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NUCLEOLAR ORGANIZATION IN THE GENUS *TRITICUM*

by

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SUMMARY

Nucleolar organizers are located in the short arms of chromosomes 1B, 6B and 5D of the complement of polyploid wheat. Numerical variation of the arms produces a corresponding variation in the maximum number of nucleoli per cell.

The short arm of chromosome 1A and the long arms of chromosomes 7A, 7D and 5D carry genes that regulate the activity of the nucleolar organizers (measured either by silver impregnation or by pulse labelling experiments with tritiated uridine). These regulators probably manifest themselves by promoting or repressing the general nucleolar organizing activity in the cells (chromosomes 1A, 1D, 2A ando 6D) or the activity of specific nucleolar organizers (chromosomes 7A or 7D). It is probable that chromosome 5D carries not only an organizer, but also a suppressor of the nucleolar activity which is cumulatively epistatic over a strong promotor located in the same chromosome. This promotor can increase the nucleolar activity in di-isosomic individuals by about 30 % relative to normal plants.

1. INTRODUCTION

Bread wheat, *Triticum aestivum* ($2n = 6x = 42$) is an allohexaploid plant that carries three different but related genomes (A, B and D) which were added through spontaneous crossing followed by the doubling of the haploid complement. It is generally accepted that the donors of A and D genomes

were a diploid wheat (2n = 14; Sax, 1922) and *Aegilops squarrosa* (2n = 14; McFadden and Sears, 1946; Riley and Chapman, 1960), respectivelly. The evidence that *Aegilops speltoides* (2n = 14) represents the donor of the B genome (Sarkar and Stebbins, 1956 and Riley, Unrau and Chapman, 1958) has been frequently questioned and several other diploid species of the sub-tribe *Triticinae* have been indicated as alternative donors (for revision see Sears, 1969).

The number of nucleolar organizers with visible production of nucleoli in polyploid wheat is less than the total expected from the donor species. In *T. aestivum,* Crosby (1957) found that only four of the five inherited organizers produced nucleoli and they were located in the short arm of chromosomes 1A, 1B, 6B and 5D. It was deduced that one of the A genome organizers was lost or became undetectable.

A very great decrease of nucleolar activity was found to occur in plants lacking the short arm of chromosome 1A (Jain et al., 1968). Aparently the reduction in nucleolar activity was much greater than that expected from the simple absence of an organizer of minor importance located in chromosome 1A. It was subsequently demonstrated that the absence of $1A^s$ also resulted in a significant decrease in total r-DNA of the cells Mohan and Flavell, 1974). It was, therefore, suspected that $1A^s$ carried a promotor of the nucleolar activity of the whole complement.

A significant decrease in nucleolar genes was also detected in tetrasomic 5D individuals in relation to normal disomic plants Mohan and Flavell, 1974). Since 5D also carries a nucleolar organizer, an increase in nucleolar activity should be expected instead. This suggested the existance of a suppressor of the nucleolar activity in chromosome 5D, which should have cummulative effects.

These facts strongly suggest that in polyploid wheat the nucleolar organizers are under the genetic control of regulatory genes distributed along the complement.

This paper presents new data that emphasize the primordial role of those genes.

2. MATERIALS AND METHODS

All the diploid and polyploid species of the sub-tribe *Triticinae* were originated from the stocks originally obtained from Dr. E. R. Sears. Tetraploids «Câmara» and «Resende» were originally produced in this laboratory from crosses *Triticum aestivum* × *Triticum durum* performed in order to obtain compensated nullisomic-tetrasomic combinations at the tetraploid level (Mello-Sampayo, 1972 and 1974). «Câmara» is a 1D-1B chromosome substitution line and «Resende» is a tetraploid wheat in which a large distal segment of chromosome 5D was distally inserted into the long arm of chromosome 5B through crossing-over between the two homoeologous chromosomes. «AAL 9» is a disomic alien chromosome addition line in which chromosome 5D of *Aegilops squarrosa* was added to *Triticum dicoccoides*. This was obtained by M. Noronha-Wagner from pentaploid «*Triticum spelta*» (synthetic) × *Aegilops squarrosa* and successive back crosses to *Aegilops squarrosa* for elimination of *Aegilops squarrosa* chromosomes. The extra *Aegilops squarrosa* chromosome was identified to be 5D through test crosses to the ditelosomic lines of *Triticum aestivum*. The strain «Zorba» (Zeller, 1972) is an alien substitution line 1R-1B supplied by the author.

Seeds were germinated in Petri dishes at 22°C.

Metaphase plates were studied after cold treatement of root tips, fixation and staining for 24 hours immersed in normal acetic-carmine stain.

The silver impregnation technique, for staining of nucleoli, was performed according the method described by Fernández-Gómez et al. (1969). The impregnation was done with 2% silver nitrate, after fixing the root tips for two hours with a 1:1 mixture of 1% hydroquinone and 10% formol. Then, after reduction with the formol-hydroquinone mixture, the roots were treated with photographic fixing solution (Kodak) for 15 minutes, to obtain more limpid preparations. In each chromosome combination the number of nucleoli per cell was counted in 100 interphase cells.

It is fair to conclude that the maximum number of nucleoli per cell corresponds to the number of active nucleolar organizers, since it was observed that it was equal to the maximum number of separate nucleolar sites observed at telophase.

Root tips for autoradiography were kept in a solution of tritium labelled uridine (5μCi/ml; specific activity 5Ci/mM) for 30 minutes. After incorporation of the label the root tips were washed in water and fixed in 1% formaldehyde in M/30 phosphate buffer (pH 7) at 2°C for 25 minutes (Evans, 1968). Squashes were made after maceration with a glass tapper on a previously cooled slide to isolate the nucleus. Cover slips were removed and the slides were digested with DNase, prior to coating with stripping-film. The autoradiographs were prepared according to the standard stripping film technique and stained with Giemsa, after an exposure time of two weeks. Silver grain counts were performed in cells with a single large nucleolus. Preliminary and extensive densitometric observations (Canas, unpublished) indicate that at this stage, nucleolar RNA amount is fairly stable and it remains at its maximum in wheat root tips.

3. RESULTS

Table 1 shows that the diploid *Triticinae* species *T. monococcoum* and *Aegilops speltoides* should carry four active nucleolar organizers whereas *Aegilops squarrosa* only two. It was also found that tetraploid wheats *(T. durum, T. dicoccoides* and *T. turgidum)* should carry four nucleolar organizers which suggests that in these wheats two of the four different organizers inherited from the parent species (probably those of the A genome) remained inactivated or were absent.

It is very likely that the two pairs of detected nucleolar organizers of tetraploid wheats correspond to those already found in the B genome chromosomes (1B and 6B) of hexaploid wheats. It was possible to substitute chromosome 1D (a non-nucleolar chromosome) for 1B in *Triticum durum.* The

corresponding strain («Câmara») war estimated to carry two nucleolar organizers. The chances are that the two remaining organizers were located in the pair of 6B chromosomes, since these were the only chromosomes carrying a secondary constriction in this strain.

TABLE 1

Nucleoli in root-tip cells at interphase in some *Triticinae* species

	2n	Genome constitution	Estimated number of nucleolar organizers	Maximum observed number of nucleoli/cell	Average number of nucleoli per cell (in 100 cells)
T. monococcum	14	AA	4	4	2.49 ± .08
Ae. speltoides	14	BB	4	4	2.09 ± .07
Ae. squarrosa	14	DD	2	2	1.39 ± .05
T. durum × T. monoc.	21	AAB	4	4	2.18 ± .08
T. durum	28	AABB	4	4	2.66 ± .08
«Câmara»	28	AABB (− 1B; + 1D)	2	2	1.43 ± .05
«Resende»	28	AABB	4	4	3.10 ± .08
T. dicoccoides	28	AABB	4	4	2.69 ± 08
«AAL 9»	30	AABB + 5D	6	6	3.40 ± .12
T. turgidum	28	AABB	4	4	2.52 ± .08
T. aestivum	42	AABBDD	6	6	3.32 ± .13
«Zorba»	42	AABB (−1B) DD (+ 1R)	4	4	2.10 ± .09
«T. spelta» (synthetic)	42	AABBDD	6	6	3.42 ± .11

Table 1 also shows that in triploid wheat hybrids, there are four estimated nucleolar organizers. This suggests that A genome organizers were not functional in triploid hybrids, as well. If the appearance of a secondary constriction denotes nucleolar activity, it is clear that chromosomes 1B and 6B which are morphologically satellited in triploids carry two of the four organizers. It is more probable that *T. monococcum* which already carries active organizers keeps them functioning at the triploid level than that the A genome organizers, if they exist, begin to work instead. Other alternative hypotheses are still less probable.

The number of active nucleolar organizers was estimated to be six in the hexaploid wheats that were studied (Tables 1 and 2). A higher number of nucleoli was never detected with reliable certainty in any cell, although minute blobs could not be excluded in extremely rare and non significant cases.

The six nucleolar organizers that were indirectly detected in «AAL 9» (Table 1) indicate that the 5D chromosome must carry the extra nucleolar organizer which is absent in tetraploid wheats, for this strain has an extra pair of 5D chromosomes added to the complement of *T. dicoccoides*, a tetraploid species, which carries only four organizers.

Also at the hexaploid level the decrease from six to four of the number of nucleolar organizers estimated in the variety «Zorba», which has a substitution of chromosome 1R for 1B (Zeller, 1972), confirms that 1B carries one of them.

Ditelosomic and aneuploid lines of *Triticum aestivum* seem to be valuable material for direct location of nucleolar organizers in the short arm of chromosomes 1B, 6B and 5D (Table 2). There is a direct numerical correspondence between the absence of one of such arms and that of one estimated nucleolar organizer.

On the other hand when a plant is homozygous defficient for the long arm of chromosomes 7A or 7D there is also a systematic absence of two estimated nucleolar organizers. This does not happen when only one chromosome 7A or 7D is absent as in the case of monosomics 7A or 7D. It can, therefore, be concluded in the best hypothesis that the homozygous deffective plants carry not a nucleolar organizer but a regulator element which operates upon a specific pair of homologous nucleolar organizers.

In its turn, in the absence of the short arms of chromosomes 1A and 1D, of the long arm of 2A and of the β arm of 6D, the number of estimated nucleolar organizers decreases from six to five which should correspond to the inactivation of one organizer. There is no indication of which organizer is inactivated but it is possible that the chromosome arms which were mentioned above carry also

genes that regulate the activity of nucleolar organizers located somewhere else.

TABLE 2

Nucleoli in root-tip cells at interphase in some ditelosomic and aneuploid lines of *Triticum aestivum* L.

	2n	Chromosome or arm defficient (—) or duplicate (+)	Estimated number of nucleolar organizers	Maximum observed number of nucleoli/cell	Average number of nucleoli per cell (in 100 cells)
Euploid	42		6	6	3.32 ± .13
Ditelosomic 1B^{L}	42	— 1B^{S}	4	4	2.77 ± .08
Monosomic 1B	41	— 1B	5	5	2.60 ± .09
Tetrasomic 1B	44	+ 1B	8	8	4.14 ± .16
Ditelosomic 6B^{L}	42	— 6B^{S}	4	4	2.92 ± .08
Monosomic 6B	41	— 6B	5	5	2.63 ± .10
Tetrasomic 6B	44	+ 6B	8	8	4.39 ± .13
Ditelosomic 5D^{L}	42	— 5D^{S}	4	4	2.93 ± .09
Monosomic 5D	41	— 5D	5	5	2.95 ± .10
Tetrasomic 5D	44	+ 5D	8	8	4.03 ± .14
Ditelosomic 7A^{S}	42	— 7A^{L}	6	4	2.50 ± .09
Monosomic 7A	41	— 7A	6	6	2.99 ± .12
Tetrasomic 7A	44	+ 7A	6	6	3.22 ± .11
Ditelosomic 7D^{S}	42	— 7D^{L}	6	4	2.52 ± .08
Monosomic 7D	41	— 7D	6	6	3.02 ± .11
Tetrasomic 7D	44	+ 7D	6	6	3.47 ± .10
Ditelosomic 1A^{L}	42	— 1A^{S}	6	5	2.94 ± .09
Monosomic 1A	41	— 1A	6	6	3.19 ± .10
Tetrasomic 1A	44	+ 1A	6	6	3.46 ± .11
Ditelosomic 1D^{L}	42	— 1D^{S}	6	5	2.93 ± .10
Monosomic 1D	41	— 1D	6	6	3.18 ± .11
Tetrasomic 1D	44	+ 1D	6	6	3.54 ± .12
Ditelosomic 2A^{S}	42	— 2A^{L}	6	5	2.98 ± .08
Monosomic 2A	41	— 2A	6	6	3.26 ± .11
Tetrasomic 2A	44	+ 2A	6	6	3.72 ± .14
Ditelosomic 6D$^{\alpha}$	42	— 6D$^{\beta}$	6	5	2.79 ± 10
Monosomic 6D	41	— 6D	6	6	3.05 ± 11
Tetrasomic 6D	44	+ 6D	6	6	3.58 ± 12

The pulse labelling experiments Table 3 were performed to give information of the overall kinetics of uridine incorporation into the r-RNA produced by the nucleolar organizers. This would indicate the total increase in nucleolar activity per cell in a short period of interphase corresponding to the given pulse, for each tested chromosome combination.

A lower mean number of silver grains was found when chromosome 1B or its short arm was absent either at the tetraploid level («Câmara») or the hexaploid level («Zorba», nullisomic 1B or ditelosomic $1B^{L}$). On the other hand, a significant increase in the mean number of silver grains was noticed when the number of 1B chromosomes increased (in trisomic 1B). Since chromosome 1B carries a nucleolar organizer, such quantitative variations in the r-RNA production were expected.

The absence of the short arm of chromosome 1A (in ditelosomic $1A^{L}$) or the long arm of chromosomes 7A or 7D (in ditelosomics $7A^{S}$ and $7D^{S}$) was associated with a decrease of the mean number of silver grains which is very pronounced in the first case. This could correspond to a lower nucleolar activity due to the absence of a regulator element for nucleolar activity located in this chromosome arm (Jain et al., 1968; Flavell and Mohan, 1973). Also, there is no reason to believe that a similar situation does not occur when the long arms of 7A or 7D are absent.

A higher dosage of chromosomes 5D or 6D (in the corresponding tetrasomics of *Triticum aestivum*) resulted in a decrease in the mean number of silver grains. The same decrease was also observed at the tetraploid level with the 5D alien chromosome addition line of *T. dicoccoides* in which a lower mean grain number than that of the tetraploid parent was found. Surprisingly, however, the increase in dosage of the long arm of chromosome 5D in hexaploid wheat (di-isosomic $5D^{L}$) as well as the inclusion of a large segment of the long arm of chromosome 5D into chromosome 5B (in «Resende») resulted in a considerable increase of silver grains. This strongly suggests that the distal segment of the long arm of chromosome 5D carries a promotor

of the nucleolar activity which cannot express itself in the presence of a strong suppressor located somewhere in the remaining portion of the chromosome. Since this suppressor is epistatic over the promotor it would explain the lower nucleolar activity in plants in which the whole chromosome 5D was added in excess.

4. DISCUSSION

Nucleolar organizers seem to carry a variable number of reiterated sequencies of genes (Ingle and Sinclair, 1972; Flavell and Mohan, 1973). These are structural genes in the sense that they code directly for the r-RNA of the cell. For this reason they may form specific regions or chromosome streches (the SAT-regions) to which nucleoli are normally attached.

The amount of reiterated sequencies in the polyploid wheats are under the control of regulatory genes. This can be inferred from the data of Flavell and Mohan (1973) who found indirect evidence of about a 50% decrease in the number of r-DNA cistrons when the short arm of chromosome 1A was absent from the complement of bread wheat (in ditelosomic $1A^{L}$). A proportional decrease in ^{3}H-uridine incorporation by the cell was observed in ditelosomic $1A^{L}$ plants (Jain et al., 1968 and this paper). This may be related to a lower r-RNA transcription. The reduction in r-DNA cistrons strongly suggests a gene amplification mechanism triggered by the short arm of chromosome 1A which in such a case could be considered to carry an effective promotor of the nucleolar activity.

The long arm of chromosome 5D must also carry an important promotor of nucleolar activity. Four doses of this chromosome arm in hexaploid di-isosomic $5D^{L}$ individuals produce a rise of almost 30% in nucleolar activity as indicated by the mean silver grain counts of ^{3}H-uridine treated cells (Table 3). A similar although not so conspicuous effect was observed in the «Resende» strain which carries a large distal segment of $5D^{L}$ at the tetraploid level.

TABLE 3

Mean number of silver grains per cell in root tip cells at interphase of different *Triticinae* species following ^{3}H-uridine incorporation

(5 μ Ci/ml-30 min)

	2n	Estimated number of nucleolar organizers	Mean number of silver grains	
			Nucleoli alone	Whole nuclei
T. monococcum	14	4	8.6 ± .18	15.8 ± .25
Ae. speltoides	14	4	12.4 ± .20	22.9 ± .35
Ae. squarrosa	14	2	13.0 ± .19	24.2 ± .36
T. durum	28	4	14.4 ± .34	26.1 ± .48
«Resende»	28	4	17.3 ± .28	31.5 ± .46
«Câmara»	28	2	11.8 ± .26	20.9 ± .42
T. dicoccoides	28	4	12.5 ± .28	21.6 ± .34
«AAL 9»	30	6	9.0 ± .29	16.4 ± .48
T. aestivum	42	6	17.9 ± .36	32.5 ± .61
Ditelosomic 1A ^{L}	42	6	9.6 ± .34	19.7 ± .72
Monosomic 1A	41	6	17.2 ± .40	31.9 ± .58
Tetrasomic 1A	44	6	16.6 ± .97	32.2 ± .40
Ditelosomic 7A	42	6	14.7 ± .11	29.2 ± .66
Monosomic 7A	41	6	16.5 ± .31	32.8 ± .59
Tetrasomic 7A	44	6	17.8 ± .15	32.5 ± .42
Ditelosomic 1B ^{L}	42	4	13.1 ± .11	26.6 ± .36
Nullisomic 1B	40	4	12.9 ± .26	24.3 ± .45
«Zorba»	42	4	14.1 ± .31	25.9 ± .54
Monosomic 1B	41	5	15.1 ± .38	26.9 ± .85
Trisomic 1B	43	7	19.7 ± .28	35.1 ± .28
Ditelosomic 6B^{L}	42	4	18.1 ± .23	31.8 ± .49
Monosomic 6B	41	5	18.2 ± .32	33.0 ± .71
Tetrasomic 6B	44	8	17.1 ± .60	33.3 ± .54
Ditelosomic 5D^{L}	42	4	18.2 ± .32	32.8 ± .46
Di-isosomic 5D^{L}	42	4	23.5 ± .34	42.6 ± .40
Monosomic 5D	41	5	13.1 ± .31	24.7 ± .44
Tetrasomic 5D	44	8	14.3 ± .30	25.4 ± .48
Ditelosomic 6D^{z}	42	6	17.8 ± .23	33.2 ± .80
Monosomic 6D	41	6	18.0 ± .39	33.4 ± .42
Tetrasomic 6D	44	6	15.9 ± .34	27.4 ± .43
Ditelosomic 7D ^{S}	42	6	15.6 ± .36	30.0 ± .73
Monosomic 7D	41	6	18.1 ± .37	32.8 ± .59
Tetrasomic 7D	44	6	18.3 ± .35	33.1 ± .89

On the other hand, the same table indicates that the absence of one complete chromosome 5D (in monosomic 5D) causes a significant decrease in mean silver grains counts. This can be due not only to the absence of the promotor but to the fact that the short arm of 5D apparently carries a nucleolar organizer whose absence in monosomic 5D decreases productions of nucleolar substance.

Chromosomes 1A and 5D, however, diverge in the fact that tetrasomic 1A of *T. aestivum* var. Chinese Spring showed no change in nucleolar activity whereas tetrasomic 5D of the same variety and the 5D alien chromosome addition line of *T. dicoccoides* («AAL 9») exhibited a decrease in their nucleolar activity relative to the activity of the corresponding normal disomic plants (Table 3).

This may indicate the presence of two different genetic mechanisms that regulate the nucleolar activity in either 1A or 5D chromosomes. These mechanisms could be composed of a promoter in the short arm of 1A and in the long arm of 5D and of an epistatic repressor in the opposite arm of 5D chromosome. Both promoter and repressor would have cumulative effect with the dosage, in 5D.

It is also very likely that the 6D chromosome carries a suppressor since tetrasomic 6D individuals exhibit a very significant decrease of nucleolar activity compared to disomic individuals. It may also be suspected that this suppressor is located in $6D^{\alpha}$ since ditelosomic $6D^{\alpha}$ does not show any change of activity in relation to the normal.

The majority of the regulatory elements that were studied are not directly associated with nucleolar organizers. However, they must induce changes in activity either upon all nucleolar organizers or on a pair of specific and identical SAT-sites. This seems to be the case for the regulatory elements of the short arm of chromosomes 7A and 7D, which must carry suppressors of the activity of a pair of specific nucleolar organizers. This repressing activity must be eliminated by the inhibitory action of the long arm of the same chromosomes.

Table 3, however, shows that the absence of the two nucleolar

organizers in ditelosomics $7A^{s}$ or $7D^{s}$ does not cause a significant decrease in nucleolar activity. This might be due to a compensatory mechanism similar to that observed in ditelosomic $6B^{L}$ (Mohan and Flavell, 1974).

From all these results it is fair to conclude that the nucleolar organization in polyploid wheat is under a very complex and intricate control by genes which are spread along the complement. These genes interact with each other and control the nucleolar activity either through the gene amplification of r-DNA cistrons or through some other unknown mechanism that regulates r-RNA transcription. Further data will be obtained after ribosomal RNA-DNA hybridization experiments are completed.

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