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Analysis of hybrids of durum wheat with *Thinopyrum junceiforme* using RAPD markers

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Abstract The objective of this study was to detect the presence of alien chromatin in intergeneric hybrids of durum wheat (*Triticum turgidum*, 2n = 4x = 28; AABB genomes) with the perennial grass *Thinopyrum junceiforme* (2n = 4x = 28; $J_1J_1J_2J_2$) using RAPD markers. The first step was to identify amplification of species-specific DNA markers in the parental grass species and durum wheat cultivars. Initially, the genomic DNA of five grass species (*Thinopyrum junceiforme*, *Th. bessarabicum*, *Lophopyrum elongatum*, *Leymus karataviensis* and *Elytrigia pycnantha*) and selected durum cultivars ('Langdon', 'Durox', 'Lloyd', 'Monroe', and 'Medora') was screened with 40 oligonucleotide primers (nano-mers). Three oligonucleotides that amplified DNA fragments specific to a grass species or to a durum cultivar were identified. Primer PR21 amplified DNA fragments specific to each of the five durum cultivars, and primers PR22 and PR23 amplified fragments specific to each of the grass species. Intergeneric hybrids between the durum cultivars 'Langdon', 'Lloyd' and 'Durox' and *Th. junceiforme*, and their backcross (BC) progeny were screened with all 40 primers. Six primers amplified parent-specific DNA fragments in the F_1 hybrids and their BC_1 progeny. Three primers, PR22, PR23 and PR41, that amplified *Th. junceiforme* DNA fragments in both F_1 and BC_1 were further analyzed. The presence of an amplified 1.7-kb *Th. junceiforme* DNA fragment in the F_1 hybrids and BC_1 progeny was confirmed using Southern analysis by hybridization with both *Th. junceiforme* genomic DNA and *Th. junceiforme* DNA amplified with primer PR41. With the exception of line BC_1F_2 no. 5, five selfed progeny of BC_1 and a BC_2 of line 3 (BC_1F_2 no. 3 × 'Lloyd') from a cross of 'Lloyd' × *Th. junceiforme* showed the presence of the 1.7-kb DNA fragment. All selfed BC_1 and BC_2 lines retained the 600-bp fragment that was confirmed after hybridization with *Th. junceiforme* DNA amplified with primer PR22. Other experiments using RFLP markers also showed the presence of up to seven *Th. junceiforme* DNA fragments in the F_1 hybrids and their BC progeny after hybridization with *Th. junceiforme* DNA amplified with primer PR41. These studies show the usefulness of molecular markers in detecting alien chromatin/DNA fragments in intergeneric hybrids with durum wheat.

Key words Alien gene transfer · Intergeneric hybrids · Molecular markers · *Thinopyrum* · *Triticum turgidum*

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Introduction

Many wild species of the tribe Triticeae of the grass family (Poaceae) are valuable sources for resistance to diseases (e.g. dwarf bunt and barley yellow dwarf virus) and insect pests (e.g. Hessian fly and greenbug) that can be used to improve the present cultivars of durum wheat (*Triticum turgidum* L., 2n = 4x = 28; AABB). Several researchers have successfully transferred useful traits

from wild relatives into hexaploid wheat by intergeneric hybridizations (e.g. Jauhar 1993; Jiang et al. 1994). Besides facilitating the improvement of crop cultivars, wide hybridization provides an excellent opportunity for studying genome relationships (Jauhar and Joppa 1996).

The identification and characterization of alien chromatin or chromosome segments introgressed into the wheat complement is important from the plant breeding standpoint. Molecular cytogenetic techniques such as in situ hybridization, DNA polymorphism by random amplified polymorphic DNA (RAPD) or restriction fragment length polymorphism (RFLP) or a combination of these methods have been employed to detect alien chromatin in intergeneric hybrids with hexaploid wheat (Friebe et al. 1991; Schwarzacher et al. 1992; Bournival et al. 1994). However, the usefulness of molecular markers in the analysis of durum wheat hybrids and their backcross progeny has not been explored so far.

The objectives of the present investigation were to: (1) identify species-specific RAPD markers for five agronomically superior durum wheat cultivars and the five grass species amplified by nano-mer oligonucleotide primers; (2) use some of *Th. junceiforme*-specific RAPD and RFLP markers for visualizing their presence in durum wheat × *Th. junceiforme* hybrids and derivatives; and (3) monitor alien chromatin/DNA fragment(s) in the backcross progeny of durum wheat hybrids using polymerase chain reaction (PCR)-amplified DNA probes. The study reported here provides data on the durum cultivars, *Th. junceiforme*, their intergeneric hybrids and backcross progeny.

Materials and methods

Intergeneric hybridization

Five durum wheat cultivars ('Langdon', 'Durox', 'Lloyd', 'Monroe', and 'Medora' — kindly provided by Dr. E. Elias of the Department of Plant Sciences, North Dakota State University, Fargo, N.D.) and five perennial grass species (obtained from USDA-ARS, Logan, Utah), viz. *Thinopyrum junceiforme* (Löve and Löve) Löve (2n = 4x = 28; $J_1J_1J_2J_2$ genomes), *Th. bessarabicum* (Săvul and Rayss) Á. Löve (2n = 2x = 14; JJ), *Lophopyrum elongatum* (Host) Á. Löve (2n = 2x = 14; EE), *Leymus karataviensis* (Hochst.) (2n = 4x = 28) and *Elytrigia pycnantha* (Godr.) Löve (2*n* = 6*x* = 42), were used for identification of species-specific RAPD markers. Hybrids between three durum wheat cultivars ('Langdon', 'Lloyd' and 'Durox') and *Th. junceiforme* were used in the present RAPD and RFLP analyses.

Hybrids between 'Langdon', 'Lloyd' and 'Durox' and *Th. junceiforme* were synthesized (with wheat as the female parent) using the embryo rescue technique. The F_1 hybrids were backcrossed to the durum parent and a BC_1 generation was raised. The florets were manually emasculated and pollinated (except for BC_1 progeny of Lloyd × *Th. junceiforme*, which was self-fertile). One day after pollination, each floret was sprayed with a hormone solution containing GA_3 (75 mg/l) and 2,4-D (5 mg/l) by the method described earlier (Jauhar and Peterson 1996). The developing embryos were rescued by in vitro culture 10–14 days after pollination. After surface sterilization, the embryos were cultured on Murashige and Skoog (1962) medium containing 3% sucrose, NAA (1 mg/l) or kinetin (2 mg/l – for small embryos) and 0.8% agar. The recovered plants were transferred first to jiffy pots and then to larger (12 cm diameter) pots in the greenhouse.

DNA extraction and PCR procedure

Six to eight young leaves were used to extract the genomic DNA by the CTAB method (Doyle and Doyle 1990). The isolated DNA was amplified using single nano-mer oligonucleotides by the PCR method described by Demeke et al. (1992), but 2.0 m*M* $MgCl_2$, 1 ng genomic template DNA and two units of *Taq* DNA polymerase were used. The following thermal cycles were used in a Perkin Elmer Cetus DNA Thermal Cycler: 95°C (2.5 min) for initial strand separation, 35 cycles of 95°C (1 min), 36°C (1.5 min) and 75°C (2.25 min). A total of 40 nine-mer oligonucleotides were tested, and 6 primers that amplified high levels of polymorphic DNA fragments were selected to screen F_1 hybrids and backcross progeny. The amplified DNA was electrophoresed on agarose gels (1%), and the amplified products were visualized under UV light by staining with ethidium bromide. Although a few faint bands were indicated, only intensive and bright amplified DNA fragments were used in the analysis.

Amplified DNA samples of parents, intergeneric hybrids and backcross progeny with RAPD primer PR41 were used to identify *Th. junceiforme* DNA fragments in the hybrids. The samples (5 μg) were electrophoresed on agarose gels (1%) and blotted on Hybond nitrocellulose paper (Amersham). Southern hybridizations were carried out as reported earlier (Bommineni et al. 1993, 1996) using standard random radiolabeling of DNA probes with [^{32}P]. Two radiolabeled probes were used: (1) sheared *Th. junceiforme* genomic DNA (sheared by passing through a 22-G needle and vortexing) and (2) *Th. junceiforme* DNA amplified with primers PR22 and PR41.

RFLP analysis

Genomic DNA from the parents, intergeneric hybrids, and backcross progeny was digested with *Hin*dIII and *Eco*RI restriction enzymes (Boehringer Mannhem). The digested samples (15 μg) were electrophoresed overnight on agarose gels (1%), blotted on to nitrocellulose paper, and Southern hybridizations were carried out using the *Th. junceiforme* probe as described above. The RAPD and RFLP experiments were repeated five times to confirm the reproducibility of DNA polymorphisms.

Results and discussion

RAPD markers specific to grass species and to durum wheat cultivars

Amplifications of genomic DNA with 6 of the 40 nano-mer oligonucleotide primers (Table 1) showed polymorphisms among the durum wheat cultivars and among the grass species. Figure 1 shows DNA polymorphisms through the amplifications of genomic DNA in the grass and durum parents with primers PR23 and PR21; Fig. 1A reveals DNA polymorphism by primer PR23. Up to six amplified DNA fragments were observed with primer PR23 in the *Thinopyrum* and *Lophopyrum* samples (Fig. 1A). A DNA fragment of approximately 230 bases (black arrow in Fig. 1A)

Table 1 Primers (nano-mer oligonucleotides) which amplified polymorphisms in durum wheat, grass species and intergeneric hybrids

Primer no.	Sequence (5′ → 3′)
PR20	ACCCGGACA
PR21	CAAACGCCA
PR22	TGGAGCAAG
PR23	GGTGCCATC
PR39	GCATCAGGT
PR41	GATCCGCTC

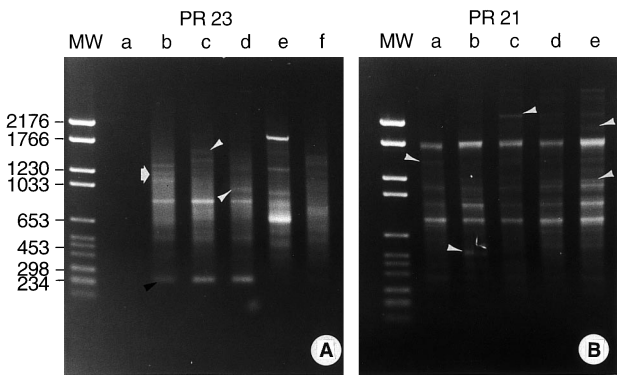

Fig. 1A, B Species-specific DNA polymorphisms in grass species and durum cultivars. **A** Grasses (primer PR23): *a* control, *b Thinopyrum junceiforme* (*wide arrow* indicates a group of specific amplified DNA fragments), *c Lophopyrum elongatum* (*arrow* indicates specific amplified DNA fragments), *d Th. bessarabicum* (*arrow* indicates specific amplified DNA fragments), *e Leymus karataviensis*, *f Elytrigia pycnantha*. *Black arrow* indicates amplified DNA fragments common to *Thinopyrum* and *Lophopyrum* species. **B** Durum wheat cultivars (primer PR21): *a* 'Langdon', *b* 'Durox', *c* 'Lloyd', *d* 'Monroe', *e* 'Medora'. *Arrows* indicate amplified DNA fragments specific to each durum cultivar. *MW* molecular-weight DNA markers

showed specificity to these species. A group of amplified DNA fragments ranging from 1.0 to 1.2 kb in size were specific to *Th. junceiforme* (wide arrow in lane b of Fig. 1A); a DNA fragment of approximately 1.5 kb showed specificity to *Lophopyrum elongatum*, and another of approximately 0.9 kb to *Th. bessarabicum*. *Leymus karataviensis* showed more than three specific amplified DNA fragments (Fig. 1A, lane e). Primer PR22 also amplified specific DNA fragments among these grass species (data not shown). No DNA fragments specific to *Elytrigia pycnantha* were observed with primer PR23.

DNA polymorphisms among the five durum cultivars by primer PR21 are shown in Fig. 1B. Up to four amplified DNA fragments were observed: One of approximately 1.4 kb was specific to 'Langdon' (Fig. 1B, lane a), another 450-bp fragment showed specificity to 'Durox' (Fig. 1B, lane b), whereas one of approximately 2.0 kb was specific to 'Medora' (Fig. 1B, lane e). Conversely, both 'Medora' and 'Monroe' showed the presence of a 1.1-kb fragment (Fig. 1B, lanes d and e) and the three cultivars 'Lloyd', 'Medora' and 'Monroe' amplified a 2.2-kb fragment (Fig. 1B, lanes c, d and e). Similar results were obtained with the durum cultivars 'Vic' and 'Rugby' (data not included).

The 6 nano-mer oligonucleotides (Table 1) amplified 20–40% polymorphic DNA fragments in durum wheat and other Triticeae species. No DNA amplifications were visualized from other oligonucleotides. Primers PR22 (data not shown) and PR23 showed differential amplification of DNA fragments specific to *Thinopyrum* and *Leymus*, each species showing the presence or absence of amplified DNA fragments (Fig. 1A); however, DNA fragments amplified by primer PR21 were specific only to some durum cultivars ('Langdon', 'Durox' and 'Medora'). These results indicate that more than 60% of the amplified DNA fragments are common among the durum cultivars or grass species. Therefore, these species-specific RAPD markers provide a cost-effective means to "fingerprint" durum cultivars or other grass species. RAPD analysis with deca-mers has been used to establish the geographical relationships among wild and cultivated tetraploid wheats (Joshi and Nguyen 1993).

In our experiments, the nine-mer oligonucleotides in a PCR reaction with related genotypes appeared to amplify several fragments that may represent different loci, and this may have useful applications in the improvement of durum wheat germplasm. The greatest advantage of the RAPD analysis is that it does not require radio-isotopes and once confirmed, the RAPD markers are relatively fast and simple to use in wide hybridization. In addition, the alien fragments from the RAPD analysis can be easily eluted from agarose gels for further applications. Therefore, these markers are not only useful to "fingerprint" durum cultivars or grass species, but also have direct applications in the assessment of genome relationships.

Detection of alien DNA fragments in durum wheat hybrids with *Th. junceiforme* by RAPD

Figure 2 shows DNA polymorphisms (with primers PR23 and PR41) among the three durum cultivars used for crossing with *Th. junceiforme*. Up to five DNA fragments with primer PR23 and up to seven with primer PR41 were amplified in the intergeneric hybrids. As expected, most of the DNA fragments common to both durum wheat and *Th. junceiforme* were visualized in the F_1 hybrids and BC_1 progeny. Genomic DNA amplified with primer PR41 also showed the presence of *Th. junceiforme* DNA fragments (of approximately 1.7 kb) in the durum hybrids and BC_1 plants. An amplified DNA fragment of 1.7 kb (shown in Fig. 2B) was closely associated (or linked) with one of the durum wheat fragments.

Southern hybridization using amplified samples with primer PR41 is given in Fig. 3. DNA samples hybridized with either *Th. junceiforme* genomic DNA or DNA

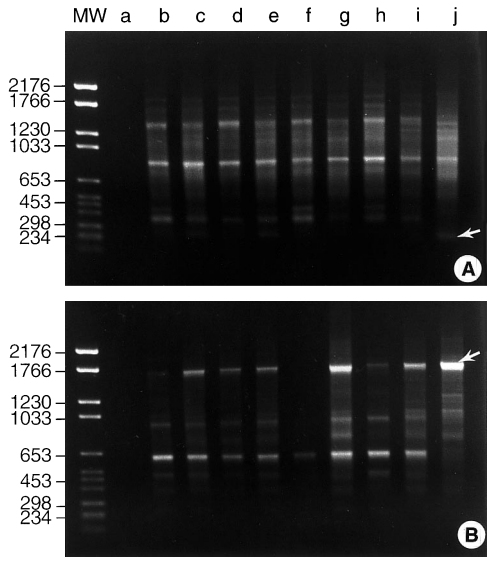

Fig. 2A, B Polymorphic DNA fragments among parents, hybrids and backcross progeny from a cross between durum wheat cultivars and *Th. junceiforme*: **A** primer PR23, **B** primer PR41. *a* Control, *b*, *c* and *d* 'Langdon', F_1 hybrid and BC_1 plant, *e*, *f* and *g* F_1 hybrid, 'Lloyd' and BC_1 plant, *h* and *i* 'Durox and F_1 hybrid, *j Th. junceiforme*. *MW* Molecular-weight DNA markers. *Arrow* indicates the amplified DNA fragments specific to *Th. junceiforme*

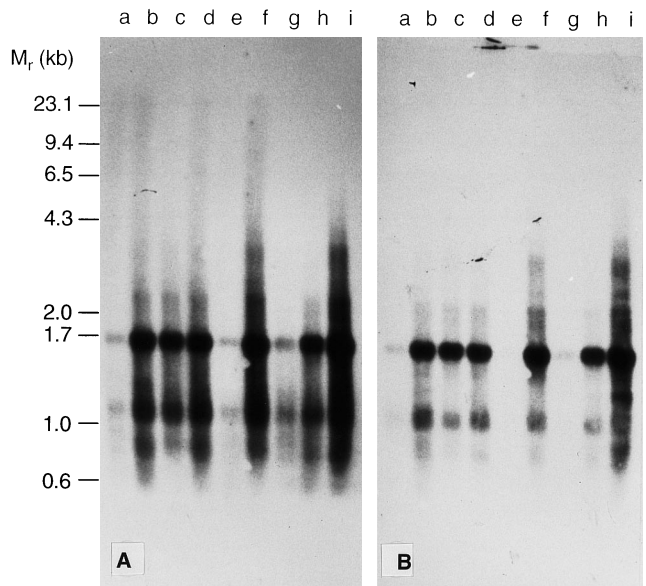

Fig. 3A, B Southern analysis of DNA amplified with RAPD primer PR41 among parents, hybrids and backcross progeny from a cross between durum wheat cultivars and *Th. junceiforme*: **A** hybridization with *Th. junceiforme* genomic DNA and **B** hybridization with *Th. junceiforme* DNA amplified with RAPD primer PR41. *a*, *b* and *c* 'Langdon', F_1 hybrid and BC_1, *d*, *e* and *f* F_1 hybrid, 'Lloyd' and BC_1, *g* and *h* 'Durox' and F_1 hybrid, *i Th. junceiforme*. M_r Molecular-weight DNA markers

amplified with primer PR41 also confirmed the presence of a 1.7-kb DNA fragment in the hybrids and BC_1 progeny (Fig. 3A, B). Subsequent analysis showed the transmission of a 1.7-kb alien DNA fragment in the progeny derived by selfing BC_1 plants of 'Lloyd' × *Th. junceiforme* hybrids (Fig. 4). With the exception of one plant (BC_1F_2 no. 5), all other five selfed BC_1 plants showed the presence of the 1.7-kb fragment. The amplified DNA fragments of plant no. 5 of BC_1F_2 were comparable to the amplified fragments of the 'Lloyd' parent (Fig. 4). Analysis of plant no. 3 of BC_1F_2 also showed transmission of the 1.7-kb fragment into BC_2 progeny (Fig. 4). Moreover, one of the *Th. junceiforme*-specific amplified DNA fragments (approximately 1.0 kb and 700 bases) with primer PR22 was transmitted to all progeny of BC_1 and BC_2 (Fig. 5). PR20 amplified a total of two faint fragments in durum cultivars and one (approximately 1 kb) in F_1 hybrid (data not included). On the other hand, primer PR39 amplified a total of three faint fragments in *Th. junceiforme* and one fragment (approximately 650 bp) in F_1 hybrid (data not shown). Further backcross generations of all the lines will be analyzed to study the inheritance and integration of the 1.7-kb, 1.0-kb and 700-bp fragments into the durum complement.

Detection of *Th. junceiforme*-specific DNA fragments in durum hybrids by RFLP analysis

To confirm our results with RAPD, we used RFLP analysis to examine genomic polymorphism in durum

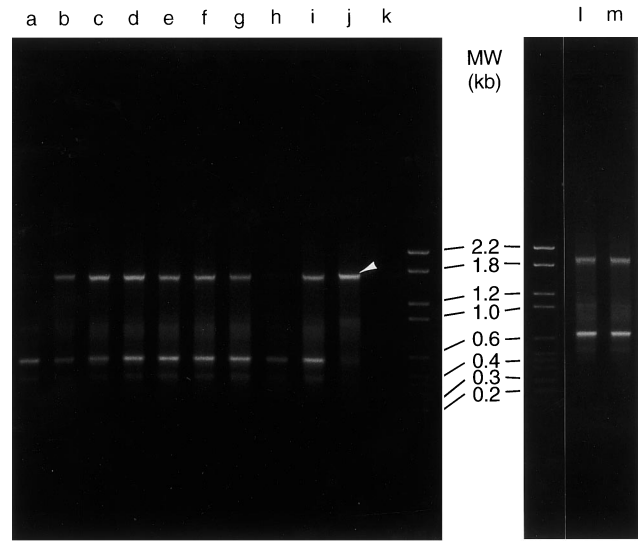

Fig. 4 Amplified DNA fragments with primer PR41 among parents, hybrid, BC_1, selfed BC_1 and BC_2 plants from a cross between durum cv 'Lloyd' and *Th. junceiforme*. *a* 'Lloyd', *b* F_1 hybrid *c* BC_1 plant, *d–i* BC_1F_2 plants (plant nos. 7, 6, 4, 3, 5 and 1, respectively), *j Th. junceiforme*, *k* control, *l* plant no. 1 of BC_2 – from a cross of (plant no. 3 of BC_1F_2) × 'Lloyd', *m* plant no 3 of BC_2 – from a cross of (plant no. 3 of BC_1F_2) × 'Lloyd'. *MW* Molecular-weight DNA markers. *Arrow* indicates the amplified DNA fragment specific to *Th. junceiforme*

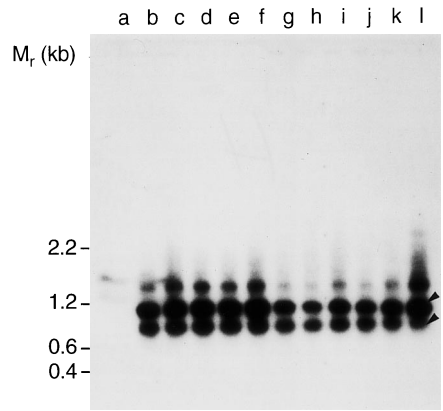

Fig. 5 Southern analysis of amplified DNA fragments with primer PR22 among 'Lloyd', hybrid, fertile BC_1, selfed BC_1 and BC_2 plants from a cross between durum cv 'Lloyd' and *Th. junceiforme*. *a* 'Lloyd', *b* F_1 hybrid, *c* BC_1, *d–g*, *j* and *k* selfed BC_1 (BC_1F_2 plants) *d–g* BC_1F_2 plant nos. 7, 6, 4, 3; *j* and *k* BC_1F_2 plant nos. 5 and 1, respectively), *h* and *i* BC_2 plants – plant no. 1 from a cross of (plant no. 3 of BC_1F_2) × 'Lloyd' and – plant no. 3 from a cross of (plant no. 3 of BC_1F_2) × 'Lloyd', *l* *Th. junceiforme*. *MW* Molecular-weight DNA markers. *Arrows* indicate the amplified DNA fragment specific to *Th. junceiforme*

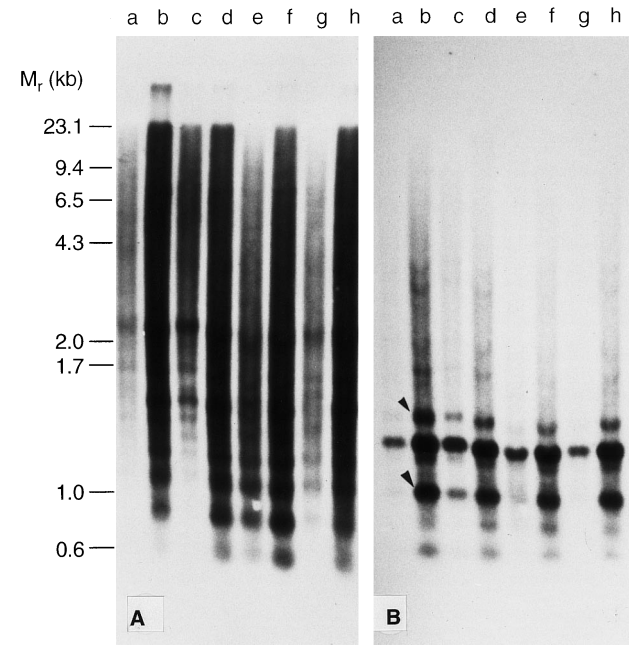

Fig. 6A, B RFLP among parents, hybrids and backcross progeny from a cross involving durum wheat cultivars and *Th. junceiforme*. Genomic DNA of all samples was digested with *Hin*dIII before Southern hybridization. **A** Hybridization with *Th. junceiforme* genomic DNA and **B** hybridization with *Th. junceiforme* DNA amplified with primer PR41. *a b* and *c* 'Langdon', F_1 hybrid and BC_1 plant, *d*, *e* and *f* F_1 hybrid, 'Lloyd' and BC_1 plant, *g* and *h* 'Durox' and F_1 hybrid. M_r Molecular weight DNA markers. *Arrows* indicate the DNA fragments specific to *Th. junceiforme*

wheat hybrids under two hybridization conditions: (1) *Th. junceiforme* genomic DNA and (2) *Th. junceiforme* amplified DNA with primer 41. Figure 6 shows, for example, polymorphic DNA fragments from genomic DNA restricted with *Hin*dIII and hybridized with *Th. junceiforme* genomic DNA (Fig. 6A) or DNA amplified by primer PR41 (Fig. 6B). Two alien DNA fragments were observed in the samples probed with the *Th. junceiforme* genomic DNA probe (Fig. 6A). Because of the non-specific nature of the total genomic probe of *Th. junceiforme*, it was not possible to identify many of the DNA fragments in the durum hybrids (Fig. 6A). Similar results on the non-specificity of the genomic probe were obtained by Anamthawat-Jónsson et al. (1990) in their studies with a hybrid of hexaploid wheat.

Seven alien DNA fragments were observed in durum hybrids and their backcross progeny using amplified *Th. junceiforme* DNA with primer PR41 as a probe (Fig. 6B). Most of the high-molecular-weight fragments were present at a lower level than the low-molecular-weight DNA fragments such as those approximately 1.1 kb in size. Genomic samples digested with *Eco*RI and hybridized with PR41-amplified *Th. junceiforme* DNA are shown in Fig. 7. Seven polymorphic DNA fragments specific to *Th. junceiforme* were detected in the RFLP analysis. High-molecular-weight DNA fragments approximately 9.0 and 3.5 kb in size were clearly detected (Fig. 7). However, no polymorphic DNA fragments were detected after hybridizing with *Th. junceiforme* genomic DNA (data not included).

Pairing among the chromosomes of the parental species could have brought about the intergeneric exchange of chromatin/DNA fragments. Our results show that both RAPD and RFLP markers are useful for the identification of *Thinopyrum* chromatin in durum wheat × *Th. junceiforme* hybrids using *Th. junceiforme*-amplified DNA as probes. Based on our cytological observations (in preparation), plants no. 4 and no. 5 of BC_1F_2, obtained by selfing BC_1 progeny, had 41 chromosomes, the BC_1 parent had 42 chromosomes, whereas BC_1F_2 no. 3 had only 38 chromosomes (Table 2). RAPD analysis showed the presence of 1.0-kb and 700-bp markers (Fig. 5) in the selfed progeny of BC_1 and BC_2 plants. It is surprising, however, that the 1.7-kb RAPD marker was completely lost in one plant (no. 5 of BC_1F_2) carrying 41 chromosomes, whereas it was present in all other plants, including plant no. 3 of BC_1F_2, with 38 chromosomes and its BC_2 progeny with only 32 chromosomes (Fig. 4). Therefore, either the *Th. junceiforme* chromosomes retained in the BC_2 plants or a chromosome segment translocated into the durum complement could have amplified the two RAPD markers that were hybridized with *Th. junceiforme*-amplified DNA with primers PR41 and PR22. Because most *Th. junceiforme* chromosomes were eliminated in the BC_2 progeny, the 1.7-kb, 1.0-kb and 700-bp RAPD markers were obviously located in either a single chromosome or on two different chromosomes.

We visualized seven RFLP fragments after hybridizing with *Th. junceiforme* DNA amplified with primer

Table 2 Chromosome number in intergeneric hybrids and fertile backcross progeny of a Lloyd × *Th. junceiforme* cross

Pedigree	Chromosome number (2n)	1.7-kb RAPD fragment[a]	1.1-kb RFLP fragment[a]
F_1	28	Yes	Yes
BC_1	42	Yes	Yes
Selfed BC_1 progeny			
BC_1F_2 (plant no. 1)	43	Yes	Yes
BC_1F_2 (plant no. 3)	38	Yes	Yes
BC_1F_2 (plant no. 4)	41	Yes	Yes
BC_1F_2 (plant no. 5)	41	No	No
BC_1F_2 (plant no. 6)	42	Yes	Yes
BC_1F_2 (plant no. 7)	41	Yes	Yes
BC_2 progeny derived from plant no. 3 of BC_1F_2			
BC_2 (plant no. 1)	32	Yes	Yes
BC_2 (plant no. 3)	33	Yes	Yes

[a] Hybridized fragment with *Th. junceiforme* DNA amplified with primer PR41

PR41 (Figs. 6B and 7), which will be useful to monitor their incorporation in reconstituted durum wheat. For example the 1.1-kb DNA fragment (Fig. 6B) was visualized in selfed progeny of BC_1 (except plant no. 5 of BC_1F_2) (Table 2). These alien fragments were more difficult to visualize when hybridized with total *Th. junceiforme* genomic DNA (Fig. 6A). Therefore, DNA hybridization using PCR (RAPD primers)-amplified alien samples may be more useful than genomic DNA probes for the clear visualization of alien DNA-specific polymorphisms by RFLP. Similarly, the amplified DNA fragments with 10-mer oligonucleotides have been shown to be useful in visualizing 5E chromatin of *Th. bessarabicum* in hexaploid 'Chinese Spring' monosomic and disomic substitution lines (King et al. 1993).

In the present study we have: (1) identified RAPD markers specific to some perennial grasses and durum wheat cultivars and (2) applied both RAPD and RFLP markers for the detection of *Th. junceiforme*-specific DNA fragments in its hybrids with durum wheat and subsequently confirmed the presence of alien fragments by Southern hybridization. RAPD analysis by nanomer oligonucleotide primers will facilitate the detection of alien DNA fragments in the derived hybrid durum and BC progeny and is, therefore, important for their isolation and cloning.

In conclusion, our results, show that RAPD and RFLP markers can be useful molecular tools for the analysis of alien chromatin in advanced generations of intergeneric hybrids of durum wheat. Depending on the fertility of the subsequent backcross progeny, future experiments will be conducted on the isolation and nucleotide sequencing of the polymorphic DNA fragments introgressed into durum wheat. These molecular markers also may be used with in situ hybridization techniques for physical identification of alien chromatin (Schwarzacher et al. 1992) and thus help elucidate genome relationships among the Triticeae species through wide hybridization.

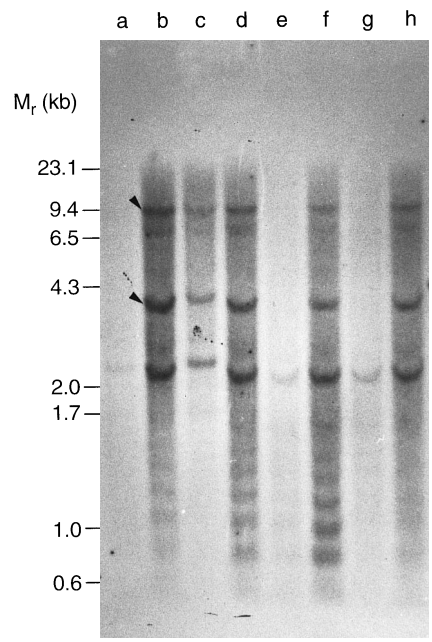

Fig. 7 RFLP among parents, hybrids and backcross progeny from a cross involving durum wheat cultivars and *Th. junceiforme*. Genomic DNA of all samples was digested with *Eco*RI before Southern hybridization and probed with *Th. junceiforme* DNA amplified with primer PR41. *a*, *b* and *c* 'Langdon', F_1 hybrid and BC_1, *d*, *e* and *f* F_1 hybrid, 'Lloyd' and BC_1, *g* and *h* 'Durox' and F_1 hybrid, M_r Molecular-weight DNA markers. *Arrows* indicate the DNA fragments specific to *Th. junceiforme*

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