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Gene flow from wheat (*Triticum aestivum* L.) to jointed goatgrass (*Aegilops cylindrica* Host.), as revealed by RAPD and microsatellite markers

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Abstract In order to estimate the potential of gene flow between wheat (*Triticum æstivum* L.) and jointed goatgrass (*Aegilops cylindrica* Host.), we carried out mixed pollinations in experimental and natural conditions. A set of species-specific RAPD (random amplified polymorphic DNA) and microsatellite markers were used to detect the presence of parental markers in the progeny of the plants used in these experiments. No hybrids were found within the offsprings of the plants used for the greenhouse experiments, while 85 *Ae. cylindrica*×*T. æstivum* hybrids were found within 2400 analyzed F_1 plants resulting from the field pollinations. The hybridization rates for individuals of different populations of the wild species differed considerably: 1% for two populations known for more than 90 years versus 7% for a newly discovered population. Most of the hybrids were completely sterile, but five of them produced 13 seeds (BC_1) by backcross with *Ae. cylindrica*. Twelve seeds germinated and generated viable and partly fertile plants. About 25% of the wheat specific RAPD markers were found in the BC_1 plants, indicating that introgression of wheat DNA into *Ae. cylindrica* is possible. In addition, one microsatellite marker, known to be situated on the D genome (a genome shared by both species), was also found in the BC_1 plants.

Keywords Gene flow · Risk assessment · Introgression · Wheat · Jointed goatgrass

Introduction

One of the most important concerns related to the field cultivation of transgenic plants is the possibility of gene transfer from crops to their wild relatives. The advantages that a wild plant would acquire in receiving resistance genes could lead to an increase of its weediness, with possible damage for agriculture and for the ecological equilibrium, e.g. increased competitiveness against other species and a consequent loss of biodiversity (Williamson 1994).

With regard to these concerns, the evaluation of the capacity of cultivated species to cross with wild relatives and to produce viable seeds is of the highest importance (Ellstrand et al. 1999). Several cases of intergeneric hybrids between wheat and wild relatives have been reported during the last few years, obtained by forced crossings (emasculation of flowers of one species) and embryo rescue (e.g. Limin and Fowler 1990; Chen et al. 1992; Sharma 1996). Such studies have focused generally on crop improvement; for example the introduction of resistances to diseases into crops. More rare and recent are studies providing evidence of this type of crossing under natural conditions (Zemetra et al. 1998). Thus, in order to determine the real range of the ecological risks related to crop-to-wild gene flow, it is necessary to determine under which conditions and at what frequency these hybridizations can occur in nature.

Most kinds of crop plants are cultivated worldwide, independently of their geographic origin. Therefore, risks existing in one region because of the presence of wild relatives may be negligible in other regions. Moreover, the genetic diversity of a wild relative itself may change completely the range of the risks, even within a relatively small region. One example of this is the case reported by Savova et al. (1996) of a wild relative (*M. falcata* L.) of tetraploid alfalfa (*Medicago sativa* L.) in Switzerland. Both species are either diploid or tetraploid, and crosses are possible and frequent only between cytotypes of the same chromosome number. In Engadin, a region in the east of Switzerland, only diploid *M. falcata* is present and, therefore, crosses with tetraploid crop alfalfa are not possible. Risk assessment studies on crop-to-wild gene flow on a regional scale are thus required.

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Several wild relatives are known to cross-fertilize with wheat (*Triticum æstivum* L.), which is one of the most important crops in the world. Its production is about 21 million tons per year worldwide, with a cultivation area of about 230 million hectares (reviewed in Ellstrand et al. 1999). Most of the hybrids are believed to be sterile (almost completely male-sterile), and this could greatly reduce the impact of genetically engineered wheat. However, some recent studies have demonstrated that some of the hybrids with wheat can produce some seeds, mostly by backcrossing with one of the parents (Li and Dang 1993; van Slageren 1994; Seefeldt et al. 1998; Zemetra et al. 1998) and only rarely by self-pollination (Li and Dong 1991).

In the study reported here, we focused on the closest relative of wheat growing in Switzerland, the tetraploid jointed goatgrass (*Aegilops cylindrica* Host.). Like hexaploid wheat (genome AABBDD) and most of the polyploid Triticeae, *Ae. cylindrica* (genome formula: CCDD) is an amphiploid, resulting from hybridization between the diploids *Ae. caudata* (CC) and *Ae. squarrosa* (DD). The presence of the D genome in both species is supposed to make the introgression of wheat genes into the jointed goatgrass genome easier after hybridization. *Ae. cylindrica* occurs as an adventive in Switzerland, which is its northwestern limit of distribution in Eurasia. This species is, however, frequent in the Mediterranean area and in the Middle East, which is its center of distribution. In North America, *Ae. cylindrica* is an introduced species. It grows within wheat fields and is considered to be a major weed, infesting about 3 million hectares (Dewey 1996) and causing significant yield losses (Ogg and Seefeldt 1999). Moreover, the control of jointed goatgrass in wheat fields using herbicides is impossible, because of its genetic similarity to wheat (Donald and Ogg 1991).

The objectives of our study were (1) to determine the hybridization rate between wheat and *Ae. cylindrica*, (2) to estimate the possibilities of introgression of wheat DNA into *Ae. cylindrica* and consequently, (3) to evaluate the relevance of the results of the present study within the framework of risk assessment of field release of transgenic wheat.

Material and methods

Plant material

Screening for wild populations of *Aegilops cylindrica* (Aec) was carried out by field excursions, on the basis of literature data, in the following regions of Switzerland: Valais, Ticino (south of the Alps), Geneva, Bern, Vaud and Zürich (Hess et al. 1967; van Slageren 1994). Only three populations, all in Valais, were found at this time (Table 1). All of the populations occupied disturbed habitats; for example, train stations and roadsides. Two populations were close to vineyards, but none were in immediate proximity of fields cultivated with cereals. In each population, a representative sample of 20–40 spikes (1 spike/plant) was collected. In addition, seeds of 13 Swiss wheat varieties [7 winter wheats and 6 spring wheats (Table 1)], obtained from Eric Schweizer, Samen AG, Thun, were included in the study. Seeds were sown and plants grown in individual pots in the Botanical Garden of Neuchâtel.

Mixed pollination experiments

Two types of mixed pollination experiments were carried out: under experimental conditions in the greenhouse and under natural conditions in the field.

Greenhouse pollinations

A total of 32 plants of *Ae. cylindrica* were used for crosses with four Swiss wheat varieties (Table 2). Experiments were performed by combining two spikes, one of each species, under a pollination bag. In order to estimate the possibility of hybridization in both directions, we did not emasculate the flowers. The bags were left on the spikes until the end of the flowering period, during which time they were shaken regularly, in order to mix the pollens. All of the

Table 1 Sampling of the species and of the individuals for genetic analyses

Species	Populations/varieties	Identification numbers of populations and species	Number of analyzed plants
Aegilops cylindrica	Brig (46°19´N; 8°00´E)	Aec1	30
	Sierre (46°18´N; 7°33´E)	Aec2	30
	Saillon (46°11´N; 8°11´E)	Aec3	20
Triticum æstivum	4 Swiss varieties		
	Arina	T.ae	1 bulk of 10
	Galaxie	T.ae	1 bulk of 10
	Boval	T.ae	1 bulk of 10
	Runal	T.ae	1 bulk of 10

Table 2 Experimental crosses in the greenhouse (*T. ae=T. aestivum*)

Crosses	Number of individuals *Ae. cylindrica* (1 spike/individual)	Number of individuals *T. æstivum* (1 spike/individual)
T. ae. var. Arina×*Ae. cylindrica*	8	8
T. ae. var. Galaxie×*Ae. cylindrica*	8	8
T. ae. var. Boval×*Ae. cylindrica*	8	8
T. ae. var. Runal×*Ae. cylindrica*	8	8

seeds obtained from these crosses (of both species) were collected and sown.

Field pollinations

Eighty individuals of *Ae. cylindrica* (30 individuals of populations Aec1 and Aec2; 20 of population Aec3) were planted in the middle of a cultivated field of *T. aestivum* var. Arina. This is the most cultivated variety in Switzerland, grown in 40–50% of the area cultivated with wheat (Valenghi 1998; Rüegger 2000). The plants were placed in four rows, each 45 m long, with 5 m between rows. In order to avoid a bias in the hybridization rate, due to possible differences in the quantity of wheat pollen in the field, individuals of each Aec population were distributed over the entire field area. This experiment was performed at the Swiss Federal Research Station for Plant Production of Changins (RAC, Nyon).

All seeds produced by *Ae. cylindrica* plants were collected. A first subset of ten seeds per individual was sown in the Botanical Garden and grown under open pollination conditions. Subsequently, in order to confirm differences in the hybridization rates of the populations observed in the first subset, we sowed an additional series of 20 seeds per mother plant. Thirteen seeds produced by the hybrids were also sown and grown in the Botanical Garden.

Hybrid detection

The different ploidy levels of *T. æstivum* (2n=42) and *Ae. cylindrica* (2n =28) enabled us to detect hybrid plants by analyzing the DNA content. The F_1 plants resulting from all of the crosses were thus analyzed by flow cytometry for quick detection of hybrids. For each individual, a small (1 cm^2) piece of fresh leaf was taken and sliced with a razor blade in a petri dish containing 0.5 ml of ice-cold PBS buffer in order to release the cell nuclei. Twenty microliters of staining solution [50% propidium iodide and 50% Triton X-100 (10%)] was then added to the solution, which was filtered through a 30-µm mesh nylon screen. Finally, the fluorescence of stained nuclei was measured with a Becton Dickinson FACStrak flow cytometer and visualized as a histogram. Samples of known ploidy (tetra- and hexaploid) were used to calibrate the flow cytometer at the beginning of each measurement session.

A morphological survey of all hybrids detected was carried out to confirm the results of the cytometry analyses. Chromosome counts of the plants produced by the hybrids were performed on root tips as described in Savova et al. (1996).

Genetic marker detection

Specific genetic markers for wheat and jointed goatgrass, obtained with random amplified polymorphic DNA (RAPD) and microsatellite (simple sequence repeats; SSRs) techniques were set up as described in Guadagnuolo (2000) and used to analyze the F_1 plants. Eight RAPD primers (OPB 6, 8, 10 and OPP 6, 7, 8, 9, 14; Operon Technologies, Alameda, Calif.) and six publicly available wheat microsatellite primers pairs (WMS 43, 44, 46, 47, 106 and 159; Plaschke et al. 1995, Röder et al. 1995) were used, as they amplified specific markers for the species. Four plants, produced by hybrids as mother plants, were analyzed with the same markers.

DNA analyses

Total DNA extraction from a single leaf was carried out on 11 samples per population for *Ae.cylindrica*. The extraction was performed on a bulk of ten plants for each wheat and spelt variety. We used a simple SDS-Na-acetate protocol (Savova-Bianchi 1996). DNA was resuspended in a TE (pH 8) solution at a concentration of 30 ng/ml and stored at –20°C.

Subsequently, DNA extraction was performed on 23 F_1 hybrids (generated by the *Ae. cylindrica* plants involved in the crosses in the field) and on the progeny (4 plants) of these hybrids.

Polymerase chain reactions (PCR) for both RAPD and SSR amplifications were performed in a volume of 25 µl under the following final concentrations:

RAPD

The amplification reaction contained 1×PCR mix, 1.5 m*M* $MgCl_2$, 0.4×Q-solution, 0.2 m*M* dNTP, 0.2 µ*M* primer, 0.03 U/µl *Taq* polymerase and 1 ng/µl template DNA. Amplifications were performed in a Biometra I thermocycler with the cycling following profile: an initial denaturation at 94°C for 5 min, followed by 35 cycles of 1 min at 93°C, 1 min at 45°C and 1 min at 72°C, with a final extension for 10 min at 72°C. PCR products were mixed with a 1/5 vol of loading buffer and separated on a 1.6% (w/v) agarose gel, containing 0.4 µg/ml ethidium bromide, in 0.5×TBE at 60 V for 2 h. The DNA fragment were then vizualized under UV light.

Microsatellites

The amplification reaction contained 1×PCR mix, 1.5 m*M* $MgCl_2$, 0.4×Q-sol, 0.2 m*M* dNTP, 0.6 n*M* of each primer, 0.03 U/µl *Taq* polymerase and 1 ng/µl template DNA. Amplifications were performed in a Biometra I thermocycler with the following cycling profile: an initial denaturation at 93°C for 3 min, followed by 45 cycles of 1 min at 93°C, 1 min at 55°C and 2 min at 72°C, with a final extension for 10 min at 72°C. PCR products were mixed with 1/5 vol of loading buffer and separated on a 6% polyacrylamide gel in 0.5×TBE at 100 V for 6 h. The gels were then stained in a 0.4 µg/ml ethidium bromide bath and the DNA fragments vizualized under UV light.

Data analysis

The data obtained with the two techniques (RAPD and microsatellites) were scored in a binary form as the presence or absence (1/0) of bands for each individual or bulk (in the case of wheat DNA samples).

We used the R4 (Beta version) package (P. Casgrain and P. Legendre, Université de Montréal) to calculate Jaccard's similarity coefficient between samples for the two sets of data: RAPD and microsatellites. Jaccard's similarity measure does not take into account double absence. This is closer to the biological reality, considering that the absence of a DNA fragment in two samples is an absence of information rather than an element of similarity.

The similarity matrices were converted into distances matrices (D=1-S) and used to perform principal coordinates analysis (PCoA) (Gower 1966).

Results

Greenhouse pollinations

The 36 *Ae. cylindrica* spikes involved in the mixed pollinations with four different wheat varieties produced 492 seeds in total (average of about 14 seeds/spike), while 853 seeds were produced by the 36 wheat spikes (an average of about 24 seeds/spike). No hybrids were detected within this progeny, neither with flow cytometry nor by observation of morphological traits.

RAPD and microsatellite analyses performed on one individual per cross from these F_1 plants (data not shown) confirmed their breeding by self-pollination because their DNA profiles corresponded exactly to those of their mother plants.

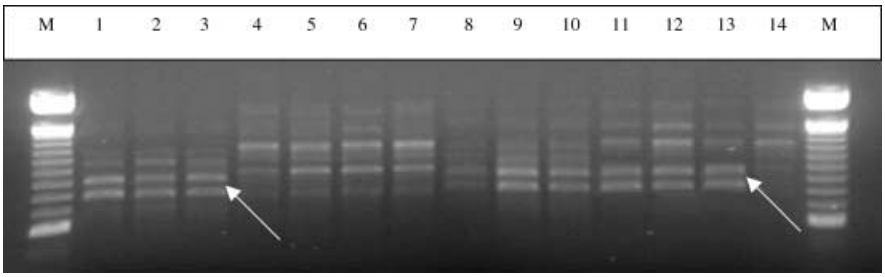

Fig. 1 Example of RAPD amplification in *T. æstivum* (*lanes 1–3*), *Ae. cylindrica* (*lanes 4–7*), hybrids *Aegilops cyl.×Triticum æst* (*lanes 8–10*) and BC_1 plants (*lanes 11–14*) with primer OPB-10. *M* 100 bp DNA ladder (GibcoBRL®, Life Technologies); *arrows* indicate two specific fragments of wheat present in three BC_1 individuals

Field pollinations

Among the 2,400 grown offsprings of *Ae. cylindrica*, 85 hybrids (2n=35) were detected. Ten were produced by plants of population Aec1 (Brig), five by plants of population Aec3 (Saillon) and 70 by plants of the newly discovered population of Sierre (Aec2). On average, the hybridization rate was 1% for the individuals of populations Aec1 and Aec3, while individuals of population Aec2 showed a hybridization rate of 7%. The morphology of the hybrids was intermediate between that of the parental species.

As expected, the hybrids were completely male-sterile and almost completely female-sterile. However, 5 of the 85 detected hybrids produced 13 seeds, of which 12 germinated. The mother plants of four from the five partially fertile hybrids were from population Aec2 and one was from population Aec3.

The DNA content of the offsprings of the hybrids was identical to that of pure *Ae. cylindrica*. This indicates that they were produced by a backross of the hybrid mother plant with *Ae. cylindrica*. Moreover, their morphology was identical to that of *Ae. cylindrica* and their chromosome numbers ranged from 28 to 29. These BC_1 plants were grown under open-pollination conditions within blocks of pure *Ae. cylindrica* individuals and produced fertile flowers and seeds.

Marker detection

The selection of molecular markers for the differentiation of the species was discussed in Guadagnuolo (2000).

RAPDs markers

The RAPD profiles of the hybrids presented most of the specific bands of both parents (Fig. 1). As expected, fragments present in both parent species were also amplified in the hybrids. Sixteen out of thirty species-specific markers of wheat (var. Arina), and 16 out of 22 species-specific markers of *Ae. cylindrica* were amplified in the hybrids as well. In addition, one fragment specific for population Aec2 of *Ae. cylindrica* was only amplified in the hybrids produced by individuals of this population.

Of the 16 specific fragments of *T. æstivum* inherited by the hybrids, 9 were amplified in the BC_1 plants as well, indicating introgression of wheat DNA into the wild species (Fig. 1). Of these fragments, five were present in all four BC_1 individuals analyzed, three were amplified in two individuals and one fragment was inherited by only one individual. On the contrary, only 1 of the 16 specific fragments of *Ae. cylindrica* was not amplified in the BC_1 plants.

Wheat microsatellite markers

All seven specific markers of *T. æstivum* were amplified in the hybrids, independently of their location on the different genomes, while this was the case for only three of the five specific bands of *Ae. cylindrica*. One fragment, only present in jointed goatgrass of populations Aec1 and Aec3, was only amplified in the hybrids produced by plants of these populations.

Five fragments in total were amplified in BC_1 plants, all of them present in the hybrids as well. One of these fragments was amplified with primer WMS 159 and was originally a specific marker of *T. aestivum*, being situated there in the D genome. Two primers of microsatellites supposed to be located in the B genome (WMS 43 and 46) amplified fragments in BC_1 plants, which, however, were already present in pure *Ae. cylindrica* and the hybrids as well. Among all the other specific fragments of wheat, neither those amplified with primers WMS 44 and 106 (supposed to be located in the D genome) nor those located in the B genome (primers WMS 43, 46 and 47) were amplified in BC_1 plants.

Species differentiation

The Principal Coordinates Analyses (PCoA) performed on both kinds of data showed a clear separation of the parental species, the hybrids and the BC_1 plants (Figs. 2 and 3). The position of the hybrids in the PCoA performed on RAPD data was perfectly intermediate between those of the parental species. In addition, the posi-

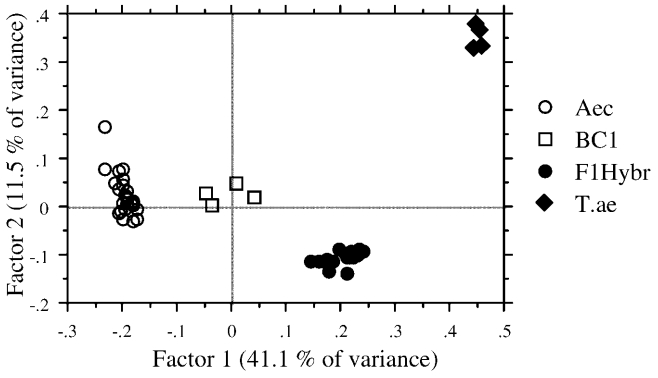

Fig. 2 Principal Coordinates Analysis (PCoA) performed on the similarity matrix obtained with RAPD data (based on Jaccard's similarity coefficient)

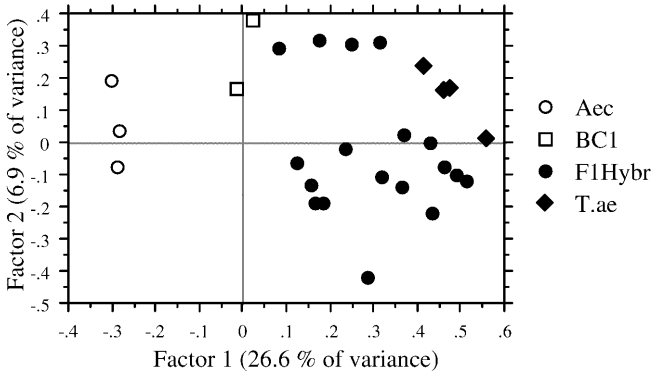

Fig. 3 PCoA performed on the similarity matrix obtained with microsatellite data (based on Jaccard's similarity coefficient)

tion of BC_1 plants was intermediate between those of hybrids and jointed goatgrass (Fig. 2). In the case of the analyses performed on wheat microsatellite data, the hybrids were dispersed and closer to wheat than to *Ae. cylindrica*. The BC_1 plants were also grouped closer to the hybrids than to *Ae. cylindrica* (Fig. 3).

The relative position of BC_1 plants, placed between hybrids and *Ae. cylindrica* by PCoA on both kinds of data, confirmed that the seeds produced by the hybrids were obtained by backcrosses with this parental species.

Discussion

Hybridization

Ae. cylindrica×wheat hybrids have already been observed, but these were believed to be sterile (van Slageren 1994). The results of the present study confirm that spontaneous hybridization between conventional (i.e. non-genetically engineered) wheat and *Ae. cylindrica* under natural conditions is possible. However, the hybrids are partly fertile, which confirms the recent results of Seefeldt et al. (1998) and Zemetra et al. (1998).

The absence of hybrids in the progeny of the experimental mixed pollinations, compared with the results of those under natural conditions, indicates that a large source of external pollen is probably necessary to induce *Ae. cylindrica* to produce hybrids. A huge amount of pollen was available in the field pollinations, where *Ae. cylindrica* was planted intermixed with wheat, but not in the greenhouse experiments, where the pollination bag limited the wheat pollen source to that of only one spike. Moreover, as shown by Fritz and Lukaszewski (1989) for wheat cultivars crosses, several pollination events are necessary to stimulate the recipient flower to allow for pollen growth on the stigma. The probability of such repeated events is certainly higher within a field with thousands of flowers producing pollen than in a pollination bag.

The spikes involved in the experimental crosses produced seeds of good quality, a result of self-fertilization, and in normal quantities. Therefore, the absence of hybrids in the progeny of these crosses was probably not caused by unfavorable environmental conditions inside the bags, which could have lowered the pollen fertility (temperature, moisture etc).

Because the hybrids were grown within a large number of pure *Ae. cylindrica* plants, all of the BC_1 plants were produced by backcrossing with the wild species and not with *T. æstivum*. Indeed, the relative DNA quantity of the BC_1 plants measured by flow cytometry was exactly the same as for pure *Ae. cylindrica* plants. Moreover, chromosome counts confirmed these results, indicating a ploidy level that was almost completely restored. This situation corresponds to the typical agricultural conditions in Europe, where crop rotation is generally applied. Therefore, hybrids produced by jointed goatgrass growing in the middle of wheat fields would grow the following year surrounded by more *Ae. cylindrica* than by wheat plants.

The BC_1 plants produced numerous seeds, indicating at least a partially restored female fertility. However, BC_1 plants were also grown within pure jointed goatgras, and thus within a large amount of pollen. Further analyses could tell whether the seeds produced by the BC_1 resulted from self pollination, thereby representing BC_1S_1 plants, or from pollination by pure *Aegilops*, consequently being BC_2 plants.

One of the most surprising results of the present study was the big difference in the hybridization rate between individuals of different populations. The plants of population Aec2 used in the crosses in the field showed an hybridization rate of 7%, while those of the two other populations (Aec1 and Aec3) hybridized with wheat at a rate of 1%. Zemetra et al. (1998) observed an hybridization rate closer to this latter, i.e. of about 2%, between wheat and *Ae. cylindrica* infesting wheat fields in USA. It is presently impossible to speculate on the reasons of these results (lower male fertility of individuals of population Aec2, partial incompatibility with own pollen etc.), but they are probably correlated with the genetic differences between this population and the two others,

as observed with RAPD and microsatellite markers (Guadagnuolo 2000). Although *Ae. cylindrica* is an adventive species in Switzerland, populations Aec1 and Aec3 have been known since at least 90 years (van Slageren 1994), while Aec2 is a newly discovered one. The important differences in the hybridization rate for populations of the same species demonstrate the need for risk assessment studies on a regional scale, especially given the fact that the three populations are located at a maximum of 100 km from each other.

Marker inheritance

The morphology of the jointed goatgrass×wheat hybrids was intermediate between those of the parental species. This was also reflected by the amplification, in hybrids DNA, of an equal number of specific RAPD markers of both species. However, a higher rate of *Ae. cylindrica*-specific RAPD markers (16 out of 22), compared to those of wheat (16 out of 30), were inherited by hybrids. This could indicate that some of these fragments originated from the cytoplasmic DNA. Indeed, the female parent of the hybrids was *Ae. cylindrica*, and in Poaceae paternal chloroplast inheritance has never been observed. Moreover, it is well-known that RAPD fragments can originate from nuclear, chloroplast and mitochondrial DNA (Lorenz et al. 1997).

The use of both RAPD and microsatellites markers allowed us to demonstrate the transfer of wheat DNA into the BC_1 plants by natural hybridization. The large number of wheat-specific RAPD markers inherited by the BC_1 plants suggests that most of them were located in the D genome, but this was not proven. Nevertheless, one fragment known to be located in the D genome, wheat microsatellite WMS 159 (Plaschke et al. 1995), was amplified in the BC_1. While these results demonstrate that introgression of wheat DNA fragments located in the D genome into *Ae. cylindrica* is possible, they can not yet prove that this is also possible from the A or B genomes. Therefore, the question of whether translocations between the A or B and C or D genomes can occur during meiosis in hybrids or subsequent backcrosses remains open, as already noticed by Seefeldt et al. (1998) and Zemetra et al. (1998).

The natural maintenance of part or the entire foreign genomes in the progeny of forced hybrids between wheat and several *Triticeae* has been recorded (Limin and Fowler 1990; Chen et al. 1992; Sharma 1996). Moreover, the genomes of the species involved in these studies were much more remotely related to those of wheat (e.g. genomes P or Ju of several *Agropyron* species) than the genomes of *Ae. cylindrica*.

The data analyses clearly separated the parental species, the hybrids and the BC_1 plants. Moreover, the persistence of parental traits in the progeny of the crosses was reflected by the relative position of each class of samples on the PCoA scattergrams. The selected DNA markers combined with such analyses would therefore be useful in detecting the putative introgression of wheat DNA into jointed goatgrass under natural conditions.

Relevance of the results for the risk assessment of transgenic wheat cultivation

In order to correctly evaluate the risks of gene or transgene escape from a crop plant to a wild relative, investigators must take several factors into account. The genetic compatibility, *i.e.* the capacity of hybridization and the fertility of the hybrids and successive backcrosses, has to be investigated. The possibility of hybridization with a wild relative and the subsequent persistence of crop genes in the wild species have already been demonstrated for several crops; for example sunflower (Arias and Rieseberg 1994; Whitton et al. 1998), sorghum (Arriola and Ellstrand 1996, 1997), radish (Lee and Snow 1998) and alfalfa (Jenczewski et al. 1999).

The results of the present study, combined with those of Zemetra et al. (1998) and Seefeldt et al. (1998), show that (1) natural hybridization between wheat and jointed goatgrass is possible with both species as female parent, (2) hybrids can produce at least some seeds; and (3) wheat traits can be transferred into the *Ae. cylindrica* genome after only two backcrosses. Therefore, the possibility of gene transfer from wheat to jointed goatgrass under natural conditions (e.g. agroecosystems) is likely.

We used here conventional instead of transgenic wheat varieties. However, the presence of a transgene is supposed to have no impact on the pollen production of wheat and thus on the hybridization rate with jointed goatgrass. Therefore, this should not have an impact on the evaluation of gene flow between the species. On the contrary, studies using transgenic crops will be necessary to evaluate the consequences of that gene flow.

Because crop-to-wild gene flow is mostly pollen-mediated (Ellstrand and Hoffman 1990), proximity and overlapping in the flowering period of both kind of species are also required (Klinger et al. 1992). Gene dispersal from crop to wild relatives growing tens or hundreds meters away from the cultivated source has been reported for insect-pollinated species as radish (Klinger et al. 1992), sunflower (Arias and Rieseberg 1994) or potato (Conner and Dale 1996). However, it is likely that for wind-pollinated species, like most Poaceae, hybridizations are restricted to a much narrower zone. Indeed, most of pollen migration of Poaceae occurs within few meters (Pedersen 1994), although pollination over 20 m (Doll 1987) and even 60 m (Wagner and Allard 1991) has been reported for crop barley. Kertesz et al. (1995) found that durum wheat pollen migrated up to 20 m but that open pollination between durum wheat varieties occured only within 3 m. In the case of wheat and jointed goatgrass in Switzerland, an overlapping of flowering periods was observed. However, the populations of the wild species were situated at least at few kilometers from fields cultivated with cereals, and all of them were in Valais, a region with a moderate cereal cultivation. It is

thus unlikely that pollination by wheat could occur in these sites. Nevertheless, as noted above, *Ae. cylindrica* is an adventive species in Switzerland, and seeds are supposed to be transportated by train or trucks from neighboring regions south of the Alps (Italy, France). Moreover, the seeds of this species exhibited a germination rate almost equal to those of the wheat varieties. The establishment of new populations in the direct proximity of wheat fields is thus not unlikely. In addition, the more recent population (Aec2) showed an hybridization rate with wheat that was much higher than those of the old ones. Therefore, the risk of gene escape from transgenic wheat to *Ae. cylindrica* in Switzerland exists but is moderate. It could be higher if new populations, with a crossing rate with wheat similar to that of population Aec2, established close to wheat cultivation areas.

Nonetheless, this risk could be dramatically higher in other countries. The geographic distribution of *Ae. cylindrica* in Europe and the Middle East, where it grows in the vicinity of or within wheat fields (van Slageren 1994), makes this species the ideal recipient for crop genes. The likelihood of this gene transfer is even higher in the USA. Jointed goatgrass is not native there but has been introduced and already infests between 2 and 3 million hectares of wheat fields, causing important yield losses (Dewey 1996; Ogg and Seefeldt 1999). Despite the low fertility of the hybrids, risks must not be underestimated. The large area occupied in parapatry by both species could lead to a great number of hybridization events and thus to a relevant number of cases of introgression. The weediness of jointed goatgrass could consequently become even higher. The extent of these hazards will be greatly influenced by the type of transgene transferred. In the case of jointed goatgrass, even a small number of plants receiving an herbicide resistance gene could have important consequences on wheat cultivation in regions where both species grow intermixed.

Conclusion

The results of the present study demonstrate that gene flow between *T. æstivum* and *Ae. cylindrica* under natural conditions is possible. The discovery that *Ae. cylindrica*×*T. æstivum* hybrids are at least partly fertile is of great importance, because it proves that introgression of wheat traits into a wild relative, and thus transgene escape, is possible. In Switzerland, jointed goatgrass has not been found in direct proximity of wheat fields and grows only in Valais, a region with a moderate cereal cultivation; the risks are thus modest. However, the high hybridization rate of one population could lead to higher risks, especially considering that jointed goatgrass is an adventive species, able to establish new populations. In the Middle East and in regions where this species has been introduced (e.g. USA), the risk is much higher, because it often grows intermixed with wheat.

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