organizer competition ability) might be tentatively stated as 6H > 6B = 1B = 7H = DM(¿¿) > 6E > 7E > 1R.

Acknowledgement. This research has been funded by the Comisión Asesora de Investigación Científica y Técnica of Spain.

References

Appels R, Gerlach WL, Dennis ES, Swift H, Peacock WJ (1980) Molecular and chromosomal organization of DNA sequences coding for the ribosomal RNAs in cereals. Chromosoma 78:293–311

Cauderon Y (1958) Etudie cytogenetique des *Agropyron* francais et de leurs hybrids avec les bles. DSc Thesis, l'Université de Paris

Cermeño MC, Orellana J, Santos JL, Lacadena JR (1984) Nucleolar competition analysis in wheat, rye and their derivatives by silver-staining procedure. Chromosoma (submitted)

Dvořák J (1975) Meiotic pairing between single chromosomes of diploid *Agropyron elongatum* and decaploid *A. elongatum* in *Triticum aestivum.* Can J Genet Cytol 17: 329–336

Dvořák J (1980) Homoeology between *Agropyron elongatum* chromosomes and *Triticum aestivum* chromosomes. Can J Genet Cytol 22:237–259

Dvořák J, Knott DR (1974) Disomic and ditelosomic additions of diploid *Agropyron elongatum* chromosomes to *Triticum aestivum*. Can J Genet Cytol 16:399–417

Evans LE (1962) Karyotype analysis and chromosome designations for diploid *Agropyron elongatum* (Host) P.B. Can J Genet Cytol 4:267–271

Gerlach WL, Miller TE, Flavell RB (1980) The nucleolus organizers of diploid wheat revealed by in situ hybridization. Theor Appl Genet 58:97–100

Giraldez R, Cermeño MC, Orellana J (1979) Comparison of C-banding pattern in the chromosomes of inbred lines and open pollinated varieties of rye, *Secale cereale* L. Z Pflanzenzücht 83:40–48

Heneen WK, Runemark H (1972) Cytology of the *Elymus (Agropyron) elongatum* complex. Hereditas 70:155–164

Hizume M, Sato S, Tanaka A (1980) A highly reproducible method of nucleolus organizing regions staining in plants. Stain Technol 55:87–90

Hutchinson J, Miller TE (1982) The nucleolar organizer of tetraploid and hexaploid wheats revealed by in situ hybridisation. Theor Appl Genet 61:285–288

Lacadena JR, Cermeño MC, Orellana J, Santos JL (1984) Evidence for wheat-rye nucleolar competition (amphiplasty) in Triticale by silver staining procedure. Theor Appl Genet 67:207–213

Matsumura S, Sakamoto S (1955) Karyotypes of diploid *Agropyron* species. Wheat Inf Serv 2:19

Miller TE, Gerlach WK, Flavell RN (1980) Nucleolus organizer variation in wheat and rye revealed by in situ hybridization. Heredity 45:377–382

Orellana J, Santos JL, Lacadena JR, Cermeño MC (1983) Nucleolar competition analysis in *Aegilops ventricosa* and its amphiploids with tetraploid wheats and diploid rye by silver staining procedure. Can J Genet Cytol (submitted)

Ramsay G, Dyer AF (1983) Nucleolar organiser suppression in barley×rye hybrids. Brandham PE, Bennett MD (eds) Kew chromosome conference II. George Allen and Unwin, London, pp 361

Santos JL, Lacadena JR, Cermeño MC, Orellana J (1983) Nucleolar organizer activity in wheat-barley chromosome addition lines. Heredity (submitted)

Sato S, Hizume M, Kawamura S (1980) Relationships between secondary constrictions and nucleolus organizing regions in *Allium sativum* chromosomes. Protoplasma 105:77–85

Schulz-Schaeffer J, Jurasits O (1962) Biosystematic investigation in the genus *Agropyron*. 1. Cytological studies of species karyotypes. Am J Bot 49:940–953