Chapter 11
Production and Molecular Cytogenetic Identification of Wheat-Alien Hybrids and Introgression Lines

Márta Molnár-Láng, István Molnár, Éva Szakács, Gabriella Linc and Zoltán Bedö

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Abstract Barley, rye, *Aegilops* and *Thinopyrum* (syn. *Agropyron*) species belonging to the *Triticeae* tribe have large genetic diversity and serve as a valuable genetic reservoir for wheat improvement. Many of these species have been used for more than a century for the production of wheat × alien hybrids and introgression lines. The most up-to-date molecular cytogenetic techniques make it possible to detect and identify alien chromosomes in the wheat genome. The first methods used to identify rye, barley, *Aegilops* and *Thinopyrum* chromosomes in the wheat genome were C- and N-banding. Genomic *in situ* hybridization (GISH) is the most accurate way of detecting the translocation breakpoint in introgression lines. Alien chromosomes can be identified in the wheat genome using fluorescence *in situ* hybridization (FISH) with the help of repetitive DNA probes. Multicolor GISH (mcGISH) was developed to demonstrate the various genomes in polyploid plant species and in interspecific and intergeneric hybrids, amphiploids and derivatives. Sequential GISH and FISH are useful methods for identifying alien translocations in the wheat genome.

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11.1 Introduction

11.1.1 Interspecific and Intergeneric Hybridization of Plant Species

For several centuries scientists have been interested in hybridizing different plant species in order to merge useful traits in a new hybrid progeny. The first known artificial interspecific hybrid was produced by Fairchild in 1717 (see Belea 1992), while systematic attempts at interspecific crossing are linked with the name of Kölreuter (1766). In 1876, Stephen Wilson presented some completely sterile ears of wheat-rye hybrids for the consideration of the Botanical Society of Edinburgh. His work was aimed at the unification of the favourable characters of the two crops. This was the first step in expanding the gene pool of wheat to include the variations carried by the many wild and cultivated related species in the *Triticeae* tribe.

The grass tribe *Triticeae* includes some of the major cereal crop species of the world, namely *Triticum aestivum* L. (bread wheat), *T. durum* L. (durum wheat), *Secale cereale* L. (rye), *Hordeum vulgare* L. (barley), the modern cereal *Triticosecale* (triticale) and about 350 other species (Knüpffer 2009). Species related to wheat in the *Triticeae* tribe have large genetic diversity and serve as a valuable genetic reservoir for wheat improvement. The majority of these species can be crossed with wheat and agronomic traits can be transferred from the hybrids into the wheat genome by backcrossing. In 1969, a new era of molecular cytogenetics began which made it possible to precisely identify the chromosomes of different species and to determine the genome composition of hybrids and derivatives.

11.1.2 Molecular Cytogenetic Techniques

11.1.2.1 Chromosome Banding Techniques

The first chromosome banding technique was developed by Caspersson et al. (1968). Alkylating fluorochromes like quinacrine (Q) and quinacrine mustard (QM) were found to differentially stain regions of C-heterochromatin in the chromosomes of *Vicia faba*. The Q and QM fluorescent patterns were chromosome-specific, thus allowing the chromosomes to be identified (see Friebe and Gill 1996). The Giemsa banding techniques originated as a by-product in an *in situ* hybridization (ISH) experiment where mouse satellite DNA was hybridized to mouse metaphase chromosomes. Pardue and Gall (1970) observed that the DNA probe preferentially hybridized to the centromeric regions, but they also observed that these regions stained darker than

other chromosome regions after counterstaining with Giemsa. This discovery led to the development of the C- and G-banding techniques for mammalian chromosomes and shortly afterwards to the development of similar techniques for plant chromosomes (Sarma and Natarajan 1973; Hadlaczky and Belea 1975). A standard karyotype and banding nomenclature system for *T. aestivum* was proposed by Gill et al. (1991). Today, it is possible to identify all 21 chromosome pairs of hexaploid wheat and also 36 of the 42 chromosome arms by C-banding. The barley chromosomes were identified by Giemsa C- and N-banding (Linde-Laursen 1975).

11.1.2.2 *In situ* Hybridization

The development of the DNA *in situ* hybridization (ISH) technique (Gall and Pardue 1969; John et al. 1969) marked the transition from the classical cytogenetics era to the modern molecular cytogenetics era (see Jiang and Gill 2006). The basic procedure of ISH is the labelling of a DNA probe and its hybridization to cytological preparations. ISH is a powerful method for localizing DNA or RNA sequences in the cytoplasm, organelles, chromosomes or nuclei of biological material (Leitch et al. 1994). Radiation-based methods were used in probe labelling and signal detection in early techniques, but they were soon replaced by fluorescence-based methodologies (Langer-Safer et al. 1982). Fluorescence *in situ* hybridization (FISH) using fluorochromes for signal detection has several advantages over ISH using isotopic probes or enzymatic detection methods. First, different DNA probes can be labelled with different haptens and simultaneously detected using different fluorochromes (multicolor FISH), thus allowing their physical order on chromosomes to be determined (Lichter et al. 1990; Leitch et al. 1991; Mukai et al. 1993). Second, fluorescence signals can be captured by special cameras or laser scanning microscopes and analysed with digital imaging systems, thus allowing more precise mapping (Jiang and Gill 1994).

Total genomic DNA probes with unlabelled blocking DNA can also be used to identify the genomes in hybrid organisms (Le et al. 1989; Schwarzacher et al. 1989). Genomic probes are used in plant breeding to detect alien translocations and substitutions in cereals (Schwarzacher et al. 1992). Genomic *in situ* hybridization (GISH) is the most efficient and accurate technique to allocate the breakpoints and estimate the amount of alien chromatin in translocation chromosomes (Anamthawat-Jonsson et al. 1993; Jiang and Gill 1994).

FISH signals derived from a single repetitive DNA probe or a cocktail containing several DNA probes can provide a hybridization pattern that allows all the chromosomes within a species to be identified. Since different probes or probe cocktails can be developed for each species, the FISH-based chromosome identification method is more versatile than the traditional chromosome banding techniques (Jiang and Gill 2006). More importantly, FISH-based chromosome identification systems can be integrated directly into the FISH mapping of other DNA sequences. Attempts to increase the detection sensitivity of very small chromosomal targets, and to improve the spatial resolution of signals derived from flanking sequences, have led to the

development of a variety of novel techniques: it is now possible to perform *in situ* hybridizations on interphase nuclei, meiotic pachytene chromosomes and isolated chromatin (DNA fibres) (de Jong et al. 1999).

11.2 Wide Hybridization of Wheat

Molecular cytogenetic techniques are applied in the selection and identification of progenies originating from distant crosses, which contain alien chromosome segments. The method for transferring genes from related species to wheat largely depends on the evolutionary distance between the species involved. Species belonging to the primary gene pool of common wheat share homologous genomes. Gene transfer from these species can be achieved by direct hybridization, homologous recombination, backcrossing and selection (Friebe et al. 1996). The secondary gene pool of common wheat includes polyploid *Triticum/Aegilops* species that have at least one homologous genome in common with *T. aestivum*. Gene transfer from these species is possible by homologous recombination if the target gene is also located on a homologous chromosome. Species belonging to the tertiary gene pool are more distantly related. Their chromosomes are not homologous to those of wheat. Other strategies need to be employed, because gene transfer from these species cannot be achieved by homologous recombination.

11.2.1 Wheat × Barley Hybridization

11.2.1.1 Production of Wheat × Barley Hybrids and Addition Lines

Bread wheat (*T. aestivum*) and barley (*H. vulgare*) are two of the most important cultivated cereals worldwide. The introgressive hybridization of barley to wheat makes it possible to transfer useful characteristics such as earliness, tolerance to drought and soil salinity, and various traits for specific nutrition quality. The first wheat × barley hybrid was produced by Kruse (1973) and the production of the first set of Chinese Spring/Betzes spring wheat-spring barley addition lines was described by Islam et al. (1978). Since then Koba et al. (1997) have reported two new 5H and 6H addition lines from a hybrid between the wheat cultivar Shinchunaga and the barley cultivar Nyugoruden. Alien additions are primarily produced to add specific desirable genes to a crop species (Gale and Miller 1987), but addition lines can be used for many other purposes, such as mapping genes and markers on introgressed alien chromosomes, examining alien gene regulation, understanding meiotic pairing behaviour and chromosome structure, and isolating individual chromosomes and genes of interest (Chang and de Jong 2005; Cho et al. 2006).

The production of wheat × barley hybrids is a difficult task because of the low crossability between the *Hordeum* and *Triticum* genera (Shepherd and Islam 1981;

Fedak and Jui 1982; Molnár-Láng and Sutka 1994). Wheat × barley hybrids can only be produced with wheat genotypes which carry recessive crossability alleles (*kr1* and *kr2*), and pollination must be carried out under favourable environmental conditions. Pollinated flowers must be given hormone treatment (2,4-dichlorophenoxyacetic acid or giberellic acid) followed by embryo rescue, but in spite of great efforts the seed set is very low (Kruse 1973; Fedak 1980; Molnár-Láng and Sutka 1994; Molnár-Láng et al. 2000a). The hybrids are male sterile, but can be pollinated with wheat (Islam and Shepherd 1990; Koba et al. 1997). However, in most cases no backcross progenies have been obtained (Wojciechowska and Pudelska 1993; Jauhar 1995).

Fourteen winter barley and three spring barley cultivars were tested as pollinators for wheat × barley hybrid production in Martonvásár, and hybrids were successfully produced with four barley genotypes: Betzes (North American two-rowed spring barley), Igri (German, two-rowed winter barley), Osnova and Manas (Ukrainan six-rowed winter barleys). The best seed set (3.3 %) was achieved with barley cv. Betzes, but less than 1 % seed set was observed when wheat was pollinated with the barley cultivars Igri, Manas and Osnova (Molnár-Láng and Sutka 1994; Molnár-Láng et al. 2000a). There was no seed set with the other thirteen barley cultivars. The Martonvásári 9 kr1 (Mv9 kr1) × Igri and Asakaze komugi × Manas hybrids were vigorous and had good tillering abillity, which made it possible to collect anthers from young inflorescences for meiotic analysis, to pollinate some spikes with wheat, and to use some developing inflorescences for *in vitro* multiplication. The hybrids were multiplied in tissue culture because of the high degree of sterility, and then pollinated with wheat to obtain backcross progenies. Meiotic analysis of the hybrids Mv9 kr1 × Igri and Asakaze × Manas and their *in vitro* regenerated progenies with the Feulgen method revealed 1.59 chromosome arm associations per cell in both initial hybrids. The number of chromosome arm associations increased after *in vitro* culture in both combinations (Molnár-Láng et al. 2000a). Wheat-barley chromosome pairing was detected in the hybrids using GISH, as in the case of wheat-rye pairing in wheat × rye hybrids (King et al. 1994; Miller et al. 1994). According to GISH analysis the number of wheat-barley chromosome arm associations increased in the *in vitro* regenerated progenies of both the wheat × barley hybrids (Molnár-Láng et al. 2000a). These results proved the possibility of producing recombinants between the two genera, and thus of transferring useful characters from barley into wheat. In vitro conditions caused an increase in chromosome arm association frequency in both combinations and in greater fertility in some regenerants. The Asakaze × Manas hybrids were maintained in tissue culture for several years and their meiotic pairing behaviour and genome composition were analysed after *in vitro* multiplication. The seven barley chromosomes were present in most cells, even after the third *in vitro* multiplication cycle, but some abnormalities were observed (Molnár-Láng et al. 2005).

The regenerated Mv9 kr1 × Igri hybrids were backcrossed with wheat and a series of novel winter wheat-winter barley disomic addition lines (2H, 3H, 4H, 6HS, 7H and 1HS isochromosome) were selected and identified from the selfed progenies of the BC_1 plants (Szakács and Molnár-Láng 2007, 2010a). GISH was used to confirm the presence of the barley chromosomes in the wheat genome. The barley chromosomes were identified by the FISH patterns (Fig. 11.1) obtained with various combinations

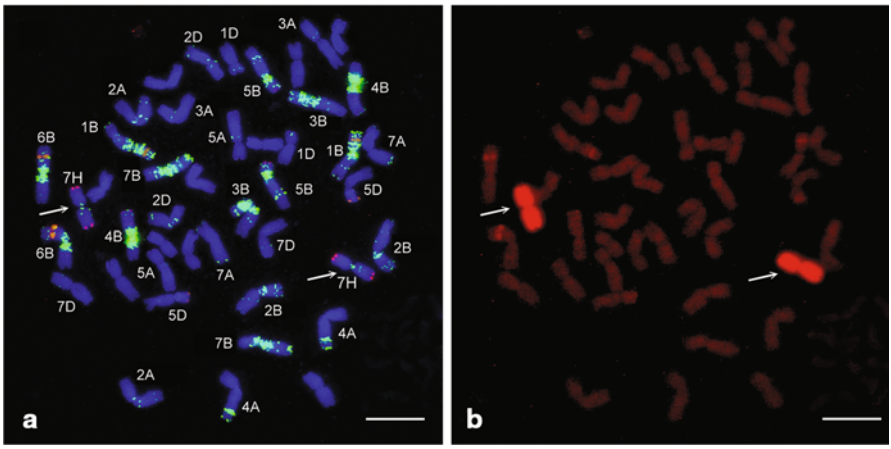

Fig. 11.1 Sequential FISH and GISH on mitotic chromosomes of 7H Mv9kr1/Igri wheat-barley disomic addition line. **a** Identification of the 7H barley chromosomes using DNA probes GAA (*green*), HvT01 (*red*) and pTa71 (*yellow*) on the FISH image. **b** Barley chromosomes are red as a result of labelling the barley DNA with digoxigenin and were detected with anti-DIG-Rhodamine on the GISH image. 7H barley chromosomes are indicated by *arrows*. Scale bar = 10 μm

of repetitive DNA probes: GAA-HvT01, pTa71-HvT01 and Afa family-HvT01 (Szakács and Molnár-Láng 2007). Various DNA probes were used earlier to characterize the barley genome using FISH. The 45S ribosomal DNA probe pTa71 hybridizes to five chromosome pairs (Leitch and Heslop-Harrison 1992). The subtelomeric regions of all barley chromosomes can be reliably identified with the barley-specific tandem repeat HvT01 (Schubert et al. 1998) or the Triticeae-specific AT-rich tandem repeat pHvMWG2315 (Busch et al. 1995). A non-random, motif-dependent distribution of tandem array trinucleotide repeats was found for barley (Cuadrado and Jouve 2007). With the exception of $(ACT)_5$ the remaining trinucleotide repeats occur predominantly in Giemsa-banding-positive heterochromatin (Pedersen and Linde-Laursen 1994; Cuadrado and Jouve 2007). The identification of the barley chromosomes in the addition lines was further confirmed with SSR markers, and the addition lines were characterized morphologically.

Disomic addition lines (2H, 3H, 4H, 6H and 7H) were also selected from selfed BC_2 plants originating from the Asakaze × Manas crosses (Molnár-Láng et al. 2012).The barley cultivar Manas is well adapted to Central European conditions, having good winter hardiness, drought tolerance and yield ability. Manas also has good tolerance of abiotic stresses such as Al and high NaCl concentration (Darkó et al. 2010; Dulai et al. 2010), so it is a suitable candidate for transfering useful agronomic traits from barley into wheat. The addition lines were identified by FISH using repetitive DNA probes (HvT01, GAA, pTa71 and Afa family), followed by confirmation with barley SSR markers. Addition lines are starting material for incorporating small segments of barley chromosomes carrying genes responsible for agronomically useful traits into the wheat genome, i.e. for producing translocation lines.

11.2.1.2 Wheat/Barley Translocations

Very few wheat/barley recombinant chromosomes have been reported (Islam and Shepherd 1992), as homoeologous pairing between the chromosomes of these species is rare (Islam and Shepherd 1988; Molnár-Láng et al. 2000a). Koba et al. (1997) reported spontaneous wheat/barley translocations originating from a new wheat × barley hybrid combination. Various methods are available for producing translocations, including irradiation (Sears 1956) or genetic methods, but the most promising way is to use the 2C *Aegilops cylindrica* addition line to induce chromosome rearrangements between wheat and barley, as described by Schubert et al. (1998). This is a unique genetic system that induces frequent chromosomal structural rearrangements in common wheat by introducing gametocidal (Gc) alien chromosomes into common wheat from wild species belonging to the genus *Aegilops* (*Ae. cylindrica* and *Ae. triuncialis*) (Endo et al. 1984). The rearranged chromosomes thus induced deletions of barley chromosomes and translocations between the barley and wheat chromosomes. Lines carrying rearranged barley chromosomes are designated as 'dissection lines'. Deletion mapping on barley chromosomes 7H, 5H and 3H was performed using barley dissection lines and barley-specific EST markers (Endo 2009). The barley dissection lines were produced from CS/Betzes addition lines, so they all carry chromosome segments from Betzes barley.

Five wheat-barley translocations (2DS.2DL-1HS, 3HS.3BL, 6BS.6BL-4HL, 4D-5HS and 7DL.7DS-5HS) were identified and characterized in Martonvásár (Molnár-Láng et al. 2000b; Nagy et al. 2002) using sequential GISH and two-colour FISH with the probes pSc119.2 and pAs1, and later by three-colour FISH with the probes pSc119.2, Afa family and pTa71 (Fig. 11.2). The barley chromatin in these lines was identified using SSR markers. The wheat/barley translocation lines were used for the physical mapping of molecular markers on barley chromosome regions (Kruppa et al. 1975).

A spontaneous interspecific Robertsonian translocation was revealed by GISH in the progenies of a monosomic 7H addition line originating from the Asakaze × Manas hybrid. FISH performed with the repetitive DNA sequences Afa family, pSc119.2 and pTa71 allowed the identification of all the wheat chromosomes, including wheat chromosome arm 4BS involved in the translocation (Cseh et al. 2011). FISH using barley telomere- and centromere-specific repetitive DNA probes (HvT01 and AGGGAG) confirmed that one of the arms of barley chromosome 7H was involved in the translocation. SSR markers identified the translocated chromosome segment as 7HL. The presence of the *HvCslF6* gene, responsible for (1,3;1,4)-β-D-glucan production, was revealed in the centromeric region of 7HL. An increased (1,3;1,4)-β-D-glucan level was also detected in the translocation line, demonstrating that the *HvCslF6* gene is of potential relevance for the manipulation of wheat (1,3;1,4)-β-D-glucan levels (Cseh et al. 2011, 2013).

Addition lines are good starting material for the incorporation of small segments of barley chromosomes, carrying genes responsible for agronomically useful traits, into the wheat genome. It will be possible to produce new barley dissection lines containing chromosome segments from Igri and Manas, which may give new information for the mapping of DNA sequences related to various agronomic traits in

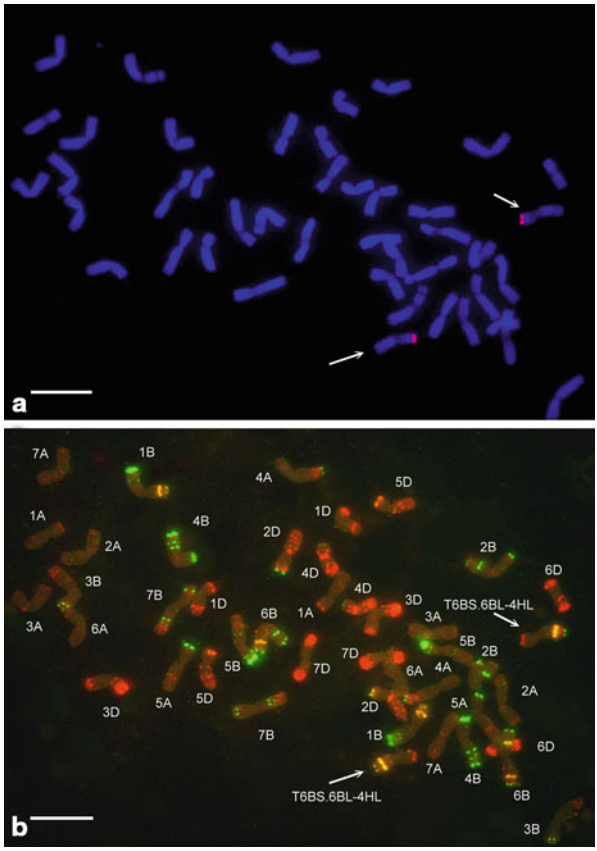

Fig. 11.2 Sequential GISH and FISH on mitotic chromosomes of 6BS.6BL-4HL wheat-barley disomic translocation line. **a** Detection of barley chromosome segments (*red*) in the translocation chromosome pairs using GISH. Total barley DNA was labelled with digoxigenin, and detected with anti-DIG-Rhodamine. The translocated chromosomes are indicated by arrows. The wheat chromosomes are blue as a result of counterstaining with DAPI. **b** Identification of the wheat chromosomes using FISH with DNA probes pSc119.2 (*green*), Afa family (*red*) and pTa71 (*yellow*). Disomic 6BS.6BL-4HL translocated chromosomes are indicated by arrows. Scale bar = 10 μm

barley. It will also be possible to introgress chromosome segments carrying genes for agronomically useful traits (nutritional parameters; Al, drought and salt tolerance) from the two-rowed and six-rowed winter barley cultivars Igri and Manas into wheat, and to determine the chromosomal location of these genes.

11.2.2 Wheat × Rye Hybrids

Secale is a small but important cereal genus that includes cultivated rye (*S. cereale* L.), weedy rye, and several wild species. As it is capable of producing higher yields than wheat under adverse conditions, rye has become a staple food grain at higher elevations and in regions with poor soils and severe winters. *Secale* spp. contain genes associated with resistance to many cereal diseases, winter hardiness, drought tolerance, sprouting, high lysine content and morphological and biochemical traits, which can be transferred to closely related cereal crops (Molski et al. 1985).

11.2.2.1 Wheat × Rye Crossability

The crossability of hexaploid wheat (*T. aestivum*) with rye is controlled by two loci, *Kr1* and *Kr2*, where the dominant alleles reduce crossability, *Kr1* having the more and *Kr2* the less potent effect. Plants which carry the *Kr1Kr1Kr2Kr2* dominant alleles give lower than 5 % seed set when pollinated with rye, but genotypes with the *kr1kr1kr2kr2* recessive homozygous genome composition may have over 50 % seed set with rye (Lein 1943). The *kr1* gene is located on the long arm of chromosome 5B, while *kr2* is located on the long arm of chromosome 5A (Riley and Chapman 1967; Lange and Riley 1973). Most European wheat varieties carry the dominant *Kr* alleles and thus have very low crossability with rye (Kiss and Rajháthy 1956; Zeven 1987). The recessive *kr* alleles are mostly present in wheat varieties from China, Japan, Siberia and other Asiatic regions, but these varieties are not suitable for production under Central European conditions. Snape et al. (1987) and Gay and Bernard (1994) transferred the recessive *kr1* allele into English and French varieties, respectively, by first incorporating the 5B chromosome from Chinese Spring or Fukuhokomugi into monosomic lines of these varieties. The major gene *Kr1* was identified on 5BL, and *SKr*, a strong QTL affecting crossability between wheat and rye, on chromosome 5BS (Tixier et al. 1998). Two SSR markers completely linked to *SKr* were used to evaluate a collection of crossable wheat progenies originating from primary triticale breeding programmes. The results confirm the major effect of *SKr* on crossability and the usefulness of the two markers for the efficient introgression of crossability into elite wheat varieties (Alfares et al. 2009).

In Hungary, the recessive crossability allele *kr1* was transferred from the spring wheat variety Chinese Spring (CS) into the winter wheat variety Martonvásári 9 (Mv9) by backcrossing Mv9 × CS hybrids with Mv9 (Molnár-Láng et al. 1996). As a result of five backcrosses with Mv9 and two selfings after each backcross, the selected progenies had over 50 % seed set with rye when tested on a large number of individual plants. These data confirmed that after the fifth backcross the selected Mv9 kr1 line carried the recessive crossability alleles *Kr1* and *Kr2*, but the genotype was 98.4 % Mv9. When the Mv9 kr1 line was pollinated with the old Hungarian rye cultivar Lovászpatonai (Molnár-Láng et al. 2002), the mean crossability percentage was fairly high, 68.4 %. The chromosome number distribution, examined in mitotic chromosome spreads of octoploid triticale obtained via colchicine treatment of the initial hybrid, was found to range from 51 to 56. All the rye chromosomes were identified with the help of C-banding and were detected using GISH (Nagy et al. 1998). The Mv9 kr1 line is now used as a maternal partner in wheat-alien hybridization experiments in Martonvásár (Molnár-Láng et al. 2002). This has the advantage that the alien genes can be transferred directly into a winter wheat line with good yielding ability and good quality, instead of into CS, which has many unfavourable features from the agronomic point of view.

11.2.2.2 Wheat-Rye Addition and Substitution Lines

Rye is most intensively used to extend the genetic variability of wheat via intergeneric hybridization and recombination (Lelley 1993). Wheat-rye addition and substitution

lines played an important role in determining the homoeologous relationship between the two genera and were extensively used to search for useful genes in rye for wheat breeding. The first wheat-rye addition lines were produced by O'Mara (1940) and since then complete series of disomic wheat-rye addition lines, including adequate disomic telocentric lines (see Shepherd and Islam 1988; Lukaszewski 1988) have been developed. The rye chromosomes in the addition lines were detected by GISH and identified using C-banding and in situ hybridization with the help of labelled repetitive DNA probes (Mukai et al. 1992).

The genetic stability of wheat/rye disomic addition lines was checked using the Feulgen method and FISH (Szakács and Molnár-Láng 2010b). Feulgen staining detected varying proportions of disomic, monosomic and telosomic plants among the progenies. The greatest stability was observed for the 7R addition line, while the most unstable lines were those with 2R and 4R additions. Chromosome rearrangements were also detected using FISH. Based on the specific hybridization patterns of repetitive DNA probes (pSc119.2 and $(AAC)_5$), and ribosomal DNA probes (5S and 45S), isochromosomes were identified in the progenies of the 1R and 4R addition lines. The results draw attention to the importance of using FISH for continuous cytological checks on basic genetic materials because this method reveals chromosome rearrangements not detected either with the conventional Feulgen staining technique or with molecular markers (Szakács and Molnár-Láng 2010b).

The first reports on the spontaneous wheat-rye chromosome substitution 5R(5A) were published by Kattermann (1937) and O'Mara (1947). Driscoll and Anderson (1967) reported the substitution of wheat chromosomes 3A, 3B, 3D and 1D by rye chromosome 3R. Since then many other wheat-rye substitutions have been produced and identified (Lukaszewski 1991; Schlegel 1997).

11.2.2.3 Wheat/Rye Translocations

The 1BL.1RS wheat/rye translocation is the most widespread alien translocation, detected in hundreds of wheat cultivars worldwide (Bedö et al. 1993; Rabinovich 1998, Lukaszewski 2000). Most varieties with a 1BL.1RS translocation contain the short arm of the 1R chromosome from Petkus rye (Zeller 1973; Schlegel and Korzun 1997). Unfortunately most of the resistance genes (*Lr26, Yr9, Pm* and, *Sr31*) located on this chromosome arm are no longer effective against new biotypes of the diseases. However, the translocation was also postulated to have a yield-enhancing effect and to improve adaptability (Rajaram et al. 1990; Villareal et al. 1998). As it is probably of single origin (Schlegel and Korzun 1997) this 1RS arm lacks any genetic variation, so new allelic variation needs to be introduced from other 1RS chromosomes in order to exploit the rich gene reservoir of diploid rye. Other rye genotypes may have new resistance genes or alleles against various diseases and may have a less deleterious effect on bread-making quality, probably the only negative consequence of the presence of the original Petkus rye chromosome arm in wheat.

Several authors have reported the production of wheat cultivars carrying 1RS chromosome arms from various rye genotypes. The 1RS.1AL translocation in wheat

cultivar Amigo carries the 1RS arm of Insave rye (Zeller and Fuchs 1983). Salmon, another 1BL.1RS wheat/rye translocation line, was derived from an F_3 seed from a hybrid between two octoploid triticale strains (Tsunewaki 1964). A 1DL.1RS translocation was derived from the rye cultivar Imperial (Shepherd 1973). Marais et al. (1994) used homologous recombination to transfer a gene from the short arm of chromosome 1R from Turkey 77 rye into the 1RS arm of the translocated chromosome in the wheat cultivar Veery. A new 1BL.1RS wheat/rye translocation line was developed by Ko et al. (2002) from the backcross of the F_1 hybrid of wheat cv. Olmil and rye cv. Paldanghomil. A fast, efficient method is urgently needed to introduce a substantial amount of allelic variation into this chromosome arm directly from diploid rye (Nagy et al. 2003; Lelley et al. 2004). Nagy et al. (2003) demonstrated that new genetic variation from the 1RS arm of rye can routinely be introduced into the 1RS of translocation wheats by crossing commercial cultivars, containing the1BL.1RS chromosome, with octoploid triticale lines.

Molnár-Láng et al. (2010) developed a wheat genotype containing both the recessive crossability alleles (*kr1kr1kr2kr2*), allowing high crossability between 6 × wheat and diploid rye, and the 1BL.1RS wheat/rye translocation chromosome. This wheat genotype was used as a recipient partner in wheat × rye crosses for the efficient introduction of new allelic variation into 1RS in translocation wheats. These wheat lines were selected after crossing the wheat cultivars Mv Magdaléna and Mv Béres, carrying the 1BL.1RS translocation, with the wheat line Mv9 kr1, which carries the *kr1kr1kr2kr2* alleles. The wheat × rye F_1 hybrids produced with new recipient wheat lines involving the rye cultivar Kriszta were analysed in meiosis using GISH. Chromosome pairing between the 1BL.1RS translocation and the 1R chromosome of the rye cultivar was detected in 62.4 % of the pollen mother cells of the wheat × rye hybrids. The use of FISH with repetitive DNA probes pSc119.2, Afa family and pTa71 allowed the 1R and 1BL.1RS chromosomes to be identified (Fig. 11.3). The presence of the 1RS arm from Kriszta as well as that of Petkus was demonstrated in the F_1 hybrids using the rye SSR markers RMS13 and SCM9. Based on GISH and SSR marker analysis it was concluded that recombination had occurred between the 1RS chromosome arms of Petkus and Kriszta in the translocated chromosome in four of the 22 plants analysed. New primary 1BL.1RS translocation lines were also created with three Chinese local rye varieties (Ren at al. 2011).

The first experimental wheat/rye translocation (4B-2R) was produced in 1967 (Driscoll and Anderson 1967), but the introgression of rye genetic information into wheat most famously occurred through a spontaneous 1RS.1BL wheat/rye translocation (Mettin et al. 1973; Zeller 1973). Another wheat/rye translocation with importance for breeding was found in the Danish variety Viking, which carries a 4B-5R interchange (Schlegel et al. 1993) causing high iron, copper and zinc efficiency compared to common wheat (Schlegel 2006). The old Portuguese wheat landrace, Barbela contains small, spontaneously occurring rye segments on the long arm of 2D. This landrace shows good productivity under the low fertility conditions often associated with acid soils (Ribeiro-Carvalho et al. 1997). A large number of wheat/rye translocations were detected among the progenies of triticale × wheat crosses (Lukaszewski and Gustafson 1983), involving all seven rye chromosomes.

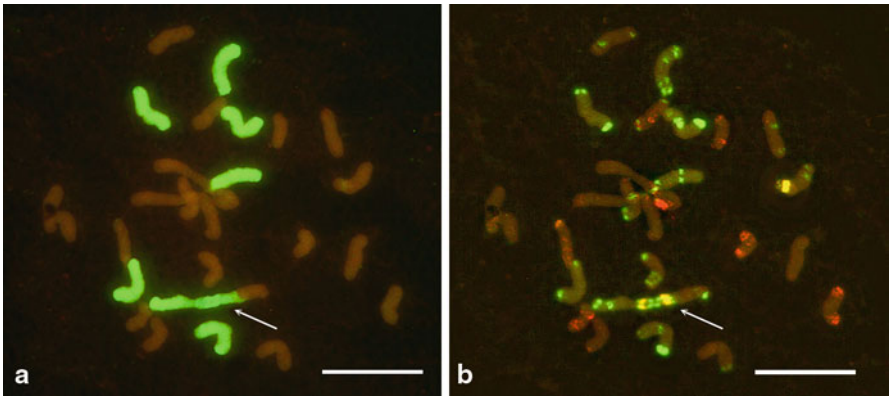

Fig. 11.3 Sequential GISH and FISH on meiotic chromosomes of a wheat × rye hybrid. **a** Genomic *in situ* hybridization (GISH) on meiotic metaphase I chromosomes of the wheat × rye F_1 hybrid produced between the Mv Béres kr1 wheat line having the 1BL.1RS translocation and the rye cv. Kriszta. Seven rye chromosomes and the 1RS arm in the 1BL.1RS translocated chromosome (*arrow*) are yellowish green. Twenty wheat chromosomes (18 univalents and 1 rod bivalent) and the 1BL arm of the 1BL.1RS chromosome are unlabelled. **b** 1BL.1RS-1R pairing was identified using FISH with repetitive DNA probes pSc119.2 (*green*), Afa family (*red*) and pTa71 (*yellow*) on the same cell. Scale bar = 10 μm

Agronomically useful wheat/rye translocations were produced by incorporating chromosome segments from the 2R (Mukade et al. 1970; Sears et al. 1992; Cainong et al. 2010), 3R (Rao 1978) and 6R (Friebe and Larter 1988; Friebe et al. 1991) rye chromosomes into the wheat genome (Friebe et al. 1996).

Triticale is the first man-made crop originating from wheat × rye hybridization (Kiss 1966; Lelley 1993). According to the FAO database it is grown on more than 4 million ha worldwide. At present, triticale is grown in Poland on 1.465 million ha, in Germany on more than 400,000 ha, in the Russian Federation on 187,000 ha and in Hungary on more than 125,000 ha.

11.2.3 Wheat × Aegilops Hybrids

11.2.3.1 *Aegilops* (goatgrass) Species

The genus *Aegilops* L. comprises 11 diploid, 10 tetraploid and 2 hexaploid species (Van Slageren 1994). Some of these species took part in the evolution of pasta and bread wheat, as *Ae. tauschii* Coss. (2n = 2 × = 14, DD) is the donor of the hexaploid wheat D genome and *Ae. speltoides* Tausch (2n = 2 × = 14, SS) exhibits the closest relationship to the B genome of wheat (Dvorak 1998). *Aegilops* species have great diversity, thus representing a large reservoir of useful traits for wheat improvement. Species belonging to this genus have been evaluated for their resistance to diseases and pests (Gill et al. 1983, 1985; Raupp et al. 1995). Many agronomically useful

traits, including disease and pest resistance, stress and salt tolerance and winter hardiness, have been transferred from these species to wheat and several of them are used in wheat improvement (Cox et al. 1994; Gill et al. 1987; Raupp et al. 1993, see Schneider et al. 2008).

The directed exploitation of this variability requires detailed knowledge of the genetic and cytogenetic structure of the *Aegilops* species. Karyotypic data including C-banding patterns and the chromosomal distribution of four repetitive DNA sequences have been reported for all the diploid *Aegilops* species (Badaeva et al. 1996a, b). This set the stage for the analysis of the genome differentiation of the polyploid *Aegilops* species, which were analysed by C-banding and FISH with repetitive DNA probes (Badaeva et al. 2002, 2004, 2011; Schneider-Linc et al. 2005; Molnár et al. 2011).

Aegilops cylindrica Host ($2n = 4 \times = 28$, $D^cD^cC^cC^c$) is an autogamous, allotetraploid wild relative of bread wheat, which is native to the Mediterranean, the Middle East and Asia, and was introduced both to the Great Plains and Pacific northwest of the United States and into Hungary (Kimber and Feldman 1987; van Slageren 1994). The genomic constitution of *Ae. cylindrica* was determined by analysing chromosome pairing (Sax and Sax 1924; Kihara 1931), storage proteins (Johnson 1967), isozymes (Jaaska 1981; Nakai 1981) and differences in the restriction length patterns of repeated nucleotide sequences (Dubcovsky and Dvorak 1994). These studies identified the diploid species *Ae. caudata* L. ($2n = 2 \times = 14$, CC) as the donor of the C genome and *Ae. tauschii* as the donor of the D genome of *Ae. cylindrica*. A detailed karyotypic analysis of *Ae. cylindrica* was performed by C-banding, GISH and FISH using the DNA clones pSc119, pAs1, pTa71, and pTa794. GISH analysis detected intergenomic translocation in three of the five *Ae. cylindrica* accessions (Linc et al. 1999).

Aegilops biuncialis Vis. [syn. *Aegilops lorentii* Hochst., *T. macrochaetum* (Shuttlev. & A. Huet ex. Duval-Jouve) K. Richt] ($2n = 4 \times = 28$, $U^bU^bM^bM^b$) is a tetraploid wild relative of wheat belonging to the section *Polyeides* of the genus *Aegilops*. *Ae. biuncialis* shares the U and M genomes with the polyploid species *Ae. geniculata* Roth. ($2n = 4 \times = 28$, $U^gU^gM^gM^g$), *Ae. columnaris* Zhuk. ($2n = 4 \times = 28$, $U^{co}U^{co}M^{co}M^{co}$) and *Ae. neglecta* REq. Ex Bertol. ($2n = 4 \times = 28$, $U^nU^nM^nM^n$). The U^b genome progenitor is the diploid *Ae. umbellulata* (syn. *Triticum umbellulatum*) Zhuk. ($2n = 2 \times = 14$, UU), while the modified M^b genome originated from *Ae. comosa* (syn. *Triticum comosum*) Sm. in Sibth. & Sm. ($2n = 2 \times = 14$, MM) (Kimber and Sears 1983; Badaeva et al. 2004). *Aegilops biuncialis* has good tolerance against biotic (Damania and Pecetti 1990; Makkouk et al. 1994) and abiotic stresses such as cold and salt stress (Colmer et al. 2006). Accessions originating from semi-desert habitats can also be used as gene sources to improve drought and heat tolerance of wheat (*T. aestivum* L.) (Molnár et al. 2004; Dulai et al. 2005). To facilitate the exact identification of the *Ae. biuncialis* chromosomes in the *T. aestivum* genetic background, FISH was carried out using repetitive DNA probes (pSc119.2, pAs1/Afa family, pTa71, $(GAA)_n$ and $(ACG)_n$) on *Ae. biuncialis, Ae. geniculata* and their two diploid progenitor species (Schneider-Linc et al. 2005; Molnár et al. 2005, 2011a, b). Differences in the hybridization patterns (Schneider-Linc et al. 2005; Molnár et al. 2011a, b) indicated that the M genome was more variable than the U

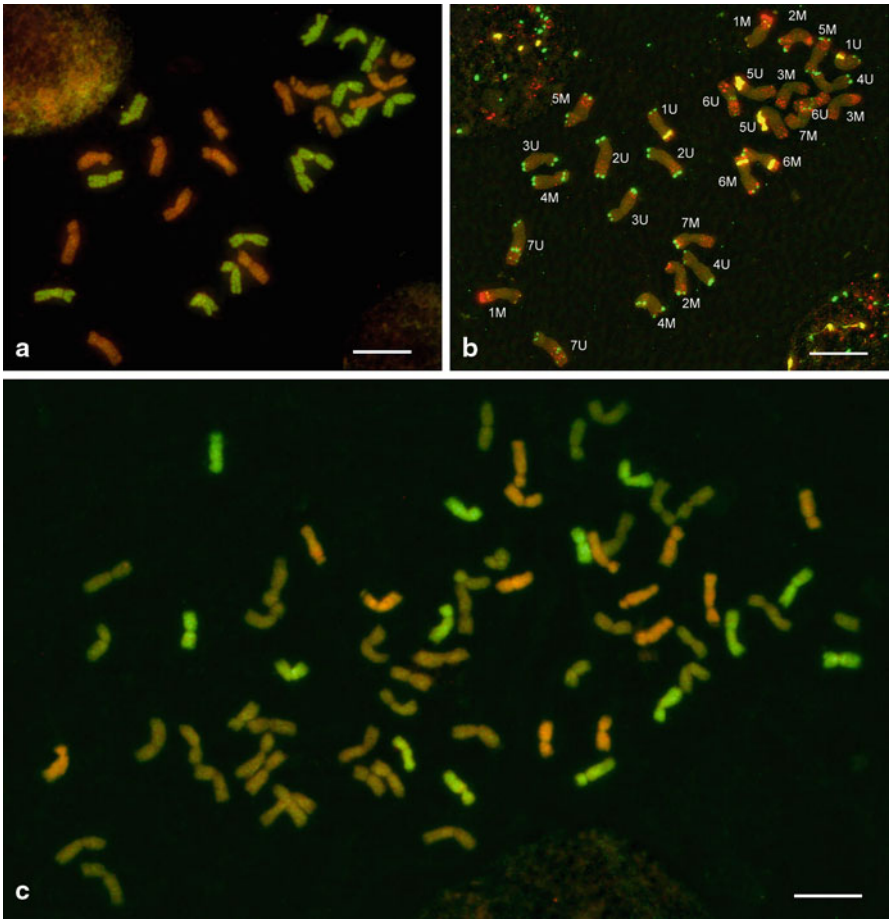

Fig. 11.4 **a, b** Two-colour genomic *in situ* hybridization (GISH) using U and M genomic probes, and FISH using Afa family, pSc119.2 and pTa71 repetitive DNA probes on mitotic chromosomes of *Aegilops biuncialis*. **a** On the GISH image the U genome is visualized in *orange* and the M genome in *green*. **b** On the FISH image pSc119.2 sites are *green*, Afa family signals are red and pTa71 signals are *yellow*. Scale bar = 10 μm. **c** Multicolor genomic in situ hybridization (mcGISH) on a partial wheat-*Ae. biuncialis* amphiploid cell having 39 wheat and 28 *Ae. biuncialis* chromosomes. The U^b genome is visualized in *orange*, the M^b genome in *green* and the wheat chromosomes in *brown*. Scale bar = 10 μm

genome which was confirmed by conserved orthologous set (COS) markers (Molnár et al. 2013). Intraspecific genetic variability was examined using two-colour GISH and FISH in 32 *Ae. biuncialis* (Fig. 11.4a, b) and 19 *Ae. geniculata* accessions. Homozygous intergenomic translocations were detected by GISH between the U and M genomes in six accessions (Molnár et al. 2011). Intergenomic translocation breakpoints were mapped to SSR-rich chromosomal regions (Molnár et al. 2011). The evolutionary changes in the karyotypes of the D, U and N genomes of diploid and polyploid *Aegilops* species have also been investigated by means of FISH and C-banding (Badaeva et al. 2002, 2004, 2011).

11.2.3.2 Production of Wheat × Aegilops Hybrids, Addition and Translocation Lines

Efforts to exploit *Aegilops* species for wheat improvement were begun more than a century ago. The results achieved to date in the field of wheat-*Aegilops* hybridization and gene transfer were reviewed by Schneider et al. (2008). Of the 23 *Aegilops* species, most of the diploids (*Ae. umbellulata* Zhuk., *Ae. mutica* Boiss., *Ae. bicornis* (Forssk.) Jaub. & Spach, *Ae. searsii* Feldman & Kislev ex Hammer, *Ae. caudata* L., *Ae. sharonensis* Eig, *Ae. speltoides* Tausch, *Ae. longissima* Schweinf. & Muschl.) and several polyploids (*Ae. ventricosa* Tausch, *Ae. peregrina* (Hack. In J. Fraser) Marie & Weiller, *Ae. geniculata* Roth, *Ae. kotschyi* Boiss., *Ae. biuncialis* L.) have been used to develop wheat-*Aegilops* addition lines while wheat-*Aegilops* substitution lines have been developed using several species, including *Ae. umbellulata, Ae. caudata, Ae. tauschii, Ae. speltoides, Ae. sharonensis, Ae. longissima* and *Ae. geniculata* (see Kilian et al. 2011). Translocations carrying genes responsible for useful agronomic traits were developed with *Ae. umbellulata, Ae. comosa, Ae. ventricosa, Ae. longissima, Ae. speltoides* and *Ae. geniculata* (see Schneider et al. 2008).

Ae. biuncialis was crossed as male parent with the winter wheat line Mv9 kr1, and F_1 hybrids were produced with great efficiency. Amphiploids were then developed and backcrossed with wheat by Logojan and Molnár-Láng (2000) who also investigated the meiotic pairing behaviour of the hybrids. The wheat-*Ae. biuncialis* amphiploids were able to maintain significantly higher water content, photosynthetic capacity and biomass production than wheat genotypes during drought stress (Molnár et al. 2008). Six different disomic addition lines, each with 22 bivalents in metaphase I of meiosis, were selected from the selfed backcross derivatives of the amphiploids (Molnár-Láng et al. 2002). Five of them were identified using FISH with repetitive DNA probes pSc119.2 and pAs1. No chromosome rearrangements between wheat and *Ae. biuncialis* were detected by GISH in these additions (Schneider-Linc et al. 2005), which can be used to study the genetic effects of individual alien chromosomes in wheat.

Since the first successful gene transfer from *Aegilops umbellulata* Zhuk. to wheat (Sears 1956), ionising irradiation (such as X- and Y-rays) has been widely applied to crop species for the production of interspecific translocations. A large number of genes were transferred from *Aegilops* species to cultivated wheat, including those for resistance to leaf rust resistance, stem rust, yellow rust and powdery mildew, and various pests (cereal cyst nematode, root knot nematode, Hessian fly and greenbug) (see Schneider et al. 2008), but there are still many untapped genetic resources in *Aegilops* species that could be used as resistance sources for plant breeding. C-banding and FISH permit the distinction of the wheat and *Aegilops* chromosomes involved in wheat-alien translocations, whereas their size and breakpoint positions can be determined by GISH analysis (see Jiang et al. 1994; Castilho et al. 1996; see Friebe et al. 1996). Biagetti et al. (1999) used two highly repetitve DNA sequences (pSc119.2 and pAs1) and one low copy 3BS-specific RFLP sequence to physically map *Ae. longissima* chromatin in wheat recombinant lines carrying *Pm13* derived from *Ae. longissima.* Using a combination of C-banding and ISH, it was possible to

identify chromosomes carrying *Aegilops*-derived chromosome segments (see Friebe et al. 1996; Nasuda et al. 1998).

Because *Aegilops* species are more closely related to wheat than rye, barley or *Agropyron* species, it is often difficult to discriminate *Aegilops*-derived chromosomes using GISH (Wang et al. 2000; Benavente et al. 2001; Molnár et al. 2005, 2009; Cifuentes et al. 2006). However, a GISH protocol combining the preannealing of the probe and blocking DNA and prehybridization with blocking DNA was successfully used both to differentiate the very closely related genomes of *Ae. uniaristata* and wheat and to distinguish the S genome of *Ae. searsii* and *Ae. longissima* from the B genome of wheat (Iqbal et al. 2000; Belyayev et al. 2001). Multicolor GISH (mcGISH) using differentially labelled total genomic DNA probes enables the parental genomes to be discriminated in allopolyploid plants (Mukai et al. 1993; Belyayev et al. 2001) can also detect intergenomic chromosome rearrangements. The simultaneous visualization of individual wheat genomes and alien chromatin in interspecific hybrids and derivatives has also been reported (Sánchez-Morán et al. 1999; Han et al. 2003). Benavente et al. (2001) individually distinguished the U^o and M^o genomes of *Aegilops ovata* L. in durum wheat-*Ae. ovata* amphiploids using the total genomic DNA of *Ae. umbellulata* and *Ae. comosa* Sm. in Sibth. & Sm. as U and M genomic probes. The simultaneous discrimination of the two constituent genomes of *Ae. biuncialis* and the wheat chromosomes by mcGISH was reported by Molnár et al. (2009). This procedure also allowed for the parallel discrimination of the U^b and M^b genomes of *Ae. biuncialis* from bread wheat chromosomes (11.4). The γ-irradiation of the wheat-*Ae. biuncialis* amphiploids yielded a large number of intergenomic translocations involving the whole of the *Aegilops* and wheat genomes (Molnár et al. 2009). Dicentric chromosomes, fragments and terminal translocations were most frequently induced by γ-irradiation. Chromosome banding and ISH techniques may fail to identify translocated chromosome segments if there are no diagnostic bands or hybridization sites. In such cases chromosome-specific molecular markers may facilitate the characterization of the *Aegilops* segment.

11.2.4 Wheat × Thinopyrum (syn. Agropyron) Hybrids

11.2.4.1 *Agropyron* Species

Wheatgrass and wildrye grasses are some of the most important grasses in the temperate regions of the world (Wang 2011). These species are important as tertiary gene pools for wheat improvement and also serve as forage crops. Many of these grasses are related to and have been hybridized with cultivated cereal crops including wheat, barley and rye as genetic sources for disease resistance, salinity tolerance and other traits.

The taxonomy of the wheatgrass and wildrye grasses has been object of considerable controversy. The wheatgrasses traditionally have been included in the genus *Agropyron* and wildrye have been largely treated as species in the genus *Elymus*

(Wang 2011). Although it is now agreed by taxonomists that *Agropyron* should be restricted to *A. cristatum* and its close relatives, in the present review, *Agropyron* is used to include species in the genera *Australopyrum* (Tzelev) A Löve, *Dasypyrum* (Coss. & Durieu) T. Durand, *Elymus* Linnaeus, *Leymus* Hochstetter, *Pascopyrum* A. Löve, *Pseudoroegneria* (Nevski) A. Löve, and *Thinopyrum* A Löve, etc. according to Wang (2011). All species in the genera *Agropyron*, *Pseudroegneria*, *Psathyrostachys*, *Thinopyrum*, *Elymus* and *Leymus* are theoretically capable of being hybridized with wheat.

Species belonging to the present *Thinopyrum* genus (formerly *Agropyron*) are known to possess genes conferring resistance to various diseases, such as leaf and stem rusts, barley yellow dwarf virus, *Fusarium* head blight, etc., making these species suitable for improving the distance resistance of wheat (Friebe et al. 1994; Zhong et al. 1994; Fedak and Han 2005; Li and Wang 2009). *Thinopyrum* genus consists of three species complexes: *Th. junceum* (L.) A. Löve, *Th. elongatum* (Host) D.R. Dewey, and *Th. intermedium*. Species in this genus possess the J-, E-genome and sometimes contains the St-genome. This genus consists of diploids, segmental allotetraploids, segmental allohexaploids, octoploids and decaploids (Wang 2011).

11.2.4.2 Exploitation of *Thinopyrum* Species for Wheat Improvement

The prospect of taking advantage of the desirable gene content of these species has urged researchers worldwide to exploit the potential of the tertiary gene pool. Up till now several resistance genes have been transferred from perennial *Triticeae*, most of them originating from species in the *Thinopyrum* genus. One of the most important alien resistance genes for biotic stress transferred from *Agropyron elongatum* (syn. *Th. elongatum*) to wheat is *Lr19* (Sharma and Knott 1966). Wide hybridization of tall wheatgrass species with wheat appears promising as an avenue to improve salt tolerance (Colmer et al. 2006; Mullan et al. 2009). Other genes conferring resistance to leaf and stem rust, scab and head blight, curl mite, powdery mildew, wheat streak mosaic virus and barley yellow dwarf virus have been introgressed successfully into wheat through chromosome engineering (see Wang 2011). The two most valuable sources are *Th. intermedium* and *Th. ponticum,* firstly due to the fact that these species have resistance to rusts, common root rot, wheat scab, wheat streak mosaic virus, green bug and curl mite, and tolerance of abiotic stress (drought, high temperature, salinity) (Liu et al. 2007) and secondly because they contain the two basic genomes E and St, which are closely related to the A and D genomes of hexaploid wheat. Their polyploid nature suggests multiple origins, with progenitors from different geographical areas. They thus possess great genetic variability and molecular polymorphism which could be exploited by researchers and breeders (García et al. 2002). An intensive hybridization programme involving annual wheat and species from the former *Agropyron* genus was successfully initiated in the early 1930s by Tsitsin (Tsitsin 1960). Several *Thinopyrum*-wheat amphiploids were obtained worldwide and used for producing addition, substitution or translocation lines such as Agrotana, OK7211542, PWM706, PWMIII and PWM 209. The

GISH and multicolor GISH (mcGISH) methodologies were used to establish the cytogenetic constitution of various partial amphiploids (Chen et al. 1995, 1999; Han et al. 2004; Sepsi et al. 2008; Georgieva et al. 2011). A set of disomic addition lines was produced in each of which a chromosome for *Agropyron elongatum* (syn. *Th. elongatum*, 2n = 14) was added to the chromosome complement of *T. aestivum* (Dvorak and Knott 1974). These were later proved to carry many agronomically useful traits (resistance to wheat streak mosaic virus, barley yellow dwarf virus, common root rot, *Fusarium* head blight, tan spot and *Stagonospora nodorum*) originating from the *Th. ponticum* progenitor and have been exploited as alien sources of disease resistance in wheat improvement (Chen et al. 1998; Thomas et al. 1998; Li et al. 2003; Fedak and Han 2005; Oliver et al. 2006).

McGISH and FISH were used to characterize the genomic composition of the wheat-*Th. ponticum* partial amphiploid BE-1. The amphiploid is a high-protein line having resistance to leaf rust and powdery mildew and has a total of 56 chromosomes per cell (Szalay 1979). Multicolor GISH identified 16 chromosomes originating from *Th. ponticum* and 14 A-genome, 14 B-genome and 12 D-genome chromosomes from wheat. Six of the *Th. ponticum* chromosomes carried segments differing from the J genome in their centromeric regions. Using the Afa family, pSc119.2 and pTa71 probes, FISH identified all the wheat chromosomes present and determined which chromosomes were involved in the translocations. On the basis of their multicolour FISH patterns, the alien chromosomes could be arranged in eight pairs and could also be unequivocally differentiated from each other (Sepsi et al. 2008). *In situ* hybridization techniques, combined with SSR marker analysis, are extremely useful in detecting and identifying intergenomic rearrangements in the wheat genome, leading to the selection of genetic materials that could be useful for future mapping studies (Somers et al. 2004; Sepsi et al. 2009).

Using the FISH technique with various repetitive DNA probes, the genes controlling agronomically important traits can be assigned to precise chromosomal regions, thus facilitating effective gene transfer. Hsiao et al. (1986) studied the karyotypes of 22 diploid species of perennial *Triticeae* representing the P, St, J (E), H, I, Ns, W and R genomes. The C-banding patterns established for 10 diploid species (Endo and Gill 1984) drew the attention to the equivalence of the J and E genomes. The two genomes are indistinguishable on the basis of the chloroplast sequence data, whereas the chromosome pairing pattern in meiosis, karyotype differences and data on the 5S DNA spacer and ITS sequences provide clear evidence that they represent different genera (Jauhar 1990; Kellogg et al. 1996). The rapid, accurate identification of these materials can only be achieved by generating detailed karyotypes of the individual genomes based on the use of molecular cytogenetic probes. A detailed FISH karyotype of the E genome of *Elytrigia elongata* (Host) Nevski (= *Agropyron elongatum*, *Thinopyrum elongatum*, 2n = 2 × = 14, EE) was generated and verified in several accessions using highly repetitive DNA sequences and the sequential GISH – mcFISH technique (Linc et al. 2012).

11.3 Conclusions

Alien chromatin originating from species in the tertiary gene pool can be detected in the wheat genome using GISH, as their genomes are not homologous with wheat. A GISH protocol for the detection of barley and rye chromosomes in a wheat background was elaborated more than twenty years ago and routinely applied in many laboratories. Differentiating the chromosomes of wheat and *Aegilops,* species previously classified in the same genus using GISH is a considerable challenge. The identification of wheat, barley, rye and *Aegilops* chromosomes was first achieved by C- and N-banding and later by in situ hybridization using various DNA probes (pSc119, pAs1, pTa71, pT794 HvT01, GAA, pSc250, pSc200, etc.). Sequential GISH and FISH, a combination of GISH and chromosome identification with the use of repetitive DNA probes is a very efficient method for detecting and identifying alien chromatin in the wheat genome.

Barley, rye, *Aegilops* and *Thinopyrum* (syn. *Agropyron*) species are important gene sources for wheat improvement, but have only been partially exploited. Very few reports have been published on gene transfer from barley, and most of these projects involved a single barley cultivar, Betzes. Rye is the related species exploited most frequently for wheat improvement. Several genes have been transferred from the *Aegilops* and *Agropyron* species into wheat, but there is still a vast reservoir of species, both in gene banks and in natural habitats, which could be tapped in future to enhance genetic diversity of wheat.

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