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A comparative study of the mitochondrial genome organization in *in vitro* cultures of diploid, tetraploid, and hexaploid *Triticum* species

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Abstract Southern-blot hybridizations of total DNA to mitochondrial DNA (mtDNA) probes were used to investigate the extent of mtDNA variability in cultures derived from immature embryos of diploid (*Triticum monococcum*, genomic formula: AA. *T. tauschii*, genomic formula: DD), allotetraploid (*T. durum* cv "Creso", genomic formula: AABB), and allohexaploid (*T. aestivum*, genomic formula: AABBDD) wheat species. Similar distinct changes in mtDNA organization were observed in *in vitro* cultures of the derived tetraploid and the hexaploid species with related genomes. The tetraploid and hexaploid species share the B genome and mtDNA variability in *in vitro* culture is known to be under nuclear control. These results suggest that a study of B genome diploids and other polyploid combinations would now shed light on whether or not mtDNA variability in tissue cultures is under B-genome control.

Key words *Triticum* · Allopolyploidy · Mitochondrial DNA · Somatic tissue culture · Chondriome variability

Introduction

Bread wheat, *Triticum aestivum*, is an allohexaploid produced from two separate hybridization events involving three species. The initial hybridization, which is believed to have occurred before 8000 BC, was between *T. urartu*, the A-genome donor, and an undetermined B-genome donor to produce the wild allotetraploid *T. turgidum* var. dicoccoides (genomic formula AABB). For many years it was speculated that *T. speltoides* (syn. *Aegilops speltoides*) was the B-genome donor. Subsequent evidence indicated, however, that *T. speltoides* could not have been the donor of the B genome (Vedel et al. 1978) and that *T. searsii* (syn. *Aegilops searsii*) is a more likely candidate (Kimber and Sears 1987; Kimber and Feldman 1987). Hexaploid wheat (AABBDD) arose as a result of a second hybridization event involving *T. turgidum* and the diploid *T. tauschii*, the donor of the D genome.

Previous work had shown that the mitochondrial genome is maternally inherited in polyploid wheats (Vedel et al.1981; Graur et al. 1989) and that the B-genome donor, and the allotetraploid species, can be regarded as the female parents of the allotetraploid and allohexaploid species, respectively (Vedel et al. 1978; Graur et al. 1989). This is consistent with both the variability observed in the mtDNA of diploid species and the stability of mtDNA in tetraploid and hexaploid species (Breiman 1987). It is possible that the mitochondrial genome organization of the hexa- and tetra-ploid *Triticum* species is unaffected by the nuclear genotype whereas the diversity observed in the diploid wheats is due to nuclear selection of particular mitochondrial genotypes. This hypothesis of some nuclear influence over control of the mitochondrial genome organization is strengthened by studies on the conversion of maize (Escote-Carlson et al. 1990) and common bean (Mackenzie et al. 1988) CMS lines to another background, in which it has been shown that nuclear genes interact with the mitochondrial genome to change its conformation. In recent work, Brown et al. (1993) have obtained some results which can be compared to those of Breiman (1987) mentioned above, in which RAPD analysis detected a noticeably higher level of polymorphism in the nuclear genome of plants regenerated from an *in vitro* culture of diploid *Triticum* species compared with hexaploid regenerants.

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Interestingly, the higher stability of both the nuclear and mitochondrial information in whole plants and regenerants of hexaploid species does not apparently apply to cultured cell lines, at least as far as the nuclear genome is concerned. Previous cytological studies comparing chromosome stability in cell suspensions of the two diploid species *T. monococcum* (AA) and *T. tauschii* (DD), the tetraploid species *T. durum* cv "Creso" (AABB), and the hexaploid *T. aestivum* cv "Sicco" (AABBDD) revealed that nuclear genome instability in cultured cells was a phenomenon common to all *Triticum* species regardless of their ploidy (Winfield et al. 1993). However, the degree of aneuploidy and chromosome restructuring was most pronounced in the hexaploid species. As far as the mitochondrial genome organization of hexaploid wheats is concerned, a number of reports describe marked changes when both somatic and gametophytic cells and tissues are cultured *in vitro* (Hartmann et al. 1987; Rode et al. 1987; Aubry et al. 1989; Morère-Le Paven et al. 1992). These changes, at least in somatic tissue cultures, are under nuclear control (Hartmann et al. 1992).

In the present work, we have determined the variability of selected coding and non-coding regions of the mitochondrial genome in short- and long-term cultures derived from two diploid (*T. monococcum* and *T. tauschii*), one tetraploid (*T. durum* cv "Creso"), and one hexaploid (*T. aestivum* cv "Sicco") wheat species. The results are consistent with those previously obtained from the nuclear genome (Winfield et al. 1993); they suggest that the organization of the mitochondrial genome in cultures derived from the diploid species is more stable than that of the tetra- and hexa-ploid species.

Materials and methods

Cultivation of plants

Seeds of the four species *T. monococcum*, *T. tauschii*, *T. durum* cv "Creso" and *T. aestivum* cv "Sicco", were supplied by S. Reader (John Innes Centre, Norwich, England). Plants were grown in a controlled environment room (16-h photoperiod of 275 $\mu E \cdot m^{-2} \cdot s^{-1}$ provided by 125-W white fluorescent tubes and 25-W tungsten bulbs; day temperature of 20°C; night temperature of 16°C).

Initiation and maintenance of callus and cell suspensions

Calluses were initiated from immature embryos dissected, with the aid of a binocular microscope, from developing seed 14–16 days post-anthesis. Dissected immature embryos, 1–2 mm long, were placed on MS medium (Murashige and Skoog 1962). This was supplemented with 30 g/l of sucrose, and 2.0 mg/l of 2,4-dichlorophenoxyacetic acid (2,4-D). Calluses were subcultured every 4 weeks. In all cases, the maintenance medium and the induction medium were identical. DNA prepared from a 3-month-old callus was used in Southern analyses.

Suspensions were initiated from 28-day-old callus by transferring all the callus from one plate into liquid MS medium, as above, supplemented with 5 g/l of 2,4-D. Liquid cultures were maintained in a rotary shaker set at 70 rpm in the dark at 25°C and were subcultured every 7 days by replacement of all the culture medium and with about 5 g fresh weight of cells per vessel. DNA prepared from 10-month-old suspensions was used in Southern analyses.

Isolation of total cellular DNA

Total cellular DNA of plants, callus, and cell suspensions was prepared as described previously (Hartmann et al. 1989).

Isolation of chloroplast DNA

Chloroplast DNA of the allohexaploid cv Sicco was prepared as previously described (Rode et al. 1985) and used in Southern-blot analysis to eventually localize hybridization signals due to sequences common to both the chloroplast genome and the mitochondrial probe used.

Molecular probes

The following cloned mtDNA fragments were used: (1) a 3.6-kb wheat *Sal*I fragment [fragment X_2 according to the nomenclature of Quétier et al.(1985)] apparently not containing any coding sequence, (2) a 10.8-kb wheat *Sal*I fragment (fragment K′, according to the nomenclature of Quétier et al. (1985)] containing the recombinogenic repeated element no. 5 and the 5′ end of the *rrn*26 gene. In hexaploid wheats, repeated element no. 5 is found in fragments J′, K_1, K′ and M, (3) a maize 1073-bp *Eco*RI-*Sst*I fragment, internal to the *atp*A gene (a generous gift of C. J. Leaver), (4) a wheat 800 bp *Xho*I-*Bam*HI fragment comprising the last 204 bp of the 3′ end of *atp*9 gene, (5) a wheat 1067-bp *Bgl*II-*Hin*dIII fragment comprising the first 900 bp of the 5′ end of the *atp*6 gene, and (6) a wheat 1279-bp *Hae*III fragment comprising the first 1238 bp of the 5′ end of the *cox*I gene.

Southern analysis

Total cellular DNA samples were digested to completion with various restriction endonucleases (*Sal*I, *Hin*dIII, *Pst*I) according to the manufacturer's specifications. Restricted DNA was electrophoresed on 0.8% agarose vertical gel slabs in TAE (0.04 M Tris-acetate, pH 8; 0.02 M EDTA) buffer stained with ethidium bromide and photographed under UV light. Gels were treated as described by Southern (1975) and DNA was transferred to Hybond C (Amersham) according to either the manufacturer's protocol or Ketner and Kelly's (1976) method. Hybridizations were performed as follows: filters were preincubated in a 2×SSC, 1×Denhardt solution for at least 4 h and allowed to hybridize at 42°C for 12–16 h in a 2×SSC, 45% formamide (v/v), 100 μg/ml of the carried DNA mixture containing the labelled probe. After hybridization, filters were washed in 2×SSC (6×15 min at room temperature) then in 0.01×SSC (2×15 min at room temperature) and dried. Autoradiography was carried out at −80°C for 1–4 days, using X-Omat AR5 Kodak film and intensifying screen.

PCR amplification

Total cellular DNA was amplified as follows. One-microgram aliquots of each DNA preparation were mixed with 25 pmol of each 5′ and 3′ oligonucleotide, 2.5 units of *Taq* polymerase (Amersham) and subjected to 30 cycles (92°C, 1 min; 55°C, 1 min; 72°C, 2 min) of amplification with 0.2 mM each of dATP, dCTP, dGTP and dTTP according to the manufacturer's conditions. The nucleotide sequences of the oligonucleotide primers were as follows:

oligonucleotide O1: $^{5'}$TGTGTGGGTGTAGATAGGAAGAAGT$^{3'}$
oligonucleotide O2: $^{5'}$AGCAACATTATCGTCGTTTATCGTA$^{3'}$
oligonucleotide O3: $^{5'}$TTTCCTGGTCTTCTCGGCTG$^{3'}$
oligonucleotide O4: $^{5'}$CTTGGCTTGTGGGTCCTGAT$^{3'}$

Results

Region of the mitochondrial genome known to be structurally unstable

Total cellular DNA was prepared from the two diploids *T. monococcum* (AA) and *T. tauschii* (DD), from the tetraploid *T. durum* cv "Creso" (AABB), from the hexaploid *T. aestivum* cv "Sicco" (AABBDD) and from corresponding short-term tissue and long-term cell-suspension cultures. DNA samples were restricted with *Sal*I and probed, after fractionation and blotting, with *Sal*I-cloned hexaploid wheat mtDNA fragments K′ and X_2. These fragments were already known to reveal changes in the molecular organization of mtDNA in tissue cultures initiated from both somatic tissues (Hartmann et al. 1987; Rode et al. 1987) and gametophytic cells (Aubry et al. 1989) of hexaploid wheat. In fact, these fragments belong to a structurally unstable region of the mitochondrial genome characterized by the presence of two unrelated sets of recombinogenic repeated sequences. This region also carries the *rrn*26 gene. In the present study, we were able to compare the organization of (1) the different *Triticum* species, (2) both types of *in vitro* culture, differing by their length of time in culture, derived from immature embryos of a given *Triticum* line, and (3) each of the *in vitro* types with the corresponding *Triticum* species. The results are shown in Fig. 1.

As expected, the banding patterns obtained when probing DNA of the hexaploid cv Sicco with probes K′ and X_2 were quantitatively and qualitatively identical to those previously obtained from mtDNA of other hexaploid varieties (Hartmann et al. 1987; Rode et al. 1987), strengthening the idea that the hexaploid cultivars are isoplasmic. The probe corresponding to fragment K′ (Fig. 1, panel A) hybridized with 4 *Sal*I fragments (J′, K_1, K′ and M) whereas probe X_2, a unique sequence, hybridized only with itself (Fig. 1, panel B). The role of the duration of *in vitro* culture was evident when the banding patterns obtained from short-term tissue culture and long-term cell-suspension cultures were compared. Both probes indicated that long-term culture resulted in a loss (or a marked decrease) of fragments J′, K′ and X_2, as has already been shown in callus derived from immature embryos of other hexaploid varieties (Rode et al. 1987). In short-term tissue culture, only a decrease in the relative amounts of these fragments could be seen. This gradual disappearance could be explained by an inability of the subgenomic organizations enclosing these fragments to replicate as *in vitro* culture is initiated. Note that the additional approximately 20-kb fragment detected in tissue culture after probing with fragment K′ is in fact a low-abundance hybridizing fragment already evident in tissue culture initiated from immature embryos of other hexaploid wheat cultivars (Hartmann et al. 1987 and unpublished data).

The banding patterns which were obtained after probing DNA prepared from the tetraploid *T. durum* with the same two probes shared several common features with those obtained with DNA from the hexaploid *T. aestivum*,

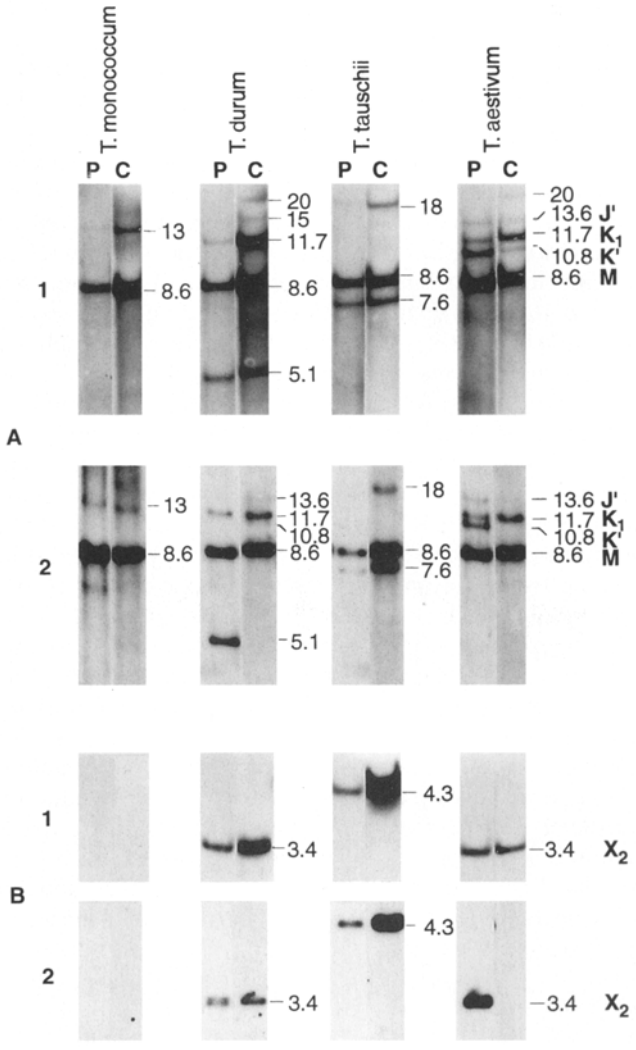

Fig. 1A, B DNA gel-blot analysis of *Sal*I-digested total cellular DNA prepared from diploid (*T. monococcum*, *T. tauschii*), tetraploid (*T. durum*, cv "Creso") and hexaploid (*T. aestivum*, cv "Sicco") *Triticum* lines (*P*) and corresponding short-term tissue culture (*C, 1*) and long-term suspension culture (*C, 2*). DNA was probed with cloned wheat mtDNA fragments K′ (panel **A**) and X_2 (panel **B**). The identity of the *T. aestivum Sal*I fragments hybridizing to the probes (panel **A:** J′, K1, K′, M; panel **B**: X_2) is indicated on the right. Fragment sizes (at the right of lanes "*C*") are in kb

namely the presence of fragment X_2 (Fig. 1, panel B), and of two out of the four fragments enclosing the repeated element no. 5 (fragments K_1 and M, Fig. 1, panel A). Indeed, in the latter case, fragments J′ and K′ were not detected. As, in hexaploid wheats, fragment X_2 is adjacent at its left border to the right border of both fragments J′ and K′, it is likely that the 5.1-kb fragment specifically present in the tetraploid line (Fig. 2) is located next to the left border of fragment X_2. Probing DNA from both tissue and cell-suspension cultures with fragment K′ (Fig. 1, panel A) strengthens this hypothesis. The 5.1-kb fragment, still present in short-term tissue culture, was apparently lost in long-term cell suspension culture. In the latter case, this

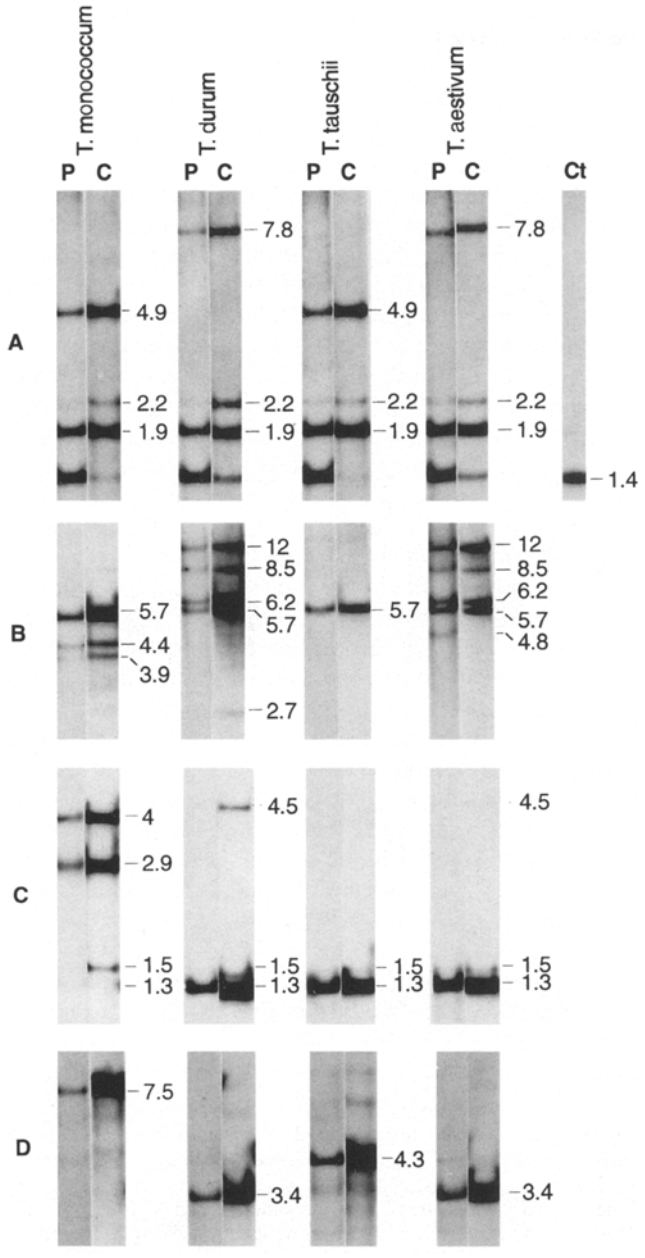

Fig. 2A–D DNA gel-blot analysis of total cellular DNA digested with either *Sal*I+*Xho*I (panels **A** and **B**) or *Sal*I + *Hin*dIII (panels **C** and **D**) and prepared from diploid (*T. monococcum, T. tauschii*), tetraploid (*T. durum*, cv "Creso") and hexaploid (*T. aestivum*, cv "Sicco") *Triticum* lines (*P*) and corresponding tissue culture (*C*). Digested DNA was probed with a series of cloned mtDNA fragments (panel **A**: a maize 1073-bp *Eco*RI-*Sst*I fragment, internal to the *atp*A gene; panel **B**: a wheat 1067-bp *Bgl*II – *Hin*dIII fragment comprising the first 900-bp of the 5′ end of the *atp*6 gene; panel **C**: a wheat 800-bp *Xho*I-*BamH*I fragment comprising the last 204-bp of the 3′ end of *atp*9 gene; panel **D**: a wheat 1279-bp *Hae*III fragment comprising the first 1238 bp of the 5′ end of the *cox*I gene). *Ct*: lane containing purified chloroplast DNA to permit identification of any fragment with homology to the mitochondrial probe used. Only the hybridization profile obtained from the probe internal to the *atp*A gene is shown, as the other three probes do not contain any DNA fragment with homology to chloroplast DNA. Fragment sizes (at the right of lanes "*C*" and lane "*Ct*") are in kb

loss was accompanied by the appearance of fragments J′ and K′ whereas the appearance of these two fragments could not be detected in short-term tissue culture. It is thus likely that *in vitro*-induced recombination events involving the 5.1-kb fragment give rise to novel molecular configurations enclosing fragments J′ and K′. Finally, two other hybridizing fragments could be seen only in short-term tissue culture (Fig. 1, panel A). The first one, about 20 kb in length, is probably the same as that already detected in tissue culture initiated from the hexaploid line. The second one is about 15 kb in length.

The situation was completely different as far as both diploid lines, *T. monococcum* and *T. tauschii*, were concerned. Whatever the probe used, the banding patterns of the whole-plant DNAs were very different both from each other and from those obtained from tetraploid and hexaploid lines (Fig. 1). Probing with X_2 (Fig. 1, panel B) showed that this sequence was absent, or considerably under-represented, in *T. monococcum* whereas it hybridized to a 4.3-kb fragment in *T. tauschii*. Probing with K′ (Fig. 1, panel A) revealed the presence of two major fragments of lengths 13 kb and 8.6 kb in *T. monococcum* and three fragments of lengths 18 kb, 8.6 kb and 7.6 kb in *T. tauschii*. Thus, both diploid lines share a common fragment (8.6 kb) which apparently is fragment M, present in both the tetraploid and hexaploid lines. As far as this particular region of the genome is concerned, this is the only common feature shared by the diploid and the hexaploid wheat lines. It should be noted that fragment M encloses the 5′ end of the *rrn*26 gene.

In vitro culture did not induce any significant reorganization of the mitochondrial genome in the diploid lines, whatever the type of culture. The apparent differences in the banding patterns of *T. tauschii* and the corresponding suspension culture are only quantitative differences due to an overloading of the DNA from the suspension culture (Fig. 1, panel A). Indeed, after a longer time of exposure, the banding patterns of the plant DNA samples appeared very similar to those of the suspension-culture DNA samples presented in Fig. 1 (data not shown). This was also true for the *T. monococcum* DNA samples (Fig. 1, panel A).

Regions of the mitochondrial genome enclosing known genes

The present study was extended to four other regions of the mitochondrial genome encoding three subunits of the ATP synthase complex (*atp*A, *atp*6 and *atp*9 genes) and one subunit of the ubiquinol-cytochrome c oxydoreductase complex (*cox*I gene). To perform this study, total DNA prepared from both the four *Triticum* species and the corresponding tissue cultures, as well as from purified *T. aestivum* chloroplast DNA, was restricted with either *Sal*I + *Xho*I (*atp*A and *atp*6 genes) or *Sal*I + *Hin*dIII (*atp*9 and *cox*I genes) and probed with DNA fragments internal to, or containing part of, these genes (Fig. 2). Only the probe internal to the *atp*A gene (Fig. 2, panel A) showed homology to the chloroplast DNA: a hybridization band co-migrating with a 1.4-kb

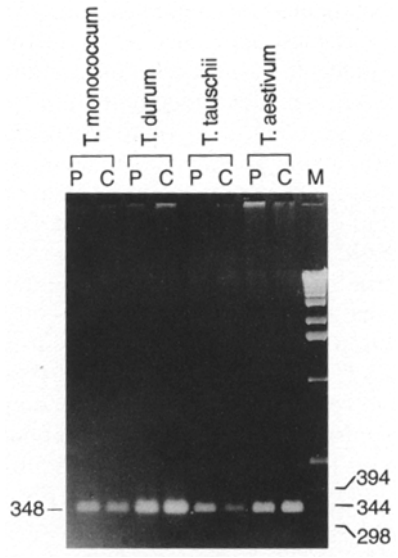

Fig. 3 PCR identification, in the diploid (*T. monococcum*, *T. tauschii*), tetraploid (*T. durum*, cv "Creso") and hexaploid (*T. aestivum*, cv "Sicco") *Triticum* lines (*P*) and corresponding tissue culture (*C*), of a mtDNA sequence containing a short (242-bp) recombinogenic repeated element (RS11) already detected in *T. aestivum*, cv "Chinese Spring" (Hartmann et al. 1994) Oligonucleotides O1 and O2 were used to amplify, by PCR, a 348-bp mtDNA fragment containing RS11. The 3′-end of O1 is 55 nucleotides from the left-side border of RS11, while the 3′-end of O2 is 3 nucleotides from the right-side border of RS11. After PCR amplification, DNA samples were fractionated on 1.2% agarose gels, stained with ethidium bromide and photographed under UV light. The size of the amplified fragment (in bp), determined from sequence analysis (Hartmann et al. 1994), is at the left. *M*: "1-kb ladder" (BRL) size standard. Fragment sizes (in bp) spanning the amplified fragment are indicated

chloroplast DNA fragment was observed in all the DNA samples and thus represents chloroplast DNA. In addition, its relative amount was higher in *Triticum* species than in corresponding tissue cultures, an observation in agreement with the fact that tissue cultures are known to contain less chloroplast DNA than plants. In all four series of experiments, tissue cultures initiated from both diploid species did not exhibit any significant rearrangement as compared with the explant source. In addition, both diploid species had the same organization in the region of the genome containing the *atp*A gene (Fig. 2, panel A), which is contrary to what was found in the regions containing the *atp*6 gene (Fig. 2, panel B), the *atp*9 gene (Fig. 2, panel C), and the *cox*I gene (Fig. 2, panel D). Conversely, the banding patterns obtained from both the tetraploid and the hexaploid lines were very similar whatever the region of the genome checked. Significant structural changes were seen in tissue culture-derived DNA. For instance, probing with a DNA fragment specific to the *atp*9 gene revealed an additional hybridizing fragment (4.5 kb long) in cultures derived from both the tetraploid and hexaploid lines (Fig. 2, panel C). Probing with the DNA fragment specific to the *atp*6 gene allowed the detection of a 4.8-kb fragment in the hexaploid line whereas it was lost, or undetectable, in the corresponding tissue culture (Fig. 2, panel B).

Recombinogenic potential of the repeated element no. 11

In a previous report (Hartmann et al. 1994), the study of two additional restriction fragments selectively amplified in the mtDNA of a single hexaploid wheat regenerant and its selfed progeny had allowed us to identify a short (242-bp) repeated element (named repeated sequence no. 11 or RS11) acting as a low-frequency recombination site in the parental plant. Here we have checked for the presence of this repeated element in the DNA prepared from the four *Triticum* lines and the corresponding tissue cultures. Two oligonucleotide primers (O1 and O2), homologous to sequences located upstream of and downstream from RS11, were used to amplify, by PCR, the resulting 348-bp fragment. The results showed (Fig. 3) that RS11 is present in all the four *Triticum* lines and the corresponding tissue cultures.

A high-frequency recombination event across RS11 should theoretically result in the detection of four fragments. In our previous work (Hartmann et al. 1994) only two fragments (19.5- and 32-kb long) were detected. The fact that the putative reciprocal recombination products have never been found is not surprising as similar observations have already been reported (Fauron et al. 1990; Newton et al. 1990) and suggest a selective elimination of the subgenomic structures enclosing these fragments. Alternately, these fragments could be present in amounts too low to be detected by conventional Southern hybridization.

To obtain further information about the recombinational potential of RS11 in both diploid and the tetraploid nuclear backgrounds, DNA prepared from the four *Triticum* lines and corresponding cultures was used to amplify, by PCR, DNA sequences specific to both of the 19.5-kb and 32-kb fragments. It was possible to define two sets of oligonucleotides (O1–O3 and O1–O4) able to amplify DNA sequences (1601- and 1614-bp) specific to the 19.5- and 32-kb fragments respectively (Fig. 4, panel A). As shown in Fig. 4, panel B, the amplified DNA sequences specific to both the 19.5- and the 32-kb fragments were detected in the tetra- and hexa-ploid lines and corresponding cultures, whereas they were not present in the diploid lines. Sequencing of the region of the 1601- and 1614-bp amplification products supposed to enclose RS11 gave evidence that these amplification products effectively contained RS11 (data not shown).

Discussion

RFLP studies using several mitochondrial probes have been performed with total cellular DNA prepared from two diploid (AA and DD), one tetraploid (AABB), and one hexaploid (AABBDD) *Triticum* species and from the corresponding *in vitro* cultures. The results principally suggest (1) that the mtDNA organization of *in vitro* cultures derived from both the tetraploid and the hexaploid lines undergoes marked changes whereas it appears to be stable in cultures derived from the diploid lines, (2) that the pat-

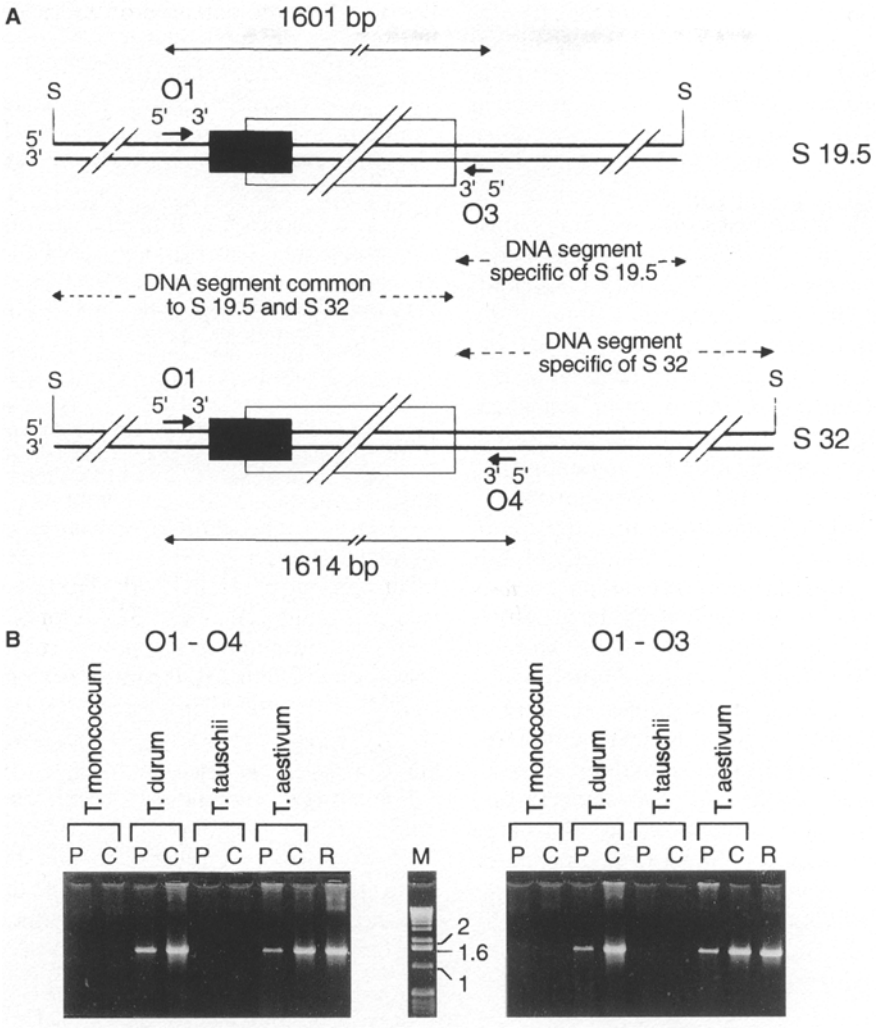

Fig. 4A, B PCR identification, in the mtDNA of the diploid (*T. monococcum*, *T. tauschii*), tetraploid (*T. durum*, cv "Creso") and hexaploid (*T. aestivum*, cv "Sicco") *Triticum* lines (*P*) and corresponding tissue culture (*C*), of the 19.5-kb (S19.5) and 32-kb (S32) *Sal*I fragments. Panel **A:** schemes showing the location, relative to both RS11 (*blackened box*) and another recombinogenic repeated element (RS7, *open broken box*), of the oligonucleotide primers (*O1*, *O3* and *O4*) used to amplify, by PCR, DNA fragments specific for S19.5 and S32. The DNA fragment upstream of RS11 is common to both S19.5 and S32 whereas, downstream from RS7, are unrelated fragments whose presence is due to a recombination event across RS7 (from Hartmann et al. 1994). Panel **B**: After PCR amplification, DNA samples were fractionated on 1.2% agarose gels, stained with ethidium bromide and photographed under UV light. *O1–O4*: identification of a 1614-bp fragment specific to S32. *O1–O3*: identification of a 1601-bp fragment specific to S19.5. *M* "1-kb ladder" (BRL) size standard. *R*: control lane loaded with the PCR amplification products obtained from DNA of the selfed progeny of the single regenerated plant in which additional 32- and 19.5-kb fragments were observed by conventional Southern-blotting (Hartmann et al. 1994). Fragment sizes are in kb

terns of mtDNA reorganization are roughly similar in the tetraploid and the hexaploid lines. Since the tetraploid and hexaploid species share the B genome, and mtDNA variability in *in vitro* cultures is known to be under nuclear control, these results suggest that a study of B-genome diploids and other polyploid combinations would now shed light on whether or not the B genome controls mtDNA instability in *in vitro* cultures. Moreover, the higher variability observed in the mtDNA of polyploid wheats in culture could be correlated with previous data (Winfield et al. 1993) showing a higher chromosomal instability in the same culture, as mtDNA reorganization in culture is influenced by nuclear genes (Hartmann et al. 1992).

Although the repeated element no. 11 has been shown, by PCR, to be present in the four *Triticum* lines, we were not able to identify its products in both diploid lines. Two hypotheses could explain these results: (1) in the diploid lines, the sequence arrangement of the regions downstream from RS11 are such that the DNA sequences specific to the recombination products cannot be amplified, (2) the nuclear information required for the expression of the recombinogenic potential of RS11 either is present, or else expressed, only in the *Triticum* lines containing the nuclear background of the B-genome donor.

Despite the apparent similarity observed in the mtDNA reorganization patterns of tetra- and hexa-ploid wheat cultures, a clear difference was apparent in the regions of the genome enclosing fragments J′ and K′ in the hexaploid line. Indeed, both fragments were undetectable in the tetraploid

species. This feature could be correlated with the specific presence, in the tetraploid species, of a 5.1-kb hybridizing fragment, undetectable in the hexaploid species. In the same way, the disappearance of this 5.1-kb fragment in long-term culture could be associated with the appearance of fragments J′ and K′. On the other hand, fragments J′ and K′ disappeared in long-term culture of the hexaploid line without any concomitant appearance of novel fragments. Thus, two different nuclear- and culture-dependent mechanisms could be at the origin of this differential behaviour. First, the disappearance in hexaploid wheat culture of the subgenomic molecules containing fragments J′ and K′ could be a consequence of their nuclear-dependent inability to replicate. Second, the appearance in tetraploid wheat culture of these subgenomic structures could correspond to the nuclear-dependent amplification (or generation) of a recombination event involving the 5.1-kb fragment.

Previous work on mtDNA variability in a hexaploid wheat culture indicated that two main patterns of variability could be obtained, depending on the variety used as explant donor (Hartmann et al. 1987; Rode et al. 1987) and, for a given variety, on the explant source (Morère-Le Paven et al. 1992). One of these patterns of mtDNA variability, obtained for instance from the *in vitro* culture of both immature embryos of cv "Aquila" and young root tips of cv "Chinese Spring", exactly corresponds to that obtained, in the present work, to the cell-suspension culture initiated from immature embryos of cv "Sicco". This type of reorganization was previously shown to be associated with the inability of *in vitro* cultures to regenerate plants (Rode et al. 1988). This hypothesis is further supported by the present work since the Sicco cultures in which this organization was observed were non-embryogenic.

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