NUCLEOLAR ACTIVITY AND COMPETITION (AMPHIPLASTY) IN THE GENUS *AEGILOPS*

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Received 20.iii.84

SUMMARY

Nucleolar activity and competition (amphiplasty) has been analysed in diploid, tetraploid and hexaploid species of *Aegilops* using a highly reproducible silver-staining procedure. A comparative analysis of somatic metaphase chromosomes by phase contrast, C-banding and Ag-staining was made to analyse the activity of the nucleolar organiser regions (NORs). With the exception of *Ae. crassa* 4x, amphiplasty occurs in all the tetraploid and hexaploid species analysed. The *U* genome from *Ae. umbellulata* suppresses completely the NOR activity of the genomes *M*^o (*Ae. ovata*), *S*^o (*Ae. variabilis*), *D* (*Ae. juvenalis*) and *C* (*Ae. triuncialis*) and that of one pair of the nucleolar organiser chromosomes of the genomes *M*^c (*Ae. columnaris*), *M*^b (*Ae. biuncialis*), *M*ⁱ (*Ae. juvenalis*) and *M*ⁱ (*Ae. triaristata* 6x). The nucleolar activity of the *D* genome is completely suppressed by the genomes *U* (*Ae. umbellulata*), *C* (*Ae. cylindrica*) and *M*^o (*Ae. ventricosa*).

1. INTRODUCTION

Nucleolus organiser regions (NORs), which are associated with secondary constrictions of satellited chromosomes, have been shown to be the sites of ribosomal RNA genes. Differential amphiplasty, that is changes affecting individual chromosomes of the complement following interspecific hybridisation (Rieger *et al.*, 1976), was first described by Navashin (1928, 1934) in *Crepis* where the secondary constriction of the SAT-chromosomes of one of the parental species was absent in hybrids. The satellite was retracted onto the chromosome and the corresponding NOR is not distinguishable.

Highly reproducible silver-staining methods have been recently developed for the differential staining of the NORs (Ag-NORs) of plant chromosomes (Hizume *et al.*, 1980; Sato *et al.*, 1980; Lacadena *et al.*, 1984a). The silver procedure can be used to visualise gene activity at the rDNA sites with conventional light microscopy since only those NORs which were functionally active during the preceding interphase are stained by silver (Miller *et al.*, 1976a, 1976b; see review by Howell, 1982). Recently, Medina *et al.* (1983) concluded that Ag-NOR staining is associated with decondensed NOR chromatin, rather than active NOR chromatin. In other words, all the active NOR chromatin is detected by silver-staining but not all the Ag-NOR-stainable structures contain active NOR chromatin.

It was recently pointed out (Lacadena *et al.*, 1984a; Cermeño *et al.*, 1984) that the Ag-staining method could be very useful for the analysis of nucleolar competition (amphiplasty) in allopolyploid species. This is the case in the genus *Aegilops*. The occurrence of amphiplasty in polyploid species of *Aegilops* has been reported both by conventional light microscopy (Chennaveeraiah, 1960) and by *in situ* hybridisation of labelled ribosomal

RNA to metaphase chromosomes (Teoh *et al.*, 1983a). These authors concluded that in the polyploid *Aegilops* species the number of sites of rRNA gene clusters was consistently higher than the number of satellited chromosomes as observed by conventional light microscopy and suggested that this discrepancy is probably due to the inactivation of some nucleolar organiser regions when different genomes combine together.

The aim of this paper is to analyse the nucleolar activity and the occurrence of nucleolar competition (amphiplasty) in the genus *Aegilops* by using a highly reproducible silver-staining procedure developed by Lacadena *et al.* (1984a).

2. MATERIAL AND METHODS

(i) Material

The following species were used:

- (a) *Diploid species* ($2n = 2x = 14$)
Aegilops umbellulata (genome constitution, UU), *Ae. comosa* (MM), *Ae. uniaristata* (M^uM^u), *Ae. caudata* (CC), *Ae. squarrosa* (DD), *Ae. speltoides* (SS), *Ae. longissima* (S^1S^1), *Ae. sharonensis* (S^1S^1) and *Ae. bicornis* (S^bS^b).
- (b) *Tetraploid species* ($2n = 4x = 28$)
Ae. ovata (UUM^oM^o), *Ae. columnaris* (UUM^cM^c), *Ae. biuncialis* (UUM^bM^b), *Ae. variabilis* (UUS^vS^v), *Ae. triuncialis* (UUCC), *Ae. cylindrica* (CCDD), *Ae. ventricosa* (DDM^vM^v) and *Ae. crassa* 4x ($DDM^{cr}M^{cr}$).
- (c) *Hexaploid species* ($2n = 6x = 42$)
Ae. triaristata ($UUM^tM^tM^{t2}M^{t2}$), *Ae. crassa* 6x ($DDD^2D^2M^{cr}M^{cr}$) and *Ae. juvenalis* ($DDUUM^jM^j$).

This material was obtained from the collections of the Plant Breeding Institute, Cambridge, UK (kindly supplied by H. Guedes-Pinto, IUTAD, Vila Real, Portugal) and from the Estación Experimental de Aula Dei, Zaragoza, Spain.

(ii) Methods

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 contract the chromosomes. The root tips were subsequently fixed in 1:3 acetic acid:ethyl alcohol.

The C-banding procedure was carried out according to Giraldez *et al.* (1979).

The silver-staining procedure for nucleolar organiser regions, based on Hizume *et al.* (1980), was described by Lacadena *et al.* (1984a).

A comparative analysis of metaphase somatic cells by phase contrast, C-banding and Ag-staining was made. The nucleolar organiser chromosomes (SAT-chromosomes) were identified both with phase contrast and C-banding from comparisons of the same metaphase cells. On the other hand, silver-stained nucleolar organiser regions (Ag-NORs) were also clearly identified, being located in close correspondence with the secondary constrictions as observed previously by phase contrast of the same cells.

Interphase nucleoli were observed by silver-staining. Contingency tests indicated that the distributions of the number of nucleoli found in the different plants of each species were homogeneous and, consequently, individual data have been combined.

3. RESULTS AND DISCUSSION

The number of silver-stained nucleolar organiser regions (Ag-NORs) and nucleoli observed, respectively, at somatic metaphase and interphase cells in diploid, tetraploid and hexaploid species of *Aegilops* are shown in table 1. The maximum number of nucleoli observed in each species agrees with that of the corresponding Ag-NORs. Only in a few cases (*Ae. comosa*, *Ae. speltoides*, *Ae. columnaris*, *Ae. biuncialis*, *Ae. juvenalis*) the Ag-staining

TABLE 1

Silver-stained nucleolar organiser regions (Ag-NORs) and numbers of observed nucleoli at somatic metaphase and interphase cells, respectively, in diploid, tetraploid and hexaploid species of Aegilops

Species	No. of plants	No. of metaphase cells scored	Ag-NORs	No. of nucleoli at interphase							
				1	2	3	4	5	6	7	8
Diploid											
<i>Ae. umbellulata</i>	5	36	4	153	292	64	12	—	—	—	—
<i>Ae. comosa</i>	2	{ 4 2	{ 4 2	38	121	42	7	—	—	—	—
<i>Ae. uniaristata</i>	5	30	2	262	269	—	—	—	—	—	—
<i>Ae. caudata</i>	4	18	4	83	227	79	18	—	—	—	—
<i>Ae. squarrosa</i>	4	12	2	240	182	—	—	—	—	—	—
<i>Ae. speltoides</i>	5	{ 2 1 20	{ 4 3 2	486	491	116	15	—	—	—	—
<i>Ae. longissima</i>	5	14	4	148	273	82	16	—	—	—	—
<i>Ae. sharonensis</i>	4	23	4	207	179	46	4	—	—	—	—
<i>Ae. bicornis</i>	4	18	4	148	152	76	14	—	—	—	—
Tetraploid											
<i>Ae. ovata</i>	3	12	4	22	175	99	15	—	—	—	—
<i>Ae. columnaris</i>	5	{ 3 13	{ 5 4	62	227	201	77	21	8	—	—
<i>Ae. biuncialis</i>	5	{ 2 11 7	{ 6 5 4	114	183	127	73	14	1	—	—
<i>Ae. variabilis</i>	5	21	4	103	277	114	28	—	—	—	—
<i>Ae. triuncialis</i>	4	23	4	115	211	88	16	—	—	—	—
<i>Ae. cylindrica</i>	4	22	4	157	191	74	9	—	—	—	—
<i>Ae. ventricosa*</i>	6	41	2	345	255	—	—	—	—	—	—
<i>Ae. crassa</i> 4x	5	26	4	108	221	167	25	—	—	—	—
Hexaploid											
<i>Ae. triaristata</i>	5	14	6	47	191	164	85	20	5	—	—
<i>Ae. crassa</i> 6x	4	12	6	5	62	146	148	51	19	—	—
<i>Ae. juvenalis</i>	5	{ 21 1	{ 6 4	57	173	193	90	13	6	—	—

* Data taken from Orellana *et al.* (1984).

TABLE 2

A comparison of the number of active NORs revealed by Ag-staining (Ag-NORs) with that of satellited chromosomes and the occurrence of amphiplasty as deduced from the expected frequency of NORs from genome combinations in the Aegilops species

Species	Haploid genome constitution*	No. of pairs of SAT-chromosomes (Chenaveeraiah, 1960)		No. of pairs of Ag-NORs		Remarks on nucleolar competition (amphiplasty)†
		This work	Obs.	Exp.		
Diploid						
<i>Ae. umbellulata</i>	U	2	2	2	2	—
<i>Ae. comosa</i>	M	2	2	2	2	—
<i>Ae. uniaristata</i>	M ^u (or U _n)	1	1	1	1	cryptic amphiplasty?
<i>Ae. caudata</i>	C	2	2	2	2	—
<i>Ae. squarrosa</i>	D	1	1	1	1	cryptic amphiplasty?
<i>Ae. speltoides</i>	S	2	2	2	2	—
<i>Ae. longissima</i>	S ^l	2	2	2	2	—
<i>Ae. sharonensis</i>	S st	2	2	2	2	—
<i>Ae. bicornis</i>	S ^b	2	2	2	2	—
Tetraploid						
<i>Ae. ovata</i>	UM ^o	2	2	2	3 or 4‡	2U chrs. active U(G) > U(D)
<i>Ae. columnaris</i>	UM ^c	3	3	3	4	2U + 1M ^c chrs. active U(G) > U(D) >> M ^c (E)
<i>Ae. biuncialis</i>	UM ^b	3	3	3	4	2U + 1M ^b chrs. active U(G) > U(D) >> M ^b (E)
<i>Ae. variabilis</i>	US ^v	3	2	2	4	2U chrs. active U(G) > U(D)
<i>Ae. triuncialis</i>	UC	3	2	2	4	2U chrs. active U(G) >> U(D)
<i>Ae. cylindrica</i>	CD	1	2	2	3	2C chrs. active C(A) >> C(C)
<i>Ae. ventricosa</i>	DM ^v	1	1	1	2	1M ^v (C) chr. active
<i>Ae. crassa</i> 4x	DM ^{cr}	2	2	2	2	no amphiplasty M ^{cr} (C) >> D(B)

Hexaploid						
<i>Ae. triaristata</i> 6x	UM^1M^{12}	2	3	3	4	$2U + 1M^{(ort_1)} \text{ chrs. active}$ $U(G) \gg U(D) \gg M^{(ort_1)}$
<i>Ae. crassa</i> 6x	DD^2M^{cr}	—	3	3	4 or 5§	$2M + 1D^2 \text{ chrs. active}$ $M^{cr}(E) > M^{cr}(F) = D^2$
<i>Ae. juvenalis</i>	DUM^1	3	3	3	5	$2U + 1M^1 \text{ chrs. active}$ $U(G) > U(D) \gg M^1(E)$

* Genome symbols are based on Kihara (1954) and Kihara *et al.* (1959) modified after Kimber and AbuBakar (1981) and Kimber *et al.*, (1983). However, according to the C-banding pattern (Cerneno, manuscript in preparation) the genomes M^c , M^b , M^{cr} of *Ae. crassa* 6x and M^1 seem to be related with the genome M of *Ae. comosa*, while the genomes M^o , M^{cr} of *Ae. crassa* 4x, M^1 , M^{12} and, probably, M^o can be related to the genome M^u (Un) of *Ae. uniaristata*.

† The letters in brackets stand for individual SAT chromosomes of the corresponding genomes according to the classification of Teoh and Hutchinson (1983) and Teoh *et al.* (1983b). The symbols $>$ and $\gg$ refer to the different sizes of the Ag-NORs which are positively correlated with the amount of rDNA (Warburton and Henderson, 1979).

‡ The expected number of pairs of Ag-NORS depends on which is the origin of the M^o genome (M or M^u).

§ The expected number of pairs of Ag-NORS depends on which is the origin of the D^2 genome (D or S^1).

seems to have insufficient resolving power to reveal all the active NORs; however, the maximum number of nucleoli fit in with the expectations.

Taking into account the genome constitutions, the occurrence of nucleolar competition (amphiplasty) becomes apparent both in tetraploid and hexaploid species (table 2). Based on the C-banding patterns (Cermeño, manuscript in preparation) it has been possible to identify which chromosomes maintain nucleolar activity (plates 1 to 3).

As previously suggested (Lacadena *et al.*, 1984a; Cermeño *et al.*, 1984), the Ag-staining method has proven to be very useful in analysing natural amphiplasty in an allopolyploid taxonomic group as *Aegilops*.

The numbers of satellited chromosomes observed by phase contrast of metaphase cells (table 2) agree with those described by Chennaveeraiah (1960) with a few discrepancies, namely, *Ae. variabilis* (2 instead of 3 pairs) *Ae. cylindrica* (2 instead of 1 pair), *Ae. triaristata* (3 instead of 2 pairs), *Ae. triuncialis* (2 instead of 3 pairs). However, it is worth mentioning that in our observations the number of satellited chromosomes, as seen by phase contrast, are in quite good agreement with the Ag-NORs and the maximum number of nucleoli observed.

All the diploid *Aegilops* species analysed show two pairs of nucleolus organiser chromosomes excepting *Ae. uniaristata* (genome M'' or Un , see below) and *Ae. squarrosa* (genome D). Certainly, the occurrence of a cryptic amphiplasty cannot be inferred since it might be possible that both genomes have actually only one pair of nucleolus organiser chromosomes. Anyway, this situation represents an additional reason to consider the genome M'' as different from the genome M of *Ae. comosa*. In fact, Kimber *et al.* (1983), on the basis of the $M-M''$ meiotic pairing in certain hybrids, suggested that the M'' genome of *Ae. uniaristata* (*Triticum uniaristatum*) be changed to Un .

With the exception of *Ae. crassa* 4x, amphiplasty occurs in all the tetraploid and hexaploid *Aegilops* species analysed. Nevertheless, in *Ae. crassa* 4x the nucleolar activity of the chromosome "C" of the M genome is much higher than that of chromosome "B" of the D genome. So, it might be considered as a partial amphiplasty. The chromosomes "C" and "B" are named according to their resemblance to the C-banding patterns of the donor diploid species as described by Teoh and Hutchinson (1983).

The identification of the satellited chromosomes and their assignment to a determined genome was made according to their morphological characteristics and C-banding patterns after Cermeño's data (manuscript in preparation).

In all the species in which the genome U from *Ae. umbellulata* is present (*Ae. ovata*, *Ae. columnaris*, *Ae. biunncialis*, *Ae. variabilis*, *Ae. triuncialis*, *Ae. triaristata* and *Ae. juvenalis*) its two pairs of nucleolar organiser chromosomes are active. In all cases the activity (measured by the NOR size, Warburton and Henderson, 1979) is higher in the "G" chromosome than in the "D" chromosome (according to the terminology used by Teoh and Hutchinson, 1983).

It is worthy of mention that in *Ae. triuncialis* and in *Ae. triaristata* 6x the size of the Ag-NORs of chromosome "G" is much larger than that of chromosome "D" (plate 4). The strong activity of the "G" chromosome resembles that of chromosomes 6H^v of *Hordeum vulgare* (Santos *et al.*, 1984) and 6B of durum and common wheats (Cermeño *et al.*, 1984).

PLATE 1. Phase contrast and C-banded somatic metaphase cells of *Aegilops umbellulata* (a and b) and *Aegilops uniaristata* (c and d). Note the very small sizes of satellites of chromosomes D in *Ae. umbellulata*. The letters representing SAT chromosomes correspond with Teoh and Hutchinson's (1983) classification.

PLATE 2. Phase contrast (a) and C-banded (b) somatic metaphase cells of *Aegilops triuncialis*. One of the D chromosomes from the U genome has a very small satellite which is usually hardly distinguishable. The letters representing SAT chromosomes stand for the genome (in brackets) and the individual chromosomes according to Teoh and Hutchinson (1983).

PLATE 3. Ag-stained somatic metaphase cells of *Aegilops umbellulata* (a) and *Aegilops triuncialis* (b) showing Ag-NORs indicated by arrows.

PLATE 4. Partial view of an Ag-stained somatic metaphase cell of *Aegilops triaristata* showing the different sizes of Ag-NORs (see the text).

FIG. 1. Diagram showing nucleolar suppression and dominance relationships between the different genomes of *Aegilops* species. Letters in brackets represent SAT chromosomes of the corresponding genomes.

It is also to be noted that the presence of the U genome seems to suppress completely the nucleolar activity of the chromosomes of the genomes M^o (*Ae. ovata*), S^v (*Ae. variabilis*) and C (*Ae. triuncialis*). In addition, in the other polyploid species in which some nucleolar organiser chromosomes show activity it is very low (small sizes of the Ag-NORs); such is the case of *Ae. columnaris*, *Ae. biuncialis*, *Ae. triaristata* 6x and *Ae. juvenalis*. This strong nucleolar activity of the U genome was suggested by Martini *et al.* (1982) reporting the partial suppression of the nucleolar organising activity of wheat chromosomes by the presence of the U chromosomes in *Triticum aestivum*-*Ae. umbellulata* chromosome addition lines.

In *Ae. cylindrica* (CCDD) the nucleolar activity of the D genome chromosomes is suppressed by the presence of the C genome as in *Ae. ventricosa* (DDM^vM^v) where the M^v genome also suppresses the nucleolar activity of the D genome chromosomes (Orellana *et al.*, 1984). The D genome chromosomes are also inhibited in the hexaploid species *Ae. juvenalis* ($DDUUM^jM^j$) and *Ae. crassa* 6x ($DDD^2D^2M^{Cr}M^{Cr}$). In the latter species, the D^2 genome might be related to the S^1 better than to the D genome according to its C-banding pattern (Cermeño, manuscript in preparation) and morphological characteristics (Kihara, 1963). As indicated above, the nucleolar organiser chromosome of the D genome only seems to be active (although at a very low level) in the tetraploid *Ae. crassa* ($DDM^{Cr}M^{Cr}$). This weak activity of the nucleolar organiser chromosome of the D genome agrees with its behaviour in hexaploid wheat, *Triticum aestivum* (Cermeño *et al.*, 1984).

Summing up, all the above data can be diagrammatically represented as indicated in the fig. 1. A graded series of nucleolar activity including chromosomes of *Triticum aestivum*, *Hordeum vulgare*, *Secale cereale*, *Agropyron elongatum* and *Aegilops ventricosa* has been suggested by Lacadena *et al.* (1984b).

The evolutionary relationships between the genomes of the diploid species of the genus *Aegilops* as well as their modified genomes present in the tetraploid and hexaploid species have been extensively analysed (Kihara,

1954, 1963; Kihara *et al.*, 1959; Zohary and Feldman, 1962; Kimber and AbuBakar, 1981; Kimber *et al.*, 1983; Kimber and Zhao, 1983). However, the comparative analysis of the C-banding pattern has not been used to study the genomic relationships of polyploid *Aegilops* species. Until now such an analysis has only been made in diploid species (Teoh and Hutchinson, 1983; Teoh *et al.*, 1983b). From our observations (Cermeno, manuscript in preparation) it can be suggested that according to their C-banding patterns, the genomes M^c , M^b , M^{cr} (of *Ae. crassa* 6x) and M^i seem to be related with the genome M of *Ae. comosa* while the genomes M^v , M^{cr} (of *Ae. crassa* 4x), M^l , M^h and, probably, M^o can be considered related to the genome M^u (*Un*) of *Ae. uniaristata*.

Acknowledgment. This work was supported by a grant from the Comisión Asesora de Investigación Científica y Técnica of Spain.

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