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The Nucleolar Organisers of Tetraploid and Hexaploid Wheats Revealed by in Situ Hybridisation

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Summary. The technique of in situ hybridisation of cloned ribosomal DNA has been used to establish the numbers of nucleolar organising sites in a range of tetraploid and hexaploid wheats.

Key words: Nucleolar organisers – In situ hybridisation – Tetraploid and hexaploid wheats

Introduction

The technique of in situ hybridisation of cloned ribosomal DNA to mitotic chromosomes has recently been used to examine the number of nucleolar organisers of some diploid and hexaploid wheats (Gerlach et al. 1980; Miller et al. 1980). This paper describes it's application to examine the nucleolar organiser sites of tetraploid and other hexaploid wheats.

Materials and Methods

1 Plant Stocks

The tetraploid (2n = 4x = 28) and hexaploid (2n = 6x = 42) *Triticum* species, together with accessions of *Aegilops squarrosa* (2n = 2x = 14) and *Ae. speltoides* (2n = 2x = 14) were all from the collection at the Plant Breeding Institute, Trumpington, Cambridge.

2 In situ Hybridisation

The technique used for in situ hybridisation to mitotic root-tip chromosomes was described by Hutchinson et al. (1980). All preparations were pre-treated with ribonuclease prior to in situ hybridisation. For all accessions except *T. militinae,* 100,000 c.p.m. of probe were applied per slide, and the autoradiographs exposed for 6½ or 12 weeks. The preparations of *T. militinae,* together with further slides of *T. zhukovskyi,* were hybridised with 350,000 c.p.m. of probe and the autoradiographs exposed for 5½ weeks.

The nucleic acid probe used was tritium labelled RNA transcribed by *E. coli* RNA polymerase from the plasmid pTA71 described by Gerlach and Bedbrook (1979). This plasmid consists of a single wheat ribosomal RNA gene repeating unit (i.e. 18S+25S rRNA genes with associated spacer DNA) in the vector plasmid pACYC184 (Gerlach and Bedbrook loc. cit.).

Results and Discussion

1 Tetraploid Wheats

All the tetraploid wheats, with one possible exception, *T. carthlicum,* showed two pairs of nucleolar organising chromosomes (Table 1). To some extent, this result might have been predicted, because examination of mitotic karyotypes of tetraploid wheats has indicated two pairs of satellited chromosomes (Giorgi and Bozzini 1969a; Bozzini and Giorgi 1969). On the other hand, it has recently been shown that the diploid A genome wheats have two pairs of rRNA gene sites (Gerlach et al. loc. cit.). Similarly, two pairs of sites, such as found in the B genome of hexaploid wheat (Miller et al. 1980), might also be expected in the B or G genomes of the tetraploid wheats. Indeed, two pairs of nucleolar organising chromosomes were identified in *Ae. speltoides* (genome SS) (Fig. 1), which is a member of the Sitopsis section of *Aegilops* and thought to be closely related to the B genome of wheat (Riley et al. 1958).

In the tetraploid wheats, as shown in Fig. 2a and b, both pairs of nucleolar organising chromosomes are clearly satellited, and appear to be karyotypically similar to the satellited B genome chromosomes, rather than to the nucleolus organiser chromosomes of the A genome diploid wheats, which have small barely detectable satellites (Gerlach et al. loc. cit.; Giorgi and Bozzini 1969b). It therefore appears that, in the tetraploid wheats, as in the hexaploid wheat cultivar 'Chinese Spring' (Miller et al. 1980) there may have been

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Table 1. Numbers of nucleolar organising chromosomes in the tetraploid and hexaploid wheats, together with their supposed diploid progenitors

Ploidy	Genome	Species	Number of pairs of sites of rRNA gene clusters	
2X	AA	*T. monococcum*	2	Data from Gerlach et al. 1980
		T. urartu	2	
		T. thaoudar	2	
		T. aegilopoides	2	
	SS	*Ae. speltoides*	2	
	DD	*Ae. squarrosa*	1	
4X	AABB	*T. dicoccoides*	2	
		T. dicoccum	2	
		T. paleocolchiccum	2	
		T. turgidum	2	
		T. durum	2	
		T. polonicum	2	
		T. carthlicum	2 (or 3)[a]	
	AAGG	*T. araraticum*	2	
		T. timopheevi	2	
		T. militinae	2	
6X	AABBDD	*T. aestivum*	2 or 3	Data from Miller et al. 1980
		T. spelta	3 (or 4)[a]	
		T. vavilovii	2	
		T. macha	2	
		T. compactum	3	
		T. sphaerococcum	3	
	AAAAGG	*T. zhukovskyi*	4	

[a] Small extra sites of hybridisation were shown in some cells only

either a diminution of the A genome ribosomal genes so that they are no longer detectable by in situ hybridisation, or, alternatively, there could have been a change in the morphology of the A genome chromosomes.

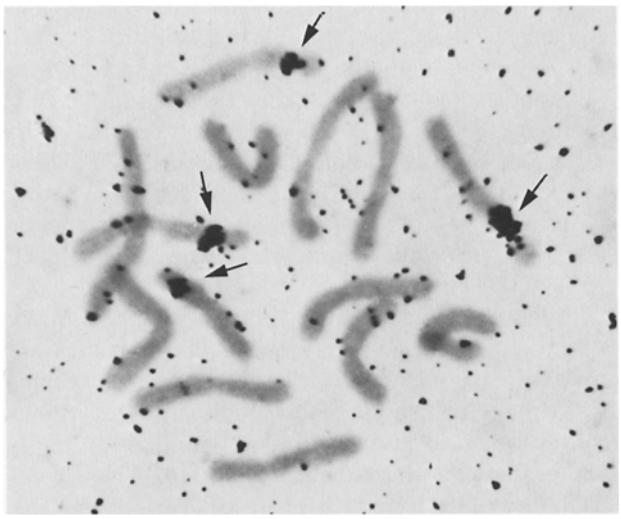

Fig. 1. In situ hybridisation of RNA complementary to nucleolar organiser DNA to mitotic metaphase chromosomes of *Aegilops speltoides*. Nucleolar organiser sites are arrowed

Unlike the other tetraploid wheats, *T. carthlicum* may show an additional pair of rRNA gene clusters in some cells after a long period of autoradiographic exposure. This third site is very small relative to the other rRNA gene sites, and is located in a terminal position. It was suggested that there may have been some introgression of *T. aestivum* into *T. carthlicum* on the evidence of the free threshing nature of *T. carthlicum* resulting from the presence of the Q gene complex found in *T. aestivum* but not in other tetraploid wheats (Morris and Sears 1967).

2 Hexaploid Wheats

2.1 Genome AABBDD

In contrast to the uniformity shown by the tetraploid wheats, the data show a range of variation in the nucleolar organising sites amongst the AABBDD hexaploids (Table 1). In all hexaploids two pairs of sites occur on chromosomes with marked satellites. However, *T. compactum* and *T. sphaerococcum* show an additional pair of minor sites located on a chromosome without an obvious satellite. Previous biochemical and in situ studies of other hexaploid wheat, *T. aestivum* and *T. spelta,* have shown that the major rRNA gene

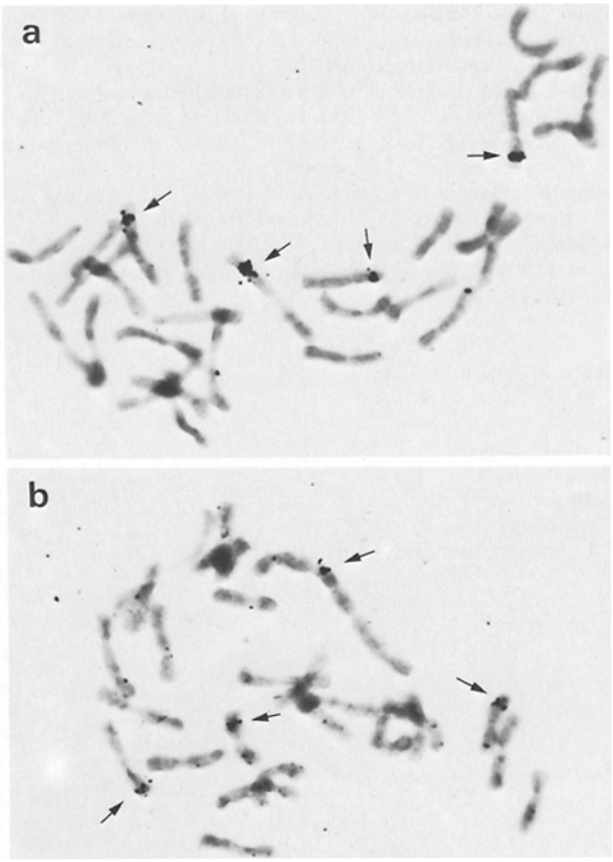

Fig. 2a and b. In situ hybridisation of the rRNA gene probe to tetraploid wheat: **a** *T. paleocolchicum;* **b** *T. timopheevi*

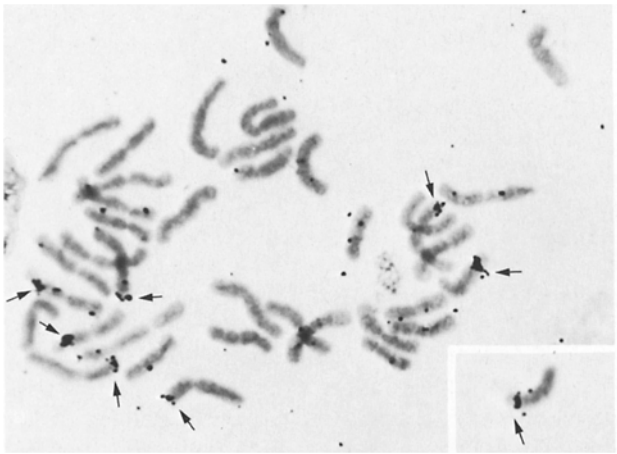

Fig. 3. In situ hybridisation of the rRNA gene probe to the hexaploid *T. zhukovskyi.* Although the spread is incomplete, all 4 pairs of nucleolar organising chromosomes are clearly shown

clusters are found on chromosomes 1 B and 6 B (Flavell and Smith 1974; Flavell and O'Dell 1976; Miller et al. 1980). Additional, smaller sites were found on chromosomes 1 A and probably 5 D of *T. spelta* using intervarietal chromosome substitution lines (Miller et al. loc. cit.), and this was confirmed in the present experiment which showed four pairs of sites in some cells of *T. spelta.* In *T. aestivum,* however, there was variation between different cultivars in that the rRNA genes were located either on chromosome 1A, or alternatively on chromosome 5 D (Miller et al. 1980). (*Ae. squarrosa,* the D genome donor, shows just one pair of nucleolar organisers). It is therefore difficult to assign the third pair of rRNA gene clusters of *T. compactum* and *T. sphaerococcum* to a particular chromosome. It should also be pointed out that variation between accessions, such as reported for *T. aestivum,* might also be found if more accessions were probed.

2.2 Genome AAAAGG.

T. zhukovskyi is thought to have the genome constitution AAAAGG, having arisen as an amphiploid from the cross *T. timopheevi* × *T. monococcum* (Bowden 1959; Upadhya and Swaminathan 1963). As described above, the tetraploid *T. timopheevi* (Genome AAGG) has two pairs of rRNA gene clusters located on satellited chromosomes, while *T. monococcum* has two pairs of terminally located sites (Gerlach et al. loc. cit.). In situ hybridisation of the rRNA gene probe to *T. zhukovskyi* shows four pairs of nucleolus organising sites (Table 1). Two of these sites are located on markedly satellited chromosomes and two are located at the ends of the chromosomes (Fig. 3). This therefore implies that, unlike the AABBDD hexaploids where one or both of the A genome rRNA gene sites have been lost, in *T. zhukovskyi* both pairs of the A genome rRNA gene sites of the supposed *T. monococcum* progenitor have been retained, together with the two pairs of sites of the *T. timopheevi* progenitor. This may simply represent a different pattern of variation, such as found amongst the different *T. aestivum* cultivars (Miller et al. 1980). Alternatively, it may imply that *T. zhukovskyi* is of relatively recent origin, as is suggested by the observation of multivalent formation at meiosis in *T. zhukovskyi* (Upadhya and Swaminathan 1963).

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