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Introgression of wheat DNA markers from A, B and D genomes in early generation progeny of *Aegilops cylindrica* Host × *Triticum aestivum* L. hybrids

N. Schoenenberger · F. Felber · D. Savova-Bianchi · R. Guadagnuolo

Abstract Introgression from allohexaploid wheat (*Triticum aestivum* L., AABBDD) to allotetraploid jointed goatgrass (*Aegilops cylindrica* Host, CCDD) can take place in areas where the two species grow in sympatry and hybridize. Wheat and *Ae. cylindrica* share the D genome, issued from the common diploid ancestor *Aegilops tauschii* Coss. It has been proposed that the A and B genome of bread wheat are secure places to insert transgenes to avoid their introgression into *Ae. cylindrica* because during meiosis in pentaploid hybrids, A and B genome chromosomes form univalents and tend to be eliminated whereas recombination takes place only in D genome chromosomes. Wheat random amplified polymorphic DNA (RAPD) fragments, detected in intergeneric hybrids and introgressed to the first backcross generation with *Ae. cylindrica* as the recurrent parent and having a euploid *Ae. cylindrica* chromosome number or one supernumerary chromosome, were assigned to wheat chromosomes using Chinese Spring nulli-tetrasomic wheat lines. Introgressed fragments were not limited to the D genome of wheat, but specific fragments of A and B genomes were also present in the BC1. Their presence indicates that DNA from any of the wheat genomes can introgress into *Ae. cylindrica*. Successfully located RAPD fragments were then converted into highly specific and easy-to-use sequence characterised amplified regions (SCARs) through sequencing and primer design. Subsequently these markers were used to characterise introgression of wheat DNA into a BC1S1 family. Implications for risk assessment of genetically modified wheat are discussed.

N. Schoenenberger (✉) · F. Felber · D. Savova-Bianchi
R. Guadagnuolo
Laboratoire de Botanique Evolutive, Institut de Botanique,
Université de Neuchâtel, rue Emile Argand 11,
2007 Neuchâtel, Switzerland
E-mail: nicola.schoenenberger@unine.ch
Tel.: +41-32-7182346
Fax: +41-32-7183001

Introduction

Gene flow by sexual reproduction from crops to their wild relatives represents one of the main concerns about commercial cultivation of genetically modified plants. It occurs through the production of first generation hybrids, followed by several backcrosses. The consequences of gene flow from conventional or transgenic crops may be severe as increased weediness and loss of biodiversity of wild taxa (Ellstrand 2003).

The wild species jointed goatgrass (*Aegilops cylindrica* Host, Poaceae) is native to a wide area from central Asia to the Mediterranean (van Slageren 1994). It has $2n = 4x = 28$ chromosomes, an allotetraploid origin, and a genomic formula notation of CCDD. *Ae. cylindrica* hybridises naturally with allohexaploid wheat (*Triticum aestivum* L.) (van Slageren 1994), which has $2n = 6x = 42$ chromosomes, and a AABBDD genome. The reason why intergeneric crosses between the two species are possible has been attributed to the fact that they share the D genome ancestor (Zemetra et al. 1998), although at least 12 *Aegilops* species acting as female parents form natural hybrids with hexaploid wheat and not all of them have a common genome with it (van Slageren 1994). *Ae. cylindrica* × *T. aestivum* hybrids are male sterile but produce viable BC1 seeds at low frequency through backcrossing to either of the parental species (Rajhathy 1960; Guadagnuolo et al. 2001; Morrison et al. 2002b).

Aegilops cylindrica was introduced as an adventive to the USA in the late nineteenth century and became a serious agricultural weed in North America, particularly in winter wheat fields (Donald and Ogg 1991). In Oregon (USA) wheat fields, *Ae. cylindrica* was found to be the predominant female parent of F1 hybrids with wheat (Morrison et al. 2002b). In fact, in

Oregon, USA (Morrison et al. 2002a, 2002b). There are probably some exceptions to this as it was observed that in different wheat lines some marker loci or chromosomes, like chromosome 2B, display segregation distortion in favour of one parental line, lowering the rate of recombination between loci (Paillard et al. 2003). It is hypothesised that segregation distortion in this chromosome involves a genetic factor. If a transgene is flanked by a particularly inefficient distorter allele, one would expect a low segregation ratio of the further. One way to identify A or B genome chromosomes or chromosome regions that have no or only reduced ability of introgression would be to construct an interspecific linkage map between wheat and *Ae. cylindrica*.

Because introgression of wheat A and B genome fragments into *Ae. cylindrica* can occur, we conclude that it is not sufficient to place a transgene on those genomes to efficiently avoid its introgression. The risk that a transgene located on the A or B genome of wheat is maintained in an *Ae. cylindrica* background depends finally on the frequency of chromosome retentions and translocations for each genomic and chromosomal region of wheat. The markers described in this study provide tools for further investigations that are needed to obtain a clear insight into the mechanisms influencing these two factors.

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RAPD analysis and chromosome assignment

Genomic DNA was extracted from fresh or frozen (−80°C) leaf tissue using the DNeasy Plant Mini Kit (Qiagen, Basel, Switzerland), following the manufacturer's protocol. Final DNA concentration was adjusted to 20–40 ng/µl, and stored at −20°C. RAPD analysis was carried out as described in Guadagnuolo et al. (2001), using primers OPB6, 8, 10, and OPP6, 8, 9, 14 (Operon Technologies, Alameda, CA, USA). Sixteen wheat-specific RAPD fragments were detected in the F1 hybrids; nine of them were present in the BC1 generation (Guadagnuolo et al. 2001). The 16 RAPD fragments were amplified in CS NT lines for chromosome assignment. A BC2 family and their BC2S1 offspring were analysed by RAPD analysis in order to test introgression to further generations.

Direct sequencing of purified RAPD products

Seven of the RAPD fragments successfully localised on wheat chromosomes were chosen for sequencing (Table 1: DP9, GP8, GB10, IB10, FP8, YP8, D1P9). The choice was based on intensity and distinctiveness of the RAPD band, on its specificity to wheat, and on its persistence in *Ae. cylindrica* × *T. aestivum* backcross derivatives. The fragments were purified from the agarose electrophoresis gel using the QIAquick Gel Extraction Kit (Qiagen), following the manufacturer's protocol. Purified RAPD fragments were directly sequenced as described by Hernandez et al. (1999). However, instead of using PCR products flanked by two different oligonucleotides as in that report, we used a purified fragment generated by only one 10-mer RAPD primer and that primer for a first cycle sequencing reaction. Usually one strand of the sequence was preferentially amplified, allowing the design and synthesis of a strand specific ≥20mer oligonucleotide (Microsynth, Balgach, Switzerland). This primer was then used in another round of cycle sequencing with the purified RAPD fragment to obtain a clean sequence of it. Reverse primers were designed to sequence the complementary strand.

Cycle sequencing was performed using the dideoxy chain dye-termination method with an ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) in a Biometra T3 thermocycler. Cycling parameters were 25 cycles of 20 s at 96°C, 20 s at 50–60°C annealing temperature depending on the primer used, and 4 min at 60°C. Cycle sequencing was performed in 5 or 10 µl reaction volume, depending on the success of cycling reactions. The products were then purified using the ethanol/EDTA/Sodium acetate precipitation method suggested by the sequencing kit's manufacturer. The purified sequencing products were resuspended in 12 µl TSR (Applied Biosystems), and run in an ABI 310 Automated Sequencer (Applied Biosystems). Basecalling was checked manually against the electropherograms, and edited if necessary, using the Sequence Navigator software (Applied Biosystems). Checked and eventually edited sequences were aligned using the Sequence Alignement Editor software (Andrew Rambaut, University of Oxford), in order to obtain the entire sequence of the RAPD fragments.

In one case (fragment DP9), both strands were equally amplified resulting in superposed electropherograms, even when the annealing temperature was increased up to 60°C. The corresponding RAPD primer elongated by each A,T,G and C nucleoside was synthesised (Microsynth) and PCR conditions optimised using each possible primer pair combination in a Biometra Tgradient thermocycler with a gradient of annealing temperatures of 45–59°C. PCR products were checked on ethidium bromide stained 1.4% (w/v) agarose gels. The optimised PCR conditions were used to perform cycle sequencing. Sequences have been deposited in GenBank; accession numbers are indicated in Table 1.

SCAR primer design and PCR

Sequences of the investigated RAPD fragments were used to design specific SCAR primer pairs 20–21 bases long. The primers were located internally or were superposed to the RAPD primer binding site resulting in

Table 1 Chromosome assignment of specific wheat fragments detected in hybrids and backcrosses with *Ae. cylindrica* and marker conversion

RAPD Fragment	Chromosome location RAPD	Presence in hybrids	Presence in BC_1	Sequencing (sequence size)	GenBank accession no.	Wheat-specific SCAR	Chromosome location SCAR
DP9	6A	Yes	Yes	Yes (644 bp)	AY842011	Yes	6A
DB6	1B	Yes	Yes	No	–	–	–
GP8	2B	Yes	Yes	Yes (950 bp)	AY842009	Yes	No/7D
HB8	5B	Yes	No	No	–	–	–
GB10	5B	Yes	Yes	Yes (673 bp)	AY842010	Yes	5B
IB10	7B	Yes	Yes	Yes (903 bp)	AY842008	Yes	No
FP8	7B	Yes	No	No	–	–	–
YP8	6D	Yes	Yes	Yes (402 bp)	–	No	–
D1P9	6D	Yes	Yes	Yes (764 bp)	AY842012	Yes	6D

PCR products usually slightly shorter than the RAPD fragment. Specific PCR was performed in 25 µl reactions containing 1 × PCR mix, 0.2 mM dNTP, 0.2 µM of each primer, 0.03U/µl Taq polymerase (Qiagen) and 20–40 ng template DNA. Amplifications were carried out in a Biometra T3 thermocycler, with the cycling profile: initial denaturation at 94°C for 10 min, then 35 cycles of 93°C for 60 s; 55–59°C for 60 s, depending on the primer pair (Table 2); 72°C for 60 s. Final extension was at 72°C for 10 min. PCR products were mixed with 1/5 vol loading buffer and loaded onto a 1.5% (w/v) agarose gel, stained with ethidium bromide. Electrophoresis was carried out at 100 V. Recovered SCAR markers were tested for specificity to wheat, for maintenance of introgressive pattern and for chromosome location. Afterwards, we analysed an introgressive series up to BC1S1 using the SCAR markers.

Results

Chromosome assignment

Among the 16 specific wheat RAPD fragments inherited by *Ae. cylindrica* × *T. aestivum* hybrids, nine were assigned to wheat chromosomes, and the remaining seven were amplified in all NT lines. It was therefore impossible to determine their chromosome location. Among the nine wheat fragments detected in BC1 plants (Guadagnuolo et al. 2001), seven were assigned to wheat chromosomes of the A, B and D genomes (Table 1). Four of these RAPD fragments originate from the B genome of wheat, two from the D genome and one from the A genome (Fig. 1). None of these fragments were amplified in the ancestor species and other wild relatives possessing a part of the wheat genome, indicating that each of the RAPD fragment arose in allopolyploid wheat, either because of mutations at one or both of the RAPD primer binding sites, because of sequence elimination in the polyploid, or by chromosome rearrangements.

Development of SCARs

Direct sequencing of the RAPD fragment using the original single 10mer primer was successful in 5 of the 7 fragments chosen. These first sequences usually had a quite high background noise represented by the sequence of the complementary strand, because in the RAPD technique, both primer binding sites consist of the same sequence. However, cycle sequencing reactions using these fragments produced directly a readable sequence allowing the design of more specific 20mer primers. These strand-specific primers were then used to sequence the whole fragment. One fragment (DP9), always produced a double sequence. It could only be sequenced using the corresponding RAPD primer elongated by one nucleoside (Cytosine), which made the primer strand-specific, thus allowing it to produce sequence in only one direction. Fragment FP8 always produced a double sequence too. The procedure of RAPD primer elongation by each nucleotide was not carried out because this fragment was not present in BC1 plants. This fragment was thus discarded from further investigations (Table 1). Based on the obtained sequences, six 20–21mer primer pairs (Table 2) were synthesised and tested on the investigated plant material in order to verify if the SCARs maintained the same pattern as the RAPD fragments. Unfortunately, by the time the SCAR primers were developed, the original DNA extracts of the BC1 from Guadagnuolo et al. (2001) were degraded and did not amplify any more. Five of the newly developed primer pairs maintained specificity to wheat. One fragment (YP8), also amplified in *Ae. cylindrica*. The sequences of the fragments amplified by the SCAR primer set in wheat and in *Ae. cylindrica* were almost identical, with some polymorphic nucleotides (data not shown). Moreover, the sequence was polymorphic even within a single wheat DNA extract indicating that it existed in more than one copy in the wheat genome. The sequence of fragment YP8 was not submitted to GeneBank because of its polymorphic nature in wheat. Marker YP8 was discarded from further analysis because of the loss of specificity to wheat. The remaining five newly developed

Table 2 SCAR primers, size of the amplified fragment, and annealing temperatures used for specific PCR

Name	Sequence (5′ → 3′)	Position in RAPD fragment (5′ → 3′)	Size SCAR (bp)	Annealing temperature (°C)
DP9F	ACGGCAATTCTTTATGGAAGT	21–41	610	55
DP9R	TAACATTCGATGATGACCGG	611–630		
GP8F	AGATTCAGTTGCACCATCAC	16–35	931	55
GP8R	CGCCCAAGGATAGCAGTCCTT	926–946		
GB10F	TGGGACCAGGATTGTGAGTAC	5–25	662	59
GB10R	GACGTGCACAAGTGGGAGGAA	646–666		
IB10F	CTGCTGGGACCCGATGAATTG	1–21	902	58
IB10R	TGCTGGGACGAAGCGTTTGAC	882–902		
YP8F	ACATCGCCCACTCTCAGAGG	1–20	402	55
YP8R	ACATCGCCCATCTTGATAACG	382–402		
D1P9F	TGGCAACAGGGTAATGATCCC	22–42	737	58
D1P9R	CGCAAAATTTGGTTTAGGGCT	738–758		

SCARs were then tested on CS NT lines to verify their chromosome location. Chromosome location was successfully identified and identical to the corresponding RAPD fragments for three of these markers, one for each wheat A, B and D genome. For two fragments, chromosome assignment was impossible after marker conversion. Fragment IB10 was amplified in all 21 NT lines, suggesting that the newly designed primers amplified at least two different regions on different chromosomes of the wheat genome. The GP8 fragment contains a 107 bp tandem repeat, the second repeat having two mutated nucleotides and one deleted compared to the first. The primer pair designed external to the repeated zone of this sequence amplified two fragments, one of the expected 950 bp length (same as the corresponding RAPD fragment) and an additional fragment of about 800 bp. The additional fragment was located on the wheat chromosome 7D, whereas the 950 bp fragment could not be localised to chromosome. We tested other primer pairs located external to the repeat but internal to the GP8 sequence. They amplified one single band in all NT lines; thus, the chromosome location of GP8 remains unknown.

Database comparisons of the obtained sequences in GenBank's nr databases, using the Basic Local Alignment Search Tool (BLAST), revealed a high degree of sequence homology for DP9, IB10 and YP8 fragments to genes from other organisms such as various diploid *Triticum* and *Aegilops* species, *Triticum turgidum*, *T. aestivum*, and other members of the Triticeae tribe like *Hordeum* and *Agropyron*. Most of the identified genes were related to metabolism, storage, structure and even putative disease resistance. Moreover, some of the identified sequences were retrotransposons.

DNA marker Introgression

Although several RAPD fragments assigned to wheat chromosomes were detected in the field-produced BC1 plants, none of these fragments were present in the analysed BC2 family and in their BC2S1 offspring (Fig. 1). This is not surprising considering the fact that they were all descendants of one single BC1 individual, which unfortunately was no longer available for analysis.

An F1 individual issued from a manual cross, its BC1 with *Ae. cylindrica*, and the selfed BC1S1 offspring were analysed using three SCARs, one for each wheat genome. Markers D1P9 (6D) and DP9 (6A) were introgressed to the BC1S1 generation whereas GB10 (5B) was only present in the hybrid and was not present in the BC1 plant (Table 3). The BC1S1 plants had 28 to 31 chromosomes, i.e. the euploid *Ae. cylindrica* chromosome number and up to 3 additional chromosomes. The BC1S1 individual 5.13.11 had 28 chromosomes and a marker from the wheat chromosome 6A (Fig. 2, lane 12).

Discussion

Marker conversion

A direct sequencing method of RAPD fragments avoids the costly and time-consuming cloning step generally used for SCAR development from RAPD markers (Paran and Michelmore 1993). Cycle sequencing of a RAPD fragment generated using one single primer would be expected always to produce a double sequence since both primer sites are identical; thus, it has been thought that only fragments flanked by two different RAPD primers can be sequenced directly (Hernandez et al. 1999). Here we report a method of direct sequencing with only one RAPD primer which produced directly a readable sequence in five out of seven fragments of interest. For one of the other fragments the RAPD primer was elongated by one nucleotide and a readable sequence was obtained, while for the other fragment, no attempt at primer elongation was under-

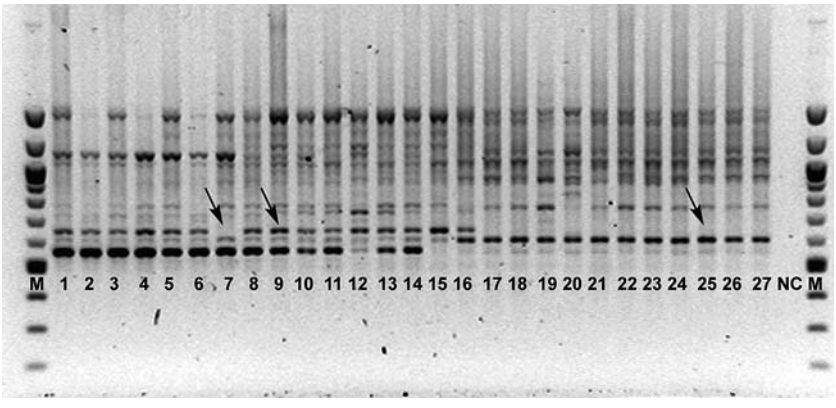

Fig. 1 RAPD amplification with primer OPP9 in euploid *T. aestivum* var. Chinese Spring (*lane 1*) and its nulli-tetrasomic lines for the seven A genome chromosomes (*lanes 2–8*), *T. aestivum* var. Arina (*lane 9*), *Ae. cylindrica* (*lane 25*), *Ae. cylindrica* × *T. aestivum* hybrids (*lanes 10–14*), BC_1 (*lanes 15–21*), BC_2 (*lanes 22–24*), and BC_2S_1 plants (*lanes 26–27*). *Arrows* indicate fragment DP9 localised on chromosome 6A, present in wheat and absent in *Ae. cylindrica*. *M* 100 bp DNA Ladder, *NC* negative control

Table 3 Chromosome numbers and presence (+) of specific wheat SCAR markers (chromosome location annotated) in the first three generations of an introgressive series from wheat var. Bobwhite to *Ae. cylindrica*

Plant	Chromosome number	D1P9 (6D)	DP9 (6A)	GB10 (5B)
F1				
5.13	35	+	+	+
BC1				
5.13	30	+	+	–
BC1S1				
5.13.1	28	–	–	NA
5.13.2	29 + t	+	+	
5.13.3	29	+	–	
5.13.4	29	–	–	
5.13.5	28	+	–	
5.13.6	NA	–	–	
5.13.7	30	+	–	
5.13.8	29	+	+	
5.13.9	31	+	+	
5.13.10	28	–	–	
5.13.11	28	+	+	
5.13.12	28	+	–	
5.13.13	30	+	–	
5.13.14	29	+	+	
5.13.15	28	+	–	
5.13.16	28	+	–	
5.13.17	28	–	–	
5.13.18	30	+	–	

NA not analysed, *t* telocentric

taken. In a case where the flanking nucleotide at the 3' end of the two primer binding sites is the same, one could elongate the primer by two nucleotides; however, the possibility of different primer design grows exponentially with every further nucleotide added to the RAPD primer, and so does the cost of the operation. To explain the high proportion of single-primer RAPD fragments generated in this study that were directly sequenceable, one must assume that the binding of the RAPD primer at each end of the successfully sequenced fragment, or the activity of the polymerase, must have been somewhat different. If template DNA preparation and cycle sequencing reactions are optimised to permit direct sequencing using a single primer RAPD product, the procedure described here is even more time and cost effective as it avoids the step of screening for markers amplified only by two different primers described by Hernandez et al. (1999).

The identification of primers and reaction conditions sufficiently specific to produce an amplified product from a marker that is a single fragment, presence/absence polymorphism can enable the amplification product to be directly stained in the PCR tubes, thus avoiding the gel electrophoresis step (Hernandez et al. 2001). This was the case of three out of four successfully located SCARs developed here. The problem of reamplification of untargeted bands (Hernandez et al. 1999) was overcome here as only RAPD fragments already localised on CS NT lines were used for sequencing. For the purpose of detecting introgressed wheat fragments localised on single chromosomes, the outcome of the overall described procedure was rather low; out of nine successfully located RAPD fragments only four useful markers were developed. This is probably due to the complexity of the allohexaploid wheat genome with several triplicated loci on homeologous chromosomes. Paradoxically, increasing PCR specificity by the use of longer oligonucleotide primers decreased genome specificity in three out of six cases. In one case (fragment YP8), PCR with 20mer primers even lost specificity to wheat. Once the primer was elongated homology of the primer at its 3′ ends was increased, causing loss of polymorphism. Similarly, a RAPD fragment specific to *Hordeum chilense*, after it was converted to a SCAR, lost its specificity to *H. chilense* and showed amplification in wheat too; mismatch of the RAPD primer rather than sequence polymorphism was considered to be the cause of the RAPD polymorphism (Hernandez et al. 1999).

Five of the six successfully sequenced fragments can be used to demonstrate hybridisation between wheat and *Ae. cylindrica*, even though chromosome location of one of these markers (IB10) remains unknown (Schoenenberger et al. 2005).

An advantage of using RAPD markers for introgression studies is that these markers occur in randomly amplified genome regions, and if sequenced and compared to nucleotide databases they may provide some information on eventual genes or traits introgressed from one species to another.

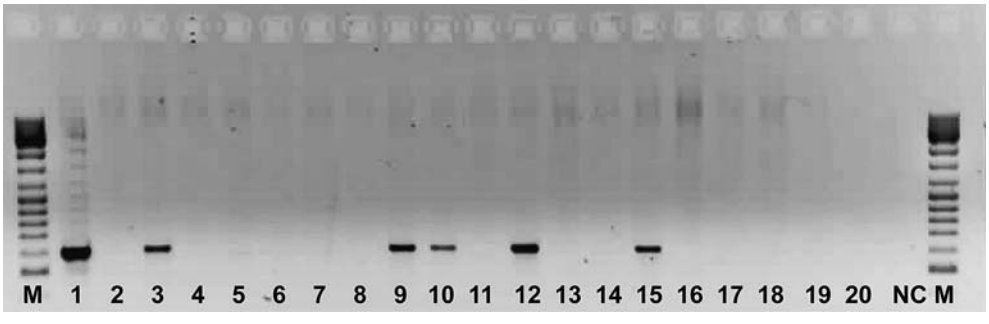

Fig. 2 Amplification of the SCAR DP9 in the wheat variety Bobwhite (*lane 1*), *Ae. cylindrica* × *T. aestivum* BC1S1 (*lane 2–19*), *Ae. cylindrica* (*lane 20*). *NC* negative control (*lane 21*), *M* 100 bp DNA Ladder

Marker introgression and implications for transgene flow

Only 9 out of 16 wheat-specific RAPD fragments investigated could be assigned to wheat chromosomes, while the remaining 7 markers were amplified in all 21 CS NT lines. This result is consistent with observations of Devos and Gale (1992) where only a fraction of RAPD fragments could be localised by aneuploid analysis. The unlocalised markers were probably present on more than one wheat chromosome, possibly members of a homeologous group, or the markers consisted of repetitive DNA. Wheat-specific markers detected in partially fertile BC1 plants having 28 or 29 chromosomes (Guadagnuolo et al. 2001) were assigned to the A, B and D genomes of wheat. Only two out of seven wheat-specific RAPD markers were located on the D genome (YP8 and D1P9), whereas four originate from the B genome. In fact, the D genome of wheat is known to be less polymorphic than the A and B genome (Nelson et al. 1995; Cadalen et al. 1997).

There are three mechanisms by which wheat genetic material can be introgressed into *Ae. cylindrica*: (1) recombination of the homologous D genome chromosomes, (2) intergenomic translocations or chromosome rearrangements, and (3) disomic chromosome retention. Recombination of homologous chromosomes is the most frequent mechanism and even though the D genome of wheat and *Ae. cylindrica* originated from different biotypes of *Ae. tauschii* (Badaeva et al. 2002) they pair successfully at meiosis forming 14 bivalents (Zemetra et al. 1998). In fact, inheritance of most microsatellites specific to the D genome of wheat fit the expected Mendelian ratio and were neither preferentially inherited nor lost in *T. aestivum* × *Ae. cylindrica* hybrids, BC1 and BC2 families (Kroiss et al. 2004). Using GISH a translocation from the A or B genome of wheat to an *Ae. cylindrica* chromosome was detected in a BC2S2 plant (Wang et al. 2000). Furthermore, the authors established that in plants with 30 chromosomes the two extra chromosomes were not a homologous pair. If they had been, one would expect the extra chromosome pair to be inherited in a stable manner (Wang et al. 2000). The field-produced BC1 (Guadagnuolo et al. 2001) plants investigated in the present study had 28 or 29 chromosomes; thus, the markers from the A and B genome detected in these plants were on a supernumerary chromosome, on a substitution A or B chromosome, or translocated. The introgressive series issued from a manual *Ae. cylindrica* × *T. aestivum* var. Bobwhite cross contains BC1S1 individuals having 28–31 chromosomes (Table 3). The supernumerary chromosomes may be present in homologous pairs, as this generation was produced by selfing of a BC1 individual having 30 chromosomes. Individual 5.13.9 had 31 chromosomes, meaning that either there was one A or B genome chromosome present in a homologous pair, and thus expected to be stably inherited, or there were three different A or B genome chromosomes present as monosomes, which presupposes the presence of a substituted chromosome in the BC1 plant. Individual 5.13.11 had a euploid *Ae. cylindrica* chromosome number and contained marker DP9. This A genome marker is likely on a translocated chromosome fragment, but may be on an A genome substitution chromosome. If it is on a translocated chromosome fragment, it may be inherited in a stable way to the next generations. All three mechanisms (recombination, translocation and chromosome retention) are well known by plant breeders who do the opposite; i.e., introgress genetic material from wild species to wheat, or produce alien addition or substitution lines to study the effect of genes of certain chromosomes or for mapping purposes (Sears 1969; Gale and Miller 1987). Although these processes usually necessitate human intervention such as manual hybridisation, post pollination hormone application or embryo rescue (Sharma 1995), some of the alien substitution lines have arisen spontaneously following hybridisation of wheat and the foreign species (Sears 1969).

It is well known that the diploid behaviour of wheat, i.e. the formation of 21 bivalents at meiosis, is controlled by the *Ph1* locus located on the long arm of chromosome 5B; if chromosome 5B is lacking, multivalents are observed at meiosis (Okamoto 1957; Riley and Chapman 1958). Therefore, in a BC1 hybrid carrying some extra A and B genome chromosomes, but not chromosome 5B, homeologous recombination may take place resulting in translocated segments. Mutants in or deletions of *Ph1* have been used to introgress genetic material into wheat by providing maximum recombination between wheat and chromosomes of alien species (e.g. Lukaszewski 2000). Furthermore, in transformed wheat using microprojectile bombardment, which is the most widely used system for wheat transformation (Janakiraman et al. 2002), transgene integration sites were often telo- or sub telomeric (Jackson et al. 2001) and thus intrinsically more prone to recombination and translocation. The same is true for transgenic barley (Salvo-Garrido et al. 2004). It was also shown that transgene integration site could correlate with chromosomal rearrangements in hexaploid *Avena sativa* L. (Svitashev et al. 2000).

The probability of transgene flow from the A and B genome of wheat to *Ae. cylindrica* is certainly reduced compared to the D genome; the extent of this reduction is unknown and difficult to hypothesise. Similarly, in allotetraploid *Brassica napus* L. (AACC genome), transgene flow to diploid *Brassica rapa* L. (AA genome) is lowered if the transgene is located on the unshared C genome, but it cannot be avoided because of intergenomic recombination between homeologous A and C genome chromosomes (Metz et al. 1997; Stewart et al. 2003). Furthermore, if a transgene confers a selective advantage to the hybrid and the subsequent introgressants, it is likely that it would be rapidly integrated into *Ae. cylindrica* on whatever genome it is placed, specially in a context of extensive hybridisation in winter wheat fields invaded by the wild species, as was observed in

Oregon, USA (Morrison et al. 2002a, 2002b). There are probably some exceptions to this as it was observed that in different wheat lines some marker loci or chromosomes, like chromosome 2B, display segregation distortion in favour of one parental line, lowering the rate of recombination between loci (Paillard et al. 2003). It is hypothesised that segregation distortion in this chromosome involves a genetic factor. If a transgene is flanked by a particularly inefficient distorter allele, one would expect a low segregation ratio of the further. One way to identify A or B genome chromosomes or chromosome regions that have no or only reduced ability of introgression would be to construct an interspecific linkage map between wheat and *Ae. cylindrica*.

Because introgression of wheat A and B genome fragments into *Ae. cylindrica* can occur, we conclude that it is not sufficient to place a transgene on those genomes to efficiently avoid its introgression. The risk that a transgene located on the A or B genome of wheat is maintained in an *Ae. cylindrica* background depends finally on the frequency of chromosome retentions and translocations for each genomic and chromosomal region of wheat. The markers described in this study provide tools for further investigations that are needed to obtain a clear insight into the mechanisms influencing these two factors.

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