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Expression of *Thinopyrum distichum* NORs in wheat × Thinopyrum amphiploids and their backcross generations

K. C. Armstrong*, H. Le and G. Fedak**

Molecular Cytogenetics, Plant Research Center, Agriculture Canada, Ottawa, Ontario K1A 0C6, Canada

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Summary. The C-banding pattern of the satellited chromosomes in *Thinopyrum distichum* and *Triticum durum* was established. Both *T. durum* and *Th. distichum* contained two pairs of satellited chromosomes, which could be distinguished from one another. In the amphiploids [*T. durum* × *Th. distichum* (2x = 56)] and in the backcross *T. durum*/(*T. durum* × *Th. distichum*)^2, BC_1F_3, and BC_1F_5 (2n = 42) the satellite was visible on only *1B* and *6B* of *T. durum*. The vector pTa 71 containing the rRNA gene from wheat hybridized to two pairs of chromosomes (four hybridization sites) in *T. durum* and *Th. distichum*, to eight sites in the amphiploid hybrid (2n = 56), and to six sites in the backcross populations BC_1F_1. BC_1F_3, and BC_1F_5 (2n = 42). The two satellite pairs in *Th. distichum* could be distinguished by the chromosomal location of the rRNA site (median or subterminal) and by the centromere position. One copy of each pair was present in the BC_1F_1, but in the BC_1F_3 and BC_1F_5 populations the pair with the subterminal location of rRNA genes was absent. Silver nitrate staining indicated that the rRNA genes of *T. durum* did not completely suppress those of *Th. distichum*. The octoploid amphiploid (2n = 56) contained a maximum of four large and four small nucleoli and the hexaploid BC_1s (2n = 42), four large and two small nucleoli.

Key words: Amphiplasty – Wheat – *Thinopyrum* – Nucleoli – In situ hybridization

* To whom reprint requests should be addressed
** Present address: Department of Plant Science, McGill University, MacDonald Campus, Box 4000, Ste. Anne de Bellevue, Quebec H9X 1CO, Canada

Introduction

The term differential amphiplasty refers to the changes that affect individual chromosomes following interspecific hybridization. The phenomenon was observed in interspecific hybrids of *Crepis*, where the secondary constriction of the satellite chromosome of one of the parent species was not expressed, rather the satellite was retracted onto its own chromosome arm (Navashin 1934). It was demonstrated that only chromosomes with a secondary constriction were active in the formation of nucleoli. Differential amphiplasty has been reported in many other species of plants and animals (Lacadena et al. 1988).

Differential expression of the nucleolus organizer regions (NORs) in interspecific and intergeneric hybrids of the Triticeae has been documented in many combinations (Lacadena et al. 1988), and there is an interesting hierarchy among the genera of suppression and dominance. For example, the NOR activity of *Secale cereale* L. is suppressed in all hybrid combinations with tetraploid or hexaploid species of *Triticum* that have been studied and in combinations with *Aegilops* and *Hordeum* (Lacadena et al. 1988). The NOR chromosomes of tetraploid and hexaploid wheat also suppress the SAT chromosomes of *Haynaldia villosa* Schur. [*Dasypyrum villosum* (L.) Candary, Friebe et al. 1987], but behave only as dominant (suppression not complete) in the presence of *Lophopyrum elongatum* (Host) D. R. Dewey [*Elytrigia elongata* (Host) Nevski., *Agropyron elongatum* (Host) Beauvois] SAT chromosomes (Lacadena et al. 1984).

Thinopyrum distichum (Thumb.) Love [*Elytrigia distycha* (Thumb.) Proludin ex Love, *Agropyron distichum* (Thumb.) P. Beauvois] is a tetraploid (2n = 28) species endemic to South Africa. It belongs to the genus *Thinopyrum* Love, section Thinopyrum, while *L. elonga-*

tum belongs to the section Lophopyrum (Dewey 1984). The amphiploid with *T. durum* was backcrossed to tetraploid wheat. *T. distichum* has tolerance to high salt and perhaps flooding, and is also known to have high protein content and to be tolerant to BYDV (Barley Yellow Dwarf Virus). Hybrids and amphiploids between both tetraploid and hexaploid wheat and *Th. distichum* have been made (Pienaar 1981).

In this study the number of NOR sites was determined by in situ hybridization with a ribosomal RNA probe, the chromosomes with secondary constrictions were defined with the C-banding techniques, and the number of nucleoli was determined with silver nitrate staining. These results were related to the expression of the rRNA genes from wheat and *Th. distichum* in hybrids.

Materials and methods

Seed stocks

Seeds were obtained from Dr. R. de V. Pienaar (Department of Genetics, University of Stellenbosch, Republic of South Africa). These included seeds from *T. turgidum* cultivars 'Calvin' and 'Nordum', *Th. distichum* collected from Betty's Bay and St. Francis Bay in South Africa, the amphiploid *T. durum* × *Th. distichum* (2n = 56), the backcross *T. durum*/(*T. durum*/*Th. distichum*) (BC_1F_3 and BC_1F_5), and the decaploid amphiploid (2n = 70) with *T. aestivum*. The production and some meiotic characteristics of the F_1 progeny and BC_1 have been described previously (Pienaar 1981).

Silver nitrate staining

The technique of Lacadena et al. (1984) was used without significant modifications. The number of nucleoli at interphase was counted to determine the number of active nucleolar organizer regions.

Giemsa C-banding

The technique employed was as described by Armstrong et al. 1983) with minor modifications to treatment times in barium hydroxide, washes, and staining times in Giemsa.

In situ hybridization

The vector pTa71 (Gerlach and Bedbrook 1979), containing a complete ribosomal RNA repeat unit from wheat, was obtained from R. Flavell (Institute for Plant Science Research, Cambridge). The in situ hybridization technique was carried out as described by Le et al. (1989) with a biotin-labelled probe.

Results

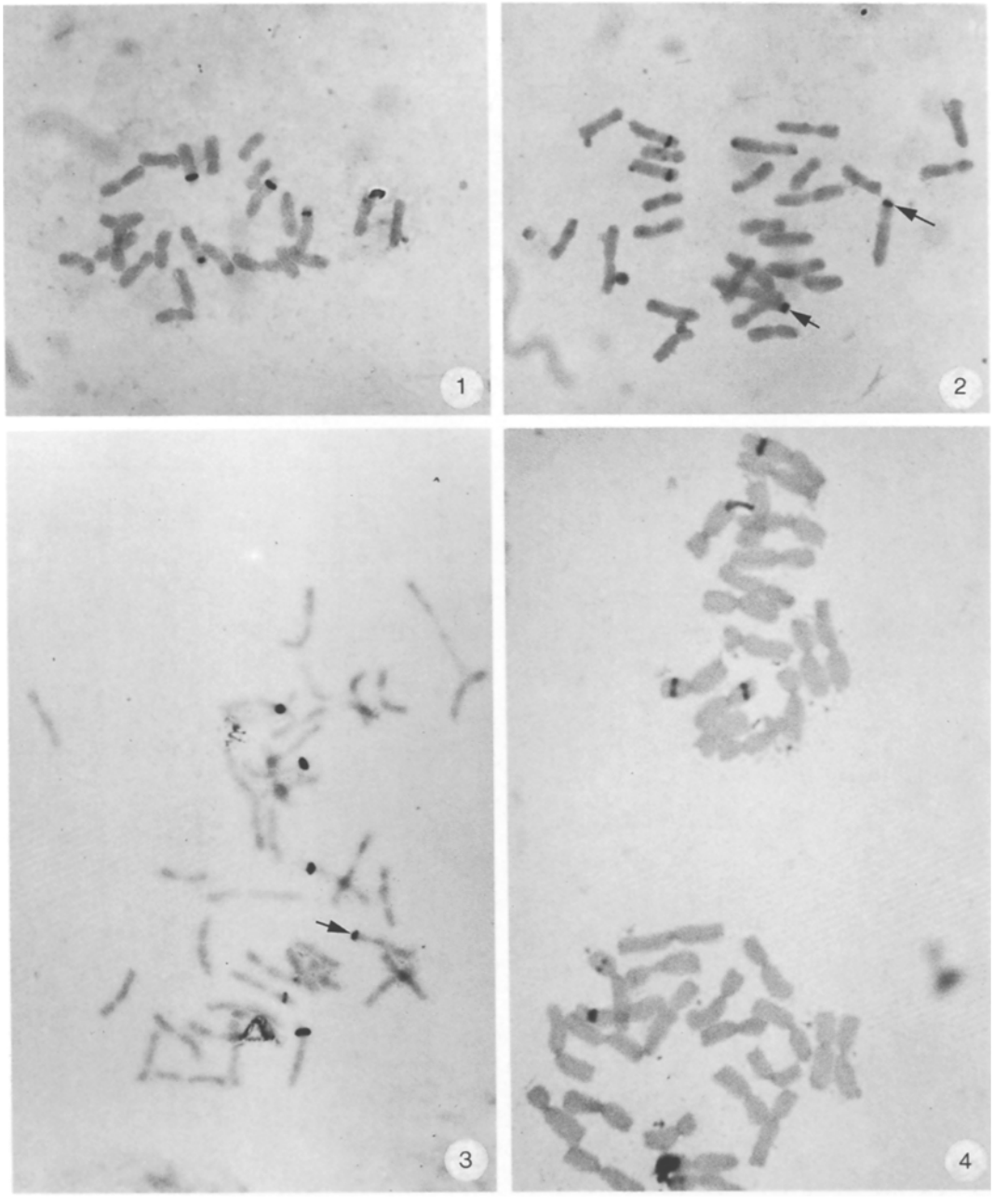
In situ hybridization

The ribosomal RNA probe binds to a NOR region, regardless of whether the NOR is active or inactive; therefore, the probe will reveal all NOR sites. In *T. durum* cultivars 'Nordum' and 'Calvin,' four rRNA sites were observed, which should correspond to the NORs of chromosome *1B* and *6B* (Fig. 1). In *Th. distichum* there were also four binding sites, corresponding to two pairs of chromosomes with SATs (Fig. 2). The amphiploid (2n = 56) contained eight hybridization sites, which corresponded to all SATs. However, the BC_1F_3 and BC_1F_5 progenies (2n = 42) contained only six hybridization sites, corresponding to only three pairs of SATs (Fig. 3). The BC_1F_1 was resynthesized by crossing the amphiploid 79S14 (2n = 56) onto *T. durum* cultivars 'Stewart 63' and 'Ma,' and the resulting plants were shown to also contain six hybridization sites (Fig. 4).

The two pairs of SAT chromosomes in *Th. distichum* could be identified by the in situ hybridization site of pTa71 and by centromere location. One pair of SAT chromosomes had a median to submedian centromere and the hybridization site was median on the short arm. The other pair had a submedian centromere and the hybridization site was subterminal on the short arm. One copy of this latter chromosome was present in the BC_1F_1 (2n = 42), but was absent in the BC_1F_3 and BC_1F_5 plants examined.

Giemsa C-banding

Giemsa C-banding patterns can be used to distinguish between the two SAT chromosome pairs of *T. durum* (*1B* and *6B*) or the two SAT pairs of *Th. distichum* (Fig. 5). The SAT pairs of *Th. distichum* were also distinguishable from those of *T. durum*. In *T. durum*, chromosome *1B* had a terminal band on the long arm, some banding at the centromere, and a median band on the short arm. Chromosome *6B* had banding on both sides of the centromere. Neither SAT pair in *Th. distichum* had a strong terminal band or strong banding around the centromere. One pair of chromosomes was a medium length submedian chromosome as described by Pienaar (1981). A weak subterminal band and two weak interstitial bands were located in the long arm of this pair, and there was a weak band proximal to the centromere in the short arm. A very weak band was also occasionally seen in the short arm proximal to the secondary constriction and another in the short satellite. The second satellited chromosome was a medium length chromosome with a more median centromere. The satellite was longer than that on the first chromosome. It had a band in the long arm submedian to the centromere and a band in the short arm proximal to the centromere. The secondary constriction in this arm was in approximately a median position. A weak band was occasionally seen in the satellite proximal to the telomere. In both the 2n = 56 amphiploid and the 2n = 42 chromosome BCs, secondary constrictions were seen only on chromosomes *1B* and *6B* of *T. durum*, and those of *Th. distichum* were contracted. The presence of the secondary constriction is correlated with functionality of the NOR; therefore, it appears that only the NORs of *T. durum* are functional.



Figs. 1–4. Metaphase chromosomes illustrating the number of hybridization sites with the rRNA probe pTa 71 from wheat. **1** Four hybridization sites in *T. durum*. **2** Four hybridization sites in *Th. distichum*. The submedian pair with the subterminal location of the rRNA genes is indicated by an *arrow*. **3** Six hybridization sites in the BC_1F_1 (2n = 42) from *T. durum*/*T. durum*/*Th. distichum*. The one subterminal rRNA site from *Th. distichum* is shown by an *arrow*. **4** Six hybridization sites in a BC_1F_3 plant. The *Th. distichum* chromosome with the subterminal site for rRNA genes is not present

Silver nitrate staining

The maximum number of nucleoli seen in *T. durum* and *Th. distichum* was four (Table 1). This correlated with the number of rRNA hybridization sites. The maximum number of nucleoli seen in the 2n = 56 amphiploid was seven (Table 1): four large and three very small ones. Presumably the small nucleoli were the NORs of *Th. distichum* which were not completely suppressed. For comparison, the number of nucleoli in the amphiploid *T. aestivum* cv 'Chinese Spring' × *Th. distichum* was also determined. This decaploid amphiploid should have eight nucleoli and, in addition, occasional expression of *5D* might be detected (Flavell and O'Dell 1976). In the decaploid,

Table 1. Number of nucleoli in root-tip cells

Species and amphiploid	2n	Number of cells with 1–8 nucleoli								Total cells	Avg. no. nucleoli	No. plants
		1	2	3	4	5	6	7	8			
T. turgidum	28	116	149	75	26	0	0	0	0	366	2.03	2
Th. distichum	28	107	127	72	21	0	0	0	0	327	2.02	2
79S14[1]	56	85	198	242	152	48	20	3	0	748	2.94	4
79S36[2]	42	51	169	107	51	2	0	0	0	380	2.43	2
78S125[3]	70	43	87	92	135	86	26	11	2	482	3.55	3
78S126[3]	70	25	123	196	156	96	29	12	4	641	3.51	5

1. (*T. durum* × *Th. distichum*)[2]
2. *T. durum* × (*T. durum* × *Th. distichum*)[2] $BC_1F_3 + BC_1F_5$
3. (*T. aestivum* cv 'C.S.' × *Th. distichum*)[2]

Table 2. Number of small nucleoli in root-tip cells

Species and amphiploid	Number of cells with 0–5 nucleoli							Total cells	Avg. no. nucleoli	No. plants
	2n	0	1	2	3	4	5			
T. turgidum	28	0	0	0	0			357	0.00	2
Th. distichum	28	0	0	0	0			327	0.00	2
79S14[1]	56	282	144	107	53	23	2	611	1.01	3
79S36[2]	42	716	73	10	0	0		799	0.12	3
78S125[3]	70	89	93	83	34	9	1	309	1.30	2
78S126[3]	70	173	69	46	22	12	1	323	0.87	2

1. (*T. durum* × *Th. distichum*)[2]
2. *T. durum* × (*T. durum* × *Th. distichum*)[2] $BC_1F_3 + BC_1F_5$
3. (*T. aestivum* cv C.S. × *Th. distichum*)[2]

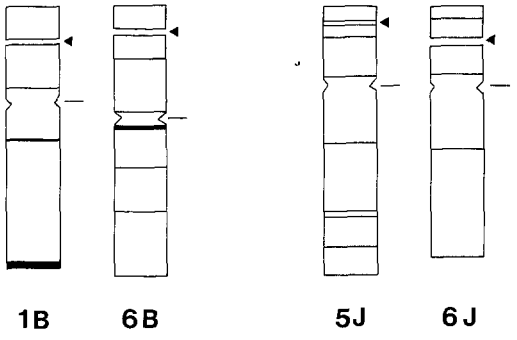


Fig. 5. Illustration of the C-banding patterns of the satellite chromosomes of *T. durum* cv 'Nordum' and *Th. distichum*. *Arrowhead* represents the secondary constriction and the horizontal line, the centromere. The reference to *5J* and *6J* is presumed on the basis of the similarity to *L. elongatum*

all eight nucleoli were seen in the same cell (four large plus four small). The average number of nucleoli seen in the two decaploid populations (79S125 and 79S126) was 3.55 and 3.51, and that in the octoploid population with *T. durum* (79S14) was 2.94. There may be a significantly higher frequency of nucleoli in the amphiploid with *T. aestivum* as compared to the amphiploid with *T. durum*. In both the BC_1F_3 and BC_1F_5, the maximum number of nucleoli was usually four, but five were seen rarely. The average number of small nucleoli in the hexaploid amphiploid BC_1F_3 was 0.12 and the maximum number was 2, as expected.

Discussion

According to Lacadena et al. (1988), the NORs of tetraploid and hexaploid wheat suppress the expression of the NORs of *Secale cereale* L., *Aegilops umbellutata*, and *Haynaldia villosa* (Friebe et al. 1987) in intergeneric combinations. However, the NORs of *L. elongatum* are not completely suppressed in intergeneric hybrids with hexaploid wheat, but the NORs of wheat are strongly dominant. Evans (1962) noted that the secondary constrictions of *L. elongtum* were not visible in hybrids with *T. durum*. This contraction of the secondary constriction is correlated with the suppression of the NOR regions.

In this study it has been shown that the secondary constrictions of *Th. distichum* are not visible in hybrids with *T. durum*, and that while the NORs of both tetraploid and hexaploid wheat are strongly dominant over the NORs of *Th. distichum*, the expression of the *Th. distichum* NORs is not completely suppressed. The latter are able to form small nucleoli in many cells.

The average number of NORs per cell in the amphiploid of *T. aestivum* vs 'Chinese Spring' – *Th. elongatum* can be calculated as 3.91 (from Lacadena et al. 1984), as compared to 2.94 for *T. durum* – *Th. distichum* and 3.55 and 3.51 for *T. aestivum* – *Th. districhum* in this study. Expression of the NOR on *5D* may account for the higher number of nucleoli in *T. aestivum* – *Th. distichum* than in *T. durum* – *Th. distichum*. The number of nucleoli in *T. aestivum* – *L. elongtum* is even higher, which suggests that a different degree of dominance is expressed in this combination.

It is not possible to compare the size of the NORs found in the two studies. It is considered that in the amphiploid with *Th. distichum*, the nucleoli formed by the NORs of *Th. distichum* are very small, indicating that their expression is strongly suppressed. There may have been a different degree of suppression in the amphiploids with *L. elongatum*. The NORs of wheat are not dominant in all wide crosses. The order of NOR dominance in addition lines to hexaploid wheat cv 'Chinese Spring' with *Hordeum vulgare* is $6B > 1B = 5HV \gg 5D$ in the *5HV* addition lines, and is $6HV > 6B > 1B \gg 5D$ in the *6HV* addition lines (Santos et al. 1984). The order of nucleolar dominance among the rDNA variants of *1U*, from *Aegilops umbellulata*, and chromosomes *1B* and *6B* of hexaploid wheat ('Chinese Spring') is $1U > 1B > 6B$ and parallels the decrease in the length of the spacer region, the decrease in the number of unmethylated CCGG sites, and the increase in the number of DNase sensitive sites (Martini et al. 1982; Flavell et al. 1986, 1988; Thompson and Flavell 1988) and in the number of spacer units (Gustafson et al. 1988). The presence of the NOR of *1U* (in addition lines of 'Chinese Spring') actually increases the methylation of the spacer regions of *1B* and *6B*. In addition, differences in sequence composition and association with high DNase sensitive repeats may affect the amount of transcript. Cytologically, the suppression of the NOR of chromosome *1R* from *Secale cereale* is correlated with the suppression of the dispersed state of these genes in the presence of *1B* and *6B* (Appels et al. 1986). The dispersed state is correlated with nucleolus formation and rDNA transcription. The cause and effect relationship of DNA dispersion and DNase sensitivity, etc., while presumably logical, has not been established, nor have the factors that promote or suppress dispersion. Presumably, enzymes such as topoisomerases may be involved (see Thompson and Flavell 1988).

The morphology of the satellited chromosomes in *Th. distichum* appears to be similar with respect to length, arm ratio, and size of satellite to those found in *L. elongatum* by Dvorak et al. (1984a). Pienaar (1981) had described only the smaller satellite in *Th. distichum*. Dvorak et al. (1984b) have shown that the nontranscribed spacer regions of the rRNA genes of *L. elongatum* differ between the two NORs. The short arm of chromosome *5E* contained a 2.4-kb variant, whereas the p arm of *6E* carried a 2.0-kb and 1.0-kb variant. Similar information is not yet available for *Th. distichum*.

The loss of the one NOR of *Th. distichum* in advanced backcrosses to *T. durum* may be of some interest. In this case, the NOR that is not present in these progenies is on the chromosome morphologically similar to *5E* of *L. elongatum* (the small satellite). This may be a random event or it may indicate that partial expression of this NOR, or another gene on the chromosome, has deleterious effects on the transmission of the chromosome or agronomic performance in a way that would result in elimination by the breeder or natural selection. The backcross progeny from *T. durum*/(*T. durum*/*Th. distichum*) would contain only one copy of each satellited chromosome and a homoeologue from which the NOR had been eliminated. The progeny of this BC would segregate for the number and type of *Th. distichum* NORs. Experiments are in progress to determine if this segregation is normal or distorted.

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