Chapter 12
Wheat–Barley Hybrids and Introgression Lines

Márta Molnár-Láng and Gabriella Linc

12.1 Wheat (*T. aestivum*) × Barley (*H. vulgare*) Hybridization

Wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) are two of the major crops cultivated in the temperate zones. Besides its agronomic importance, barley has been an important material for genetic and genomic studies on the *Triticeae*, a tribe of the grass family. The advantages of barley as an experimental material reside basically in its diploid nature. Wheat/barley intergeneric hybridization could make it possible to incorporate the major agronomical traits of barley (earliness, β-glucan content, favourable amino acid composition, salt and drought tolerance, good tillering ability, etc.) into the bread wheat genome (Molnár-Láng et al. 2014). Although experiments had already begun in the early twentieth century, the first demonstrably successful cross between the two species was reported by the Danish scientist Kruse in 1973. Encouraged by this success, attempts were made worldwide, and at first hybrids were produced with relatively greater frequency when barley was used as the female parent (Islam and Shepherd 1990). Barley × wheat hybrids were developed in numerous combinations by several scientists: Islam et al. (1975), Fedak (1977), Thomas et al. (1977), Mujeeb-Kazi (1981), Clauss (1980), Shumny et al. (1981), Wojciechowska (1985) and Molnár-Láng et al. (1985). In crosses between a total of 18 barley varieties and 15 wheat varieties, the highest seed set was achieved when the wheat variety Chinese Spring (CS) was hybridized with the barley varieties Betzes and Ketch. A seed set of 15.4 % was reported by Islam et al. (1975), while Fedak (1980) achieved 49 % seed set, though only 2 % developed into plants. Fedak and Jui (1982) used the CS-Hope substitution line series to determine the chromosomal location of genes in CS that permit

M. Molnár-Láng (✉) • G. Linc
Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Brunszvik 2, Martonvásár 2462, Hungary
e-mail: molnar.marta@agrar.mta.hu

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crossability with Betzes. No progeny were obtained from substitution lines 5A, 5B, 5D, indicating that the homoeologous group 5 chromosomes of CS are the chromosomes chiefly responsible for permitting crossability with Betzes. All the hybrid plants obtained were raised in embryo culture, since hybrid seeds have no endosperm (Islam and Shepherd 1990). The barley × wheat hybrids exhibited complete male sterility, but when backcrossed with wheat it proved possible to produce BC_1 and BC_2 plants. The seed set in the first backcross was extremely low (0.5–1.2 seeds/spike) (Islam and Shepherd 1990). Due to the pistilloidy observed in the BC_1 and BC_2 plants the progeny remained sterile despite several backcrosses, making it impossible to develop fertile addition lines. In order to eliminate pistilloidy, attempts were made to make reciprocal crosses, using wheat as the female parent. Far fewer laboratories were able to report successful crosses and these involved a much smaller number of combinations (Islam and Shepherd 1990; Fedak 1980; Wojciechowska and Pudelska 1993; Molnár-Láng and Sutka 1994; Molnár-Láng et al. 2000b; Jauhar 1995; Taketa et al. 1998). Altogether about 100 combinations between 29 wheat cultivars and 55 barley accessions were tested, of which about 45 combinations produced 1 % of hybrid embryos. It was found by Islam et al. (1978, 1981) that crosses between CS and Betzes gave the highest seed set, but this was only 1.3 %. In experiments carried out under controlled conditions in the Martonvásár phytotron, 3.3 % seed set was achieved with the same combination (Molnár-Láng and Sutka 1994). A relatively short time after the development of the first wheat × barley hybrids, addition lines (2H, 3H, 4H, 5H, 6H, 7H) were also produced for the first time between CS wheat and the spring barley Betzes (Islam et al. 1978, 1981) (Table 12.1). By crossing this addition series with the relevant monosomic lines, substitution lines were developed for all the chromosomes except 1H and 5H (Islam and Shepherd 1992b, 1995; Ya-Ping et al. 2003). Despite many attempts, it proved extremely difficult to expand the number of genotypes that could be successfully crossed, and very few hybrids were developed from genotypes with satisfactory agronomic traits (Wojciechowska and Pudelska 1993; Jauhar 1995; Taketa et al. 1998). It proved impossible to develop BC_1 seed on a substantial proportion of the new hybrids, so no fertile progeny could be obtained from the new combinations (Wojciechowska and Pudelska 1993; Jauhar 1995). The efficiency of hybrid development was greatly improved by Koba et al. (1991), who used the 2,4-D treatment that had been successfully applied in wheat × maize crosses and also proved that F_1 hybrids could be produced from most embryos through embryo culture. A number of Japanese wheat varieties were included in the crosses, among which Norin 12, Norin 61 and Shinchunaga gave better seed set than CS × Betzes crosses. The highest seed set (8.25 %) was obtained from the Norin 12 × Betzes combination. Addition lines containing the barley chromosomes 5H and 6H were developed from a cross between Shinchunaga and Nyugoruden. Translocation lines were produced containing the 5HS.5BL translocation chromosome pair in addition to 42 wheat chromosomes (Koba et al. 1997). However, it has recently been reported that, despite resulting in several new combinations, the application of 2,4-D treatment also led to a significantly higher frequency of maternal haploids (Polgári et al. 2014). Backcross progenies (BC_1 and BC_2) were developed from the Shinchunaga × barley line

Table 12.1 Wheat (*Triticum aestivum*)—barley (*Hordeum*) disomic addition lines

Triticum aestivum genotype	*Hordeum* genotype	Added barley chromosome	References
Chinese Spring	*Hordeum vulgare* cv. Betzes (2-row, spring, German)	2H, 3H, 4H, 5H, 6H, 7H, 1H/1HS + 6H 1HS, 2HS, 2HL, 3HS, 3HL, 4HS, 4HL,5HS, 5HL, 6HS, 6HL, 7HS, 7HL	Islam et al. (1978, 1981) Islam and Shepherd (2000) Islam (1983), Islam and Shepherd (1990)
Shinchunaga (Japanese, spring)	*H. vulgare* cv. Nyugoruden (New Golden) (2-row, spring, Japanese)	6H, 7H	Koba et al (1997)
Martonvásári 9 kr_1 (Mv9kr1) (Hungarian, winter)	*H. vulgare* cv Igri (2-row, winter, German)	2H, 3H, 4H, 7H, 1HS, 6HS,	Molnár-Láng et al. (2007) Szakács and Molnár-Láng (2007; 2010)
Asakaze komugi (Japanese, facultative)	*H. vulgare* cv. Manas (6-row, winter, Ukrainian)	2H, 3H, 4H, 6H, 7H 2HS, 2HL, 3HS, 3HL, 5HS, 5HL, 6HS, 6HL, 7HS, 7HL	Molnár-Láng et al. (2012) Türkösi et al. (2014b)
Chinese Spring	*Hordeum chilense*	$1H^{ch}$, $4H^{ch}$, $5H^{ch}$, $7H^{ch}$	Miller et al. (1981)
Shinchunaga (Japanese, spring)	*Hordeum vulgare* ssp. *spontaneum* OUH602	2H, 3H, 4H, 5H, 6H, 7H, 1HS, 5HS, 6HS, 6HL,	Taketa and Takeda (2001)
Chinese Spring	*Hordeum marinum*	1 H^m, $2H^m$, $4H^m$, $5H^m$, $6H^m$, $7H^m$	Islam and Colmer (2008)

T3-7aai combination by Malysheva et al. (2003). The genome composition of the backcross progenies was analysed using genomic in situ hybridization (GISH) and microsatellite markers. Some of the barley chromosomes (2H, 4H) were entirely eliminated from the BC_2 plants, and the presence of 1H caused sterility, while chromosome segments from other barley chromosomes (3H, 5H, 6H, 7H) were detected in some BC_2 plants. The development of disomic addition lines from this combination was not reported.

Barley has great genetic diversity for many agronomically important traits (spring or winter habit, two-rowed or six-rowed, tolerance to abiotic stresses, yield ability, earliness, quality, adaptation, etc.). In order to utilize these traits, it would be worth producing wheat/barley addition and introgression lines with agronomically adaptable winter barley cultivars. Two new additions were reported from the wheat × barley hybrids produced using winter barley cultivars in Martonvásár (Molnár-Láng et al. 2000b) (Fig 12.1). Backcross progenies were developed on the hybrids at very low frequency (Molnár-Láng et al. 2000b, 2005). Wheat–barley disomic addition lines (2H, 3H, 4H, 6HS, 7H, 1HS isochromosome) were pro-

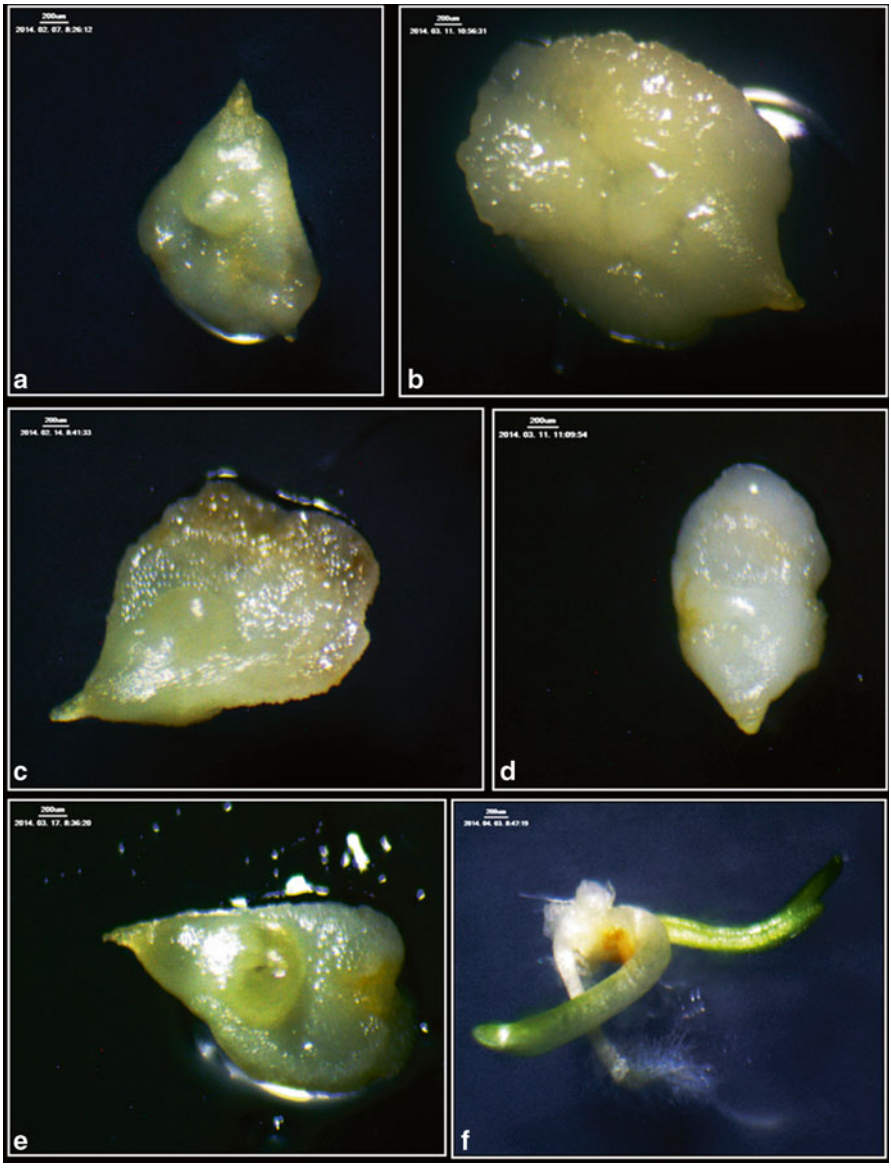

Fig. 12.1 (**a**–**e**) Wheat × barley hybrid embryos excised 3 weeks after pollination from developing seeds with no endosperm (each bar = 200 μm). (**f**) Developing plantlet, maintained in vitro

duced from hybrids between winter wheat line Mv9kr1 and the German two-rowed winter barley Igri (Molnár-Láng et al. 2007; Szakács and Molnár-Láng 2007; Szakács and Molnár-Láng 2010) and were identified using molecular cytogenetic methods. In order to increase the allelic variation in wheat/barley introgressions, new wheat/barley disomic addition lines were developed containing the 2H, 3H,

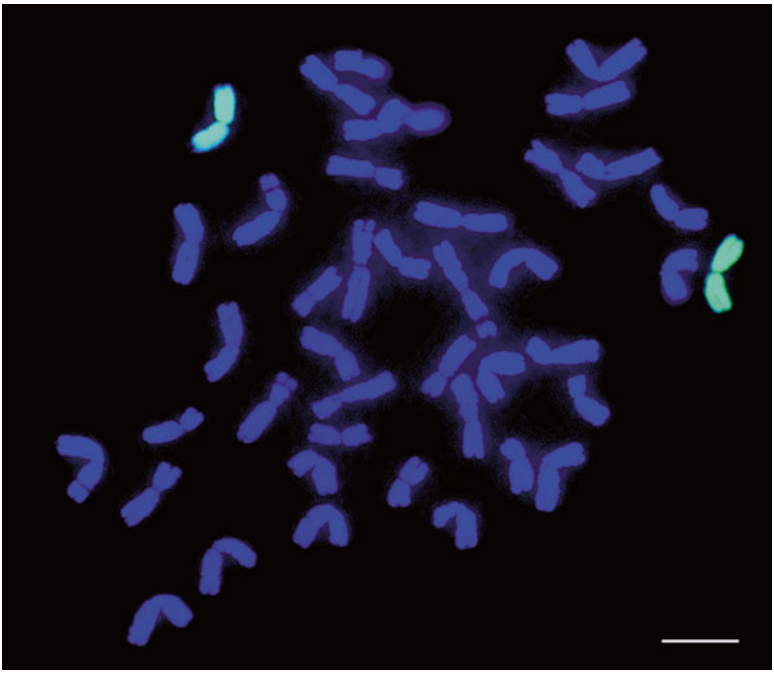

Fig. 12.2 Mitotic chromosomes of the 2H disomic addition line after GISH. Total genomic *H. vulgare* DNA was labelled with biotin-11-dUTP and detected with streptavidin-FITC (*green*), while wheat chromosomes were counterstained with DAPI (*blue*)

4H, 6H and 7H chromosomes of the six-rowed Ukrainian winter barley cultivar Manas (Molnár-Láng et al. 2012) (Figs. 12.2 and 12.3). This cultivar is agronomically much better adapted to Central European environmental conditions than the two-rowed spring barley cultivar Betzes previously used.

12.2 Production of Wheat × Barley Hybrids Using Alternative *Hordeum* Species

In addition to hexaploid wheat (*T. aestivum* L.) and diploid barley (*Hordeum vulgare* L.), hybrids have also been developed between other *Triticum*and *Hordeum* species. The most successful of these is hexaploid tritordeum, which arose from a cross between *Triticum turgidum* L. ssp. durum (Desf.) Husn. (synonym: *T. durum*) and *Hordeum chilense* Roem. et Schulz. (Martín and Sanchez-Monge Laguna 1980, 1982) (Table 12.2). *Hordeum chilense* was first pollinated with hexaploid *T. aestivum* to produce an F_1 hybrid (Martín and Chapman 1977), and a partially fertile amphidiploid was produced by means of colchicine treatment (Chapman and Miller 1978). The amphidiploid was then backcrossed to wheat to develop wheat/*H. chilense*

Fig. 12.3 Spike morphology of the Asakaze/Manas disomic addition lines compared with that of the parental Asakaze (wheat) and Manas (barley) genotypes

Table 12.2 Wheat (*Triticum*) – barley (*Hordeum*) amphidiploids

Wheat genotype	Hordeum species	Amphidiploid	References
Triticum timopheevii	*Hordeum bogdanii*	$2n=42$	Kimber and Sallee (1976)
Triticum aestivum	*Hordeum chilense* (maternal plant)	$2n=56$ designated as octoploid *Tritordeum*	Martín and Chapman (1977) Martin et al. (1987)
Triticumturgidum	*H. chilense* (maternal plant)	$2n=42$ designated as hexaploid *Tritordeum*	Martín and Sanchez-Monge Laguna (1982) Martín and Cubero (1981)
T.aestivum	*H. californicum* (maternal plant)	$2n=56$	Gupta and Fedak (1985)
T.aestivum	*Hordeum marinum* (maternal plant)	$2n=56$	Islam and Colmer (2008)
T. aestivum	*Hordeum vulgare*	–	Not reported

addition lines (Miller et al. 1981). Later, *H. chilense* was pollinated with *T. durum*, after which the hybrid was treated with colchicine to develop fertile amphidiploids with 42 chromosomes (Martín and Sanchez-Monge Laguna 1980, 1982). As this new amphidiploid exhibited fewer meiotic chromosome pairing anomalies and fertility problems than the primary triticale, it was assumed that, like triticale, it could be cultivated as a new plant species, and was named tritordeum. Hexaploid

tritordeum was found to have a protein content of 19–24 % (Martín and Cubero 1981), so numerous analyses were made to provide a detailed description of the quality parameters. After 6–7 years of self-fertilization in field experiments, it was established that the new species yielded only 20–40 % as much as cultivated wheat, but had a protein content amounting to 17.6–25.2 % of the dry matter (Cubero et al. 1986). Its other quality parameters (fibre, lignin, cellulose and hemicellulose contents, and amino acid composition) were similar to those of cultivated wheat. A multi-location study under diverse growth conditions revealed important information about the effect of water availability on the yield of tritordeum. In the lowest yielding environments tritordeum and triticale had similar yields (Villegas et al. 2010). However, under better growth conditions tritordeum was found to yield less than wheat and triticale. It is suggested that tritordeum could be a new option for cultivation in very dry environments. In the course of cytological analyses, the chromosome number and chromosome pairing of the new species were first monitored using the Feulgen method, which revealed a high level of chromosome stability (Martín and Cubero 1981). Later the *H. chilense* chromosomes were studied by means of C-banding (Fernandez et al. 1985) and fluorescence in situ hybridization (FISH) using repetitive DNA sequences (Cabrera et al. 1995). Hybridization with the pAs1 DNA clone (isolated from *Aegilopstauschii* Coss.) gave a hybridization pattern similar to that of the D genome chromosomes of wheat for the *H. chilense* chromosomes, with strong hybridization signals on the telomeres. This DNA probe gives diffuse signals on the *H. vulgare* chromosomes and cannot be identified. The hybridization pattern obtained with C-banding bore more resemblance to that of the wheat chromosomes and strong telomeric bands were observed on the *H. chilense* chromosomes, while in the case of *H. vulgare* chromosomes, C-banding revealed interstitial bands near the centromere (Cabrera et al. 1995). Molecular cytogenetic analysis showed that *H. chilense* was genetically distant from cultivated barley. Numerous papers have been published on the taxonomical classification of *Hordeum* species (Löve 1982, 1984; Dewey 1984), which were first classified on the basis of morphological observations, then on the basis of chromosome pairing in interspecific hybrids, and later in terms of the conclusions drawn from molecular genetic analysis. Bothmer et al. (1986, 1987) used the data of chromosome pairing analysis to divide the *Hordeum* species into four basic genomes (I, Y, X and H). Molecular genetic analysis later confirmed this classification (Svitashev et al. 1994), showing that *H. vulgare* and *H. bulbosum* contained genome I and *H. murinum* genome Y, while *H. chilense* was one of the species carrying the H genome, and *H. marinum* Huds. had an X genome. This classification confirmed the relatively distant relationship between *H. vulgare* and *H. chilense.*

In an effort to improve the agronomic traits of tritordeum, further crosses were made, primarily with triticale. The progeny were then analysed using various cytogenetic methods (Fernandez-Escobar and Martín 1985; Lima-Brito et al. 1996). The chromosomes of both *H. chilense* and rye could be identified by means of FISH (Lima-Brito et al. 1996). The hexaploid tritordeum was also crossed with *H. vulgare*, but the amphidiploid developed by treating the F_1 hybrid with colchicine proved to be sterile (Martín et al. 1995). Transgenic lines were developed by transforming tritordeum

(Barcelo et al. 1994) and these were later studied in nursery experiments (Hernandez et al. 2001). A new cytoplasmic male sterility (CMS) source designated msH1 was reported in bread wheat by Martín et al. (2009). This system uses the cytoplasm of *H. chilense*. The male sterility of alloplasmic wheat containing *H. chilense* cytoplasm is stable under various environmental conditions, and the plants exhibit no developmental or floral abnormalities, except for slightly reduced height and some delay in heading. There is thus real potential for the development of a viable technology for hybrid wheat production. The addition of chromosome $6H^{ch}S$ from *H. chilense* accession H1 was able to restore the pollen fertility of the CMS phenotype induced by the presence of *H. chilense* cytoplasm in wheat. An optimal combination for fertility restoration was the translocation $T6H^{ch}.6DL$, developed by Martín et al. (2009). In addition to *H. chilense*, the following *Hordeum* species have been hybridized with wheat:

- *H. spontaneum* [syn.: *H. vulgare* ssp. *spontaneum* (C. Koch) Thell] (Islam and Shepherd 1990; Taketa et al. 1995).
- *H. bulbosum* L. (Barclay 1975; Blanco et al. 1986).
- *H. bogdanii* Wil. (Kimber and Sallee 1976).
- *H. pussillum* Nutt. (Finch and Bennett 1980).
- *H. geniculatum* All. (Clauss 1983; Pershina et al. 1988).
- *H. pubiflorum* Hook. f. (Fedak 1983).
- *H. californicum* Covas & Stebbins [syn.: *H. brachyantherum* Nevski ssp. *californicum* (Covas & Stebbins)] (Gupta and Fedak 1985).
- *H. marinum* Huds. (Jiang and Dajun 1987; Islam et al. 2007; Pershina et al. 2009).
- *H. depressum* (Scribn. & Smith) Rydb. (Jiang and Dajun 1987).

A complete set of wheat–wild barley (*Hordeum vulgare* ssp. *spontaneum)* chromosome addition lines was developed by Taketa and Takeda (2001). The chromosome constitution of the addition lines was confirmed by C-banding and GISH hybridization. Addition lines for the entire 1H chromosome and its long arm are only available as monosomic and monotelosomic additions, respectively, because of sterility. Disomic additions involving individual chromosomes of sea barleygrass (*Hordeum marinum* Huds.) in CS were obtained by Islam and Colmer (2008). The salt tolerance of the wheat–*H. marinum* amphiploid was intermediate to that of its parents (Islam et al. 2007). Alloplasmic wheat–barley substitution and addition lines were produced by Pershina et al. (2009) from *H. marinum* ssp. *gussoneanum* Huds. × *T. aestivum* hybrids.

12.3 Maintenance of Wheat × Barley Hybrids in Tissue Culture

Sterile interspecific and intergeneric hybrids can be maintained and multiplied in a vegetative manner through callus formation in tissue culture (in vitro) (Fedak 1985). Interspecific and intergeneric hybrids produced in wide crosses are often

not only male sterile, but also have such a low level of female fertility that seeds are only set at extremely low frequency even when pollinated with one of the parents. Tissue culture makes it possible to multiply hybrids and produce enough progeny for backcrossing. The in vitro multiplication of interspecific and intergeneric hybrids developed between cereal species has been reported for various combinations: barley × rye (Shumny and Pershina 1979); wheat × rye (Armstrong et al. 1983; Doré et al. 1988); *Aegilops crassa* (*Triticum crassum*) × *Hordeum vulgare* (Nakamura et al. 1981); wheat × *Agropyron* hybrids (Sharma et al. 1984; Bai and Knott 1993); *Elymuscanadensis* L. × *Psathyrostachys juncea* (Fisch.) Nevski (Park et al. 1990). When the progeny were subjected to cytological analysis, deviations were observed in all cases compared with the initial hybrids. It was established that the somaclonal variability (SV) observed during the in vitro multiplication of plants (Larkin and Scowcroft 1981) could lead to useful rearrangements during the maintenance of interspecificand intergeneric hybrids in tissue culture (Fedak 1985). Amphidiploids with a doubled chromosome number have been successfully produced from F_1 hybrids (Doré et al. 1988; Ter Kuile et al. 1988), translocations have been observed (Sharma et al. 1984), and in some cases the regenerants have been found to have increased fertility (Sharma et al. 1984; Fedak and Grainger 1986; Molnár-Láng et al. 1991). Wheat–barley hybrids were multiplied in tissue culture by Pershina and Shumny (1981), Chu et al. (1984), Junming et al. (1985), Galiba et al. (1986), Surikov and Kissel (1988) and Koba et al. (1988). Detailed cytological analyses on the regenerants were published by Junming et al. (1985), Fedak and Grainger (1986), Shimada et al. (1987), Fedak et al. (1987) and Molnár-Láng et al. (1991). Chromosome numbers differing from that of the initial hybrid (28) were observed by Junming et al. (1985) and Koba et al. (1988) in some regenerants (21–27) and all the authors recorded the occurrence of amphidiploid cells. The appearance of telocentric chromosomes in the regenerants was observed in several experiments (Junming et al. 1985; Koba et al. 1988; Molnár-Láng et al. 1991). A detailed analysis was made of the meiosis of regenerant hybrids by Molnár-Láng et al. (1991), who found an increase in the rate of homoeologous chromosome pairing. A similar conclusion was drawn by Dahleen (1999) when investigating the progeny regenerated from barley × wild rye hybrids in tissue culture. As no backcross seeds were obtained from the initial hybrid of facultative wheat cv. Asakaze × winter barley cv. Manas, young inflorescences of the hybrids were used for in vitro multiplication in three consecutive cycles until a backcross progeny was developed. The chromosome constitution of the regenerated hybrids was analysed using GISH after each in vitro multiplication cycle (Molnár-Láng et al. 2005). The seven barley chromosomes were present even after the third cycle, but abnormalities were observed. Due to chromosome breakages, the number of barley telocentrics became significantly higher after the third cycle and amphidiploid cells with 56 chromosomes were counted. The number of wheat–barley chromosome arm associations, i.e. the homoeologous pairing frequency, increased after in vitro multiplication (Molnár-Láng et al. 2005).

12.4 Meiotic Pairing Behaviour of Wheat × Barley Hybrids

At first, the Feulgen technique was used to analyse the meiotic pairing behaviour of wheat × barley hybrids. In most cases Islam and Shepherd (1980) observed 28 univalent chromosomes when analysing pollen mother cells, though chromosome pairing could be seen in a few cells, with an average of 0.7 bivalents per pollen mother cell. A higher rate of chromosome pairing was recorded by Fedak (1977), resulting in a chiasma frequency of 1.82 per pollen mother cell. This was higher than the rate reported earlier in wheat haploids (Riley and Law 1965), suggesting that pairing also took place between barley and wheat chromosomes. Fedak (1977) drew attention to the phenomenon of homoeologous pairing between the chromosomes of two distantly related genera, and suggested that this should be confirmed with the Giemsa technique, the best method available at the time. Later Jauhar (1995) demonstrated a chiasma frequency of 2.16–6.72 per pollen mother cell in wheat × barley hybrids developed using the barley variety Luther. These data pointed to pairing between wheat and barley chromosomes, but as the chromosomes were analysed in meiosis using the Feulgen method, it was not possible to identify the individual chromosomes. An average of 5.03–6.63 bivalents per pollen mother cell could be observed in wheat × barley hybrids produced using the *Ph* mutant of CS, together with a small number of trivalents and quadrivalents (Sethi et al. 1986), but pairing between wheat and barley could not be demonstrated with the Feulgen method. Wojciechowska (1985) performed detailed meiosis analysis in several barley × wheat hybrid combinations and found a chiasma frequency of 1.17–1.98 per pollen mother cell in hybrid cells containing 28 chromosomes. Islam and Shepherd (1988) elaborated a method for the detection of pairing between wheat and barley chromosomes. They crossed ditelosomic wheat/barley addition lines with a high-pairing strain of an *Ae. speltoides* genotype carrying the *Ph* suppressor gene. F_1 hybrids possessing 28 + 1 telocentric somatic chromosomes (21 wheat + 7 *Ae. speltoides* + 1 barley telocentric) were grown. Pairing between telocentric and non-telocentric chromosomes was observed in 1.2–4.5 % of the pollen mother cells. Triple monosomic addition lines were developed in a wheat monosomic background, one of which contained 19 pairs of wheat chromosomes together with one 5B *Ph* mutant chromosome, one 3HL barley chromosome arm and a 3A wheat chromosome (Islam and Shepherd 1992a). In another line the 5B *Ph* mutant was accompanied by one 6HS and one 6B chromosome. In the triple monosomic addition lines, plants carrying the 3HL and 6HS barley chromosome arms only exhibited pairing in 0.3–0.7 % of the cells. These experiments proved that chromosomes of the distantly related species wheat and barley are capable of pairing with each other, thus allowing recombinations to occur. The meiotic instability of wheat × barley hybrids was noted by a number of authors, who found many cells with hypo- or hyperploid chromosome numbers in addition to cells with 28 chromosomes (Fedak 1980; Mujeeb-Kazi and Rodriguez 1983; Islam and Shepherd 1980; Wojciechowska 1985). Islam and Shepherd (1980) observed that the chromosome number became doubled in some cells during meiosis (restitution nuclei). In these hybrids the univalent chromosomes assembled in

the equatorial plate during meiotic metaphase I, but in many cells, instead of migrating to the two poles in anaphase I, the chromosomes remained together, forming a chromatin mass, thus leading to the formation of cells with a doubled chromosome number. In these cells, however, it was often observed that the second phase of meiosis did not take place, preventing the development of microspores with the full chromosome complement, which would restore the fertility of the hybrids (Islam and Shepherd 1980). It can be assumed that the egg-cells, which became fertilized and set seed when the hybrids were backcrossed arose from megaspores with a doubled chromosome number. Wheat–barley chromosome pairing was first detected using GISH by Molnár-Láng et al. (2000b). Meiotic analysis of the wheat × barley hybrid Mv9 kr1 × Igri revealed 1.59 chromosome arm associations per cell using the Feulgen method (Molnár-Láng et al. 2000b). The number of chromosome arm associations increased to 4.72 after in vitro culture. According to GISH analysis, wheat–barley chromosome arm associations made up 3.6 % of the total in the initial hybrid and 16.5 % of the total in progenies of the Mv9 kr1 × Igri hybrids regenerated in vitro. The meiotic pairing behaviour of a wheat–winter barley hybrid (Asakaze × Manas) was analysed using GISH after long-term maintenance in tissue culture (Molnár-Láng et al. 2005) (Fig. 12.4a). As no backcross seeds were obtained

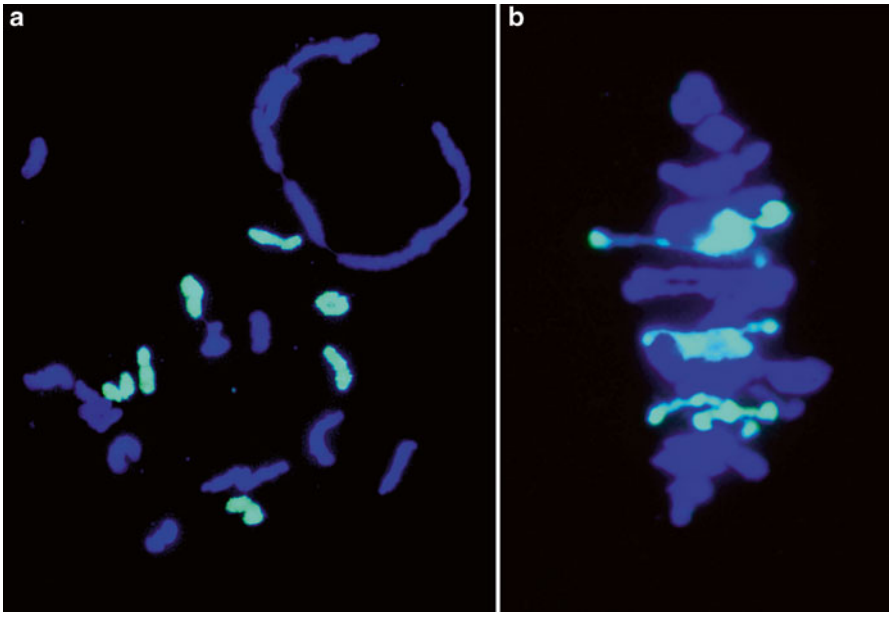

Fig. 12.4 GISH on meiotic chromosomes from the wheat × barley (Asakaze × Manas) hybrid multiplied in vitro. Total barley genomic DNA was labelled with Fluorogreen and used as probe. Barley chromosomes are *green*, and wheat chromosomes are *blue* as a result of counterstaining with DAPI. (**a**) Seven barley univalents + 14 wheat univalents + 2 wheat rod bivalents and 1 wheat trivalent. (**b**) An amphidiploid cell. Five barley rod bivalents + 2 barley ring bivalents + 4 wheat rod bivalents + 17 wheat ring bivalents

from the initial hybrid, young inflorescences were used for in vitro multiplication in three consecutive cycles until a backcross progeny was developed. The chromosome constitution of the regenerated hybrids was analysed using GISH after each in vitro multiplication cycle. The number of wheat–barley chromosome arm associations increased after the second and third cycles. Amphidiploid cells containing seven barley bivalents were counted after the third cycle (Fig. 12.4b). The use of the GISH technique to demonstrate wheat–barley chromosome pairing in the hybrids, and especially in their in vitro-regenerated progenies, proved the possibility of producing recombinants between these two genera, and thus of transferring useful characters from barley into wheat (Molnár-Láng et al. 2000b, 2005). In some regenerants in vitro conditions caused an increase in chromosome arm association frequency and in fertility.

12.5 Wheat–Barley Introgression Lines

Recombinant lines are the vehicles for transferring barley chromatin and its characters into the wheat genome. Islam and Shepherd (1992a) were the first to develop recombinations from wheat × barley crosses. Triple monosomics were developed from crosses between wheat/barley ditelosomic substitution lines and the *Ph* mutant of CS wheat. In addition to 19 wheat chromosome pairs, the triple monosomic additions contained one barley telocentric chromosome, the homoeologous wheat chromosome and one 5B *Ph* mutant chromosome. With this method six wheat/barley recombinations involving 6HL and 3HL chromosome segments were detected among the progeny. The presence of the recombinations was proved by isoenzyme analysis: the progeny were found to contain isoenzymes located either on the 6A and 6H or on the 3A and 3H chromosomes. Sherman et al. (2001) also utilized the effect of the *Ph* mutant gene to develop recombinations from the 4H and 5H wheat/barley addition lines produced by Islam et al. (1981). The presence of the recombinations was confirmed with PCR-based molecular markers. The use of GISH to detect the occurrence of wheat–barley translocations was first reported by Schwarzacher et al. (1992). The translocation line was developed by Islam and Shepherd (unpublished data) and isoenzyme analysis proved that at least the segment of the 4HL barley chromosome arm carrying the gene coding for the barley β-amylase isoenzyme had been incorporated into this line. GISH analysis then demonstrated that the whole of the 4HL chromosome arm was present in the translocation line, i.e. a centric fusion had occurred between wheat and barley. The occurrence of spontaneous translocations was observed by Koba et al. (1997) in the progeny of a cross between Shinchunaga wheat and Nyugoruden barley. The translocation chromosome was identified with C-banding and, using an earlier nomenclature, it was found that it involved the short arm of barley chromosome 7H and the long arm of wheat chromosome 5B. When the names of the barley chromosomes were later revized, the old chromosome 7H was renamed 5H (Linde-Laursen et al. 1997) and it became clear that a homoeologous translocation had indeed taken place.

Various methods are available for producing translocations, including irradiation (Sears 1956; Szakács et al. 2010) or the induction of homoeologous pairing (Riley and Chapman 1958; Sears 1972; Griffiths et al. 2006). A number of genes from common wheat promote chromosome pairing and several act as inhibitors (Sears 1976). The pairing homoeologous gene, *Ph1*, on the long arm of chromosome 5B, has the most decisive effect. In its presence, pairing is restricted to homologues; in its absence, homoeologues also pair, albeit less frequently than homologues. The simple deletion of *Ph1*, or the counteraction of its effect by high-pairing types of *Ae. speltoides* or *Ae. mutica*, can induce many *Triticinae* chromosomes to pair with their wheat homoeologues. Such induced homoeologous pairing is usually the method of choice for transferring genes from alienchromosomes to those of wheat. The "*Ph* system" was used by Islam and Shepherd (1992a) and by Sherman et al. (2001) to produce wheat–barley translocations. A unique genetic system exists in common wheat, which induces frequent chromosomal structural rearrangements. The gametocidal (Gc) system involves alien chromosomes called Gc chromosomes, which were introduced into common wheat from certain wild species belonging to the genus*Aegilops* (Endo 2007). This system proved to be effective in inducing structural rearrangements in the barley chromosomes added to common wheat, as well as in common wheat chromosomes (Endo 2009).

The rearranged chromosomes thus induced include deletions of barley chromosomes and translocations between the barley and wheat chromosomes. Lines carrying rearranged barley chromosomes are designated as "dissection lines" (Endo 2009). Schubert et al. (1998) developed wheat–barley translocations from wheat/barley disomic addition lines by exploiting the gametocidal effect of the 2C chromosome of *Aegilops cylindrica*. The 7H wheat/barley addition line was crossed with the 2C wheat/*Ae. cylindrica* addition line and the resulting line, containing two different alien chromosomes, was self-fertilized. Lines carrying barley deletions and wheat–barley translocations were selected from the progeny. More than ten translocation lines carrying segments of the 7H barley chromosome were produced. The incorporation of the barley chromosome segments was detected by means of GISH, and with FISH using the repetitive probe HvT01. These 7H deletion and translocation lines were then used for the physical mapping of the 7H barley chromosome (Serizawa et al. 2001).

The Gc system was used for the dissection of several barley chromosomes using CS/Betzes disomic addition lines. When the barley chromosome 5H added to common wheat was dissected, chromosomes with structural chromosomal changes involving 5H were selected. Barley-specific EST markers were screened and the authors proved the usefulness of the 5H dissection line for the physical mapping of DNA markers (Ashida et al. 2007). A chromosome 3H addition was used to establish 50 common wheat lines carrying single rearranged (or dissected) 3H chromosomes of independent origin. The dissected 3H chromosomes were either deletions or translocations with wheat chromosomes. These lines were used to map EST markers, after which then polymorphic markers were selected to construct a 3H genetic map (Sakai et al. 2009). The Gc chromosome induced chromosomal structural rearrangements in the progeny of the 4H addition line of common wheat, and

the rearranged chromosomes were characterized by sequential C-banding and in situ hybridization. 4H chromosome-specific EST markers were used for cytological mapping (Sakata et al. 2010), while Nasuda et al. (2005) performed chromosomal assignment and deletion mapping of barley EST markers. EST markers were demonstrated to be amplified differently on wheat and barley was assigned to all seven barley chromosomes. By using a set of Betzes ditelosomic additions of CS, the chromosome arm location of 90 % of the EST markers assigned to each barley chromosome was determined. Barley chromosomes 1H and 6H were dissected by thegametocidal system and structural changes were identified by means of GISH and FISH (Ishihara et al 2014). Five aberrations of chromosome 1H were found and 33 dissection lines carrying single aberrant 6H chromosomes were established. PCR analysis of the aberrant barley chromosomes was conducted using 75 and 81 EST markers specific to chromosomes 1H and 6H, respectively. A cytological map of chromosome 6H was compared to the previously reported genetic and physical map. The cytological map had better resolution in the proximal region than the corresponding genetic map (Ishihara et al 2014). The agronomical value of the dissection lines have not been analysed in the above mentioned reports. The barley dissection lines were produced from CS-Betzes addition lines, so they all carried chromosome segments from Betzes barley.

Molnár-Láng et al. (2000a) developed translocations from wheat–barley hybrids multiplied in tissue culture, using GISH for confirmation (Fig. 12.5). The origin of the barley chromosome segments involved in the selected homozygous translocation lines was determined using molecular markers (Nagy et al. 2002). Segments of various sizes from the 1H, 3H, 4H and 5H chromosomes were found to have been incorporated in the translocation lines. These lines were then used for the physical mapping of microsatellite markers previously located on the barley chromosomes. Sepsi et al. (2006) produced wheat/barley translocations as the result of induced homoeologous chromosome pairing in a 4H(4D) wheat–barley substitution line by crossing with the line CO4-1, which carries the *Ph* suppressor gene from *Aegilopsspeltoides*. Kruppa et al. (2013) reported the development of a 4HL.5DL Robertsonian translocation line after crossing the 4H(4D) wheat–barley substitution line with the CS*ph1b* mutant. The rearrangement was confirmed with sequential GISH, FISH and SSR markers. A spontaneous wheat–barley translocation was identified using sequential GISH, FISH and SSR markers by Cseh et al. (2011) in the progenies of the Asakaze × Manas hybrid. This translocation line carries a 4BS wheat chromosome arm and a 7HL chromosome arm from the Ukrainian six-rowed winter barley. Another spontaneous wheat/barley translocation line, identified as 5HS-7DS.7DL, was detected among the progenies of the Mv9kr1 × Igri wheat–barley hybrid (Kruppa et al. 2013) (Fig. 12.6). Despite the non-compensating nature of the translocation, the plants showed good viability. Of the 45 microsatellite markers analysed, ten failed to amplify any 7DS-specific fragments, signalling the elimination of a short chromosome segment in the telomeric region. The breakpoint of the 5HS-7DS.7DL translocation appeared to be more distal than that of reported deletion lines, thus providing a new physical landmark for future deletion mapping studies.

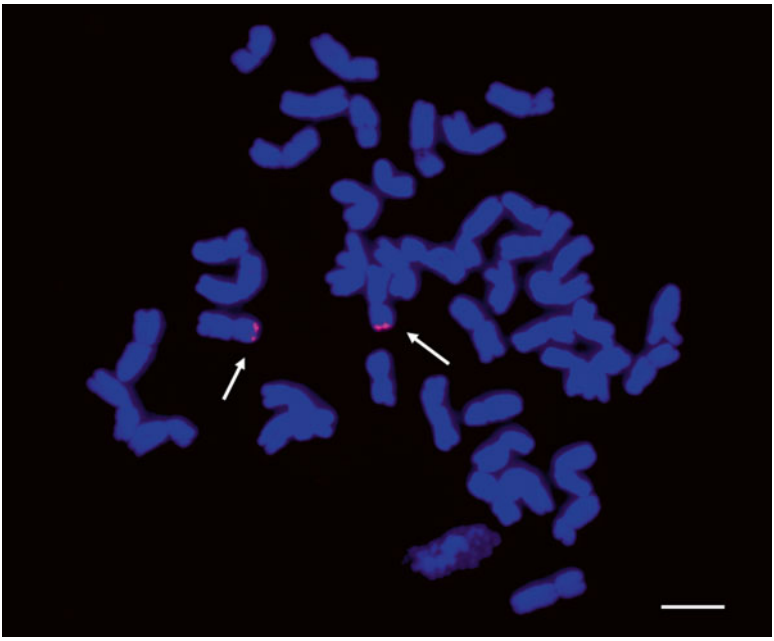

Fig. 12.5 Detection of barley chromosome segments in the 6BS.6BL-4HL translocation (arrows) using GISH. Total barley DNA was labelled with digoxigenin and detected with anti-DIG-Rhodamine (*red*). Wheat chromosomes were counterstained with DAPI (*blue*)

12.6 Morphological and Agronomic Traits of Wheat–Barley Introgressions

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 wheat–barley addition lines produced in various cultivar combinations (CS × Betzes, Mv9 kr1 × Igri, Asakaze × Manas) had several morphological traits in common (Molnár-Láng et al. 2012). The 4H additions had the best fertility and 7H the lowest in all three combinations. The 2H addition line had a lax spike structure each of the cultivar combinations. The 3H addition had the shortest, most compact spike of all the addition lines in the Mv9kr1-Igri and CS-Betzes sets (Szakács and Molnár-Láng 2007; Aranyi et al. 2014a). The 3H Asakaze–Manas addition also had a short spike, but it was not as dense as in the other two combinations. This addition line showed a high level of genetic instability, which cannot yet be explained. The 4H addition line had the tallest plants and

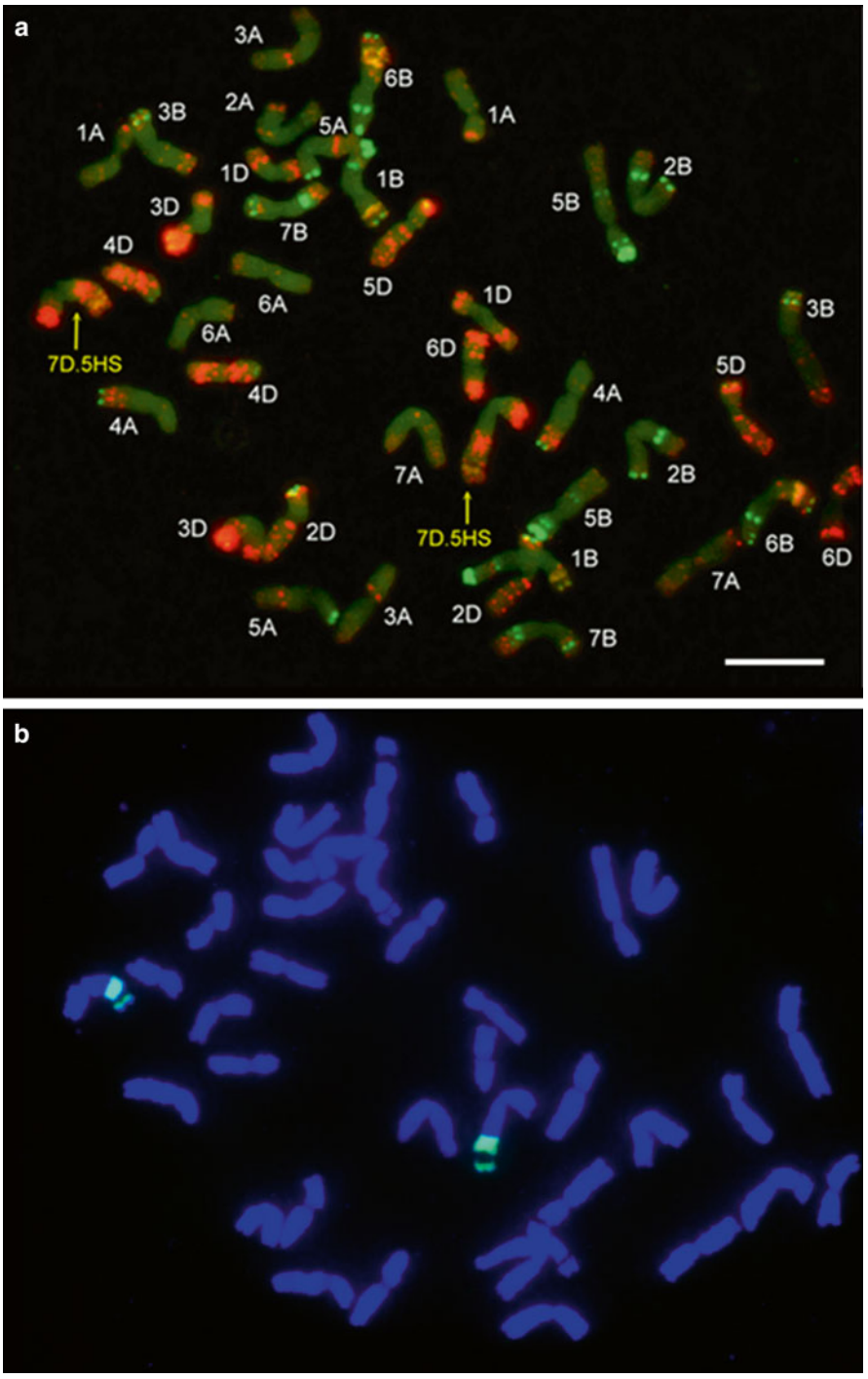

Fig. 12.6 Sequential GISH and FISH on mitotic chromosomes from 7DL.7DS-5HS wheat–barley disomic translocation line. (**a**) Identification of the chromosomes using DNA probes pSc119.2 (*green*), Afa family (*red*) and pTa71 (*yellow*). (**b**) Barley total genomic DNA was labelled with biotin and detected with streptavidin-FITC (*green*). Wheat chromosomes were counterstained with DAPI (*blue*)

3H the shortest. The 6H and 7H additions were shorter than 4H, as also observed in the Mv9kr1-Igri addition lines. Unfortunately no 5H additions could be selected from the Mv9 kr1 × Igri and Asakaze × Manas combinations, as this chromosome was eliminated most frequently from the backcross progenies (Molnár-Láng et al. 2005). Barley chromosome 1H caused sterility even in the presence of other barley chromosomes such as 2H, 3HS, 4H, 5HS and 7H. A fertile addition line involving the entire barley chromosome 1H could not be produced by Islam et al. (1978), because a gene on the long arm of this chromosome caused sterility when present in a wheat background. The double monosomic 1H and 6H addition became partly female fertile and a few backcross seeds were produced after pollinating them with normal wheat (Islam and Shepherd 1990). Partially self-fertile plants disomic for 6H and monosomic for 1H were developed (Islam and Shepherd 2000). Unfortunately none of the BC_2 plants from the Asakaze × Manas and Mv9 kr1 × Igri combinations carried the barley chromosomes 6H and 1H together. Hart et al. (1980) used differences between wheat and barley isozymes to determine the chromosomal locations of barley structural genes for these isozymes. Genes controlling more than 58 isozymes have been allocated to specific barley chromosomes or to the arms thereof using wheat–barley addition lines (Islam and Shepherd 1990). The effect of the added barley chromosomes on heading characters was studied by Murai et al. (1997) using the CS-Betzes addition lines produced by Islam et al. (1978) together with the 5H and 6H Shinchunaga/New Golden additions produced by Koba et al. (1997). The earliest flowering was observed for the CS-Betzes 5H addition line and for the Shinchunaga/New Golden 5H addition lines. Murai et al. (1997) demonstrated that the heading characters of wheat may be altered by barley genes. The Mv9kr-Igri and Asakaze-Manas wheat/winter barley addition lines made it possible to study the effects of chromosomes from winter barley cultivars on flowering time in the wheat genetic background under various environmental conditions (Farkas et al. 2014) (Table 12.3). The winter barley chromosome additions significantly influenced the flowering time of wheat both in a controlled environment test and under field-sown conditions. Unfortunately the 5H addition was missing from both combinations, because 5H was the first chromosome to be eliminated from the backcross derivatives (Molnár-Láng et al. 2005, 2012; Szakács and Molnár-Láng 2010). Of all the barley addition lines, the effect of the 4H and 7H additions was the most characteristic. The 7H addition lines were the earliest in both cultivar combinations in each treatment (Farkas et al. 2014). In the Mv9kr1-Igri combination the 4H addition was the latest under all the environmental conditions (Aranyi et al. 2014a). In the Asakaze–Manas combination the 4H addition was the latest under short-day and long-day illumination in the phytotron, but the 6H addition was the latest without vernalization and in the field in several years (2012, 2013, 2014). There was 12 and 11 days' difference between the flowering times of the 7H and 4H Mv9kr1-Igri and Asakaze-Manas addition lines in the field in 2012, which increased to 52 and 44 days under short-day illumination in the phytotron (Farkas et al. 2014). Only two days' difference was observed between the CS-Betzes 7H and 4H addition lines by Murai et al. (1997) under short-day illumination in the phytotron, which could be

Table 12.3 Flowering time of wheat (*T. aestivum*) -barley (*H. vulgare*) addition lines (Mv9 kr1/Igri, Asakaze/Manas) in the field in Martonvásár in three consecutive years (2012, 2013, 2014)

Genotype	2012 May	2013 May	2014 May
Mv9 kr1	11th	13th	8th
Igri	6th	5th	1st
2H Mv9kr1/Igri	20th	20th	18th
3H Mv9kr1/Igri	18th	24th	16th
4H Mv9kr1/Igri	22nd	21st	20th
6HS Mv9kr1/Igri	16th	14th	9th
7H Mv9kr1/Igri	10th	12th	11th
Asakaze	8th	9th	4th
Manas	4th	6th	1st
2H Asakaze/Manas	13th	10th	7th
3H Asakaze/Manas	16th	10th	8th
4H Asakaze/Manas	17th	14th	12th
6H Asakaze/Manas	22nd	24th	20th
7H Asakaze/Manas	6th	8th	2nd

primarily due to the fact that Betzes, being a spring barley, did not carry the *ZCCT-H* genes at the *Vrn-H2* locus, giving further indirect proof that the effect of *Vrn-H2* was detected in these addition lines.

The dietary fibre (1,3;1,4)- β-D-glucan (β-glucan), is a major quality parameter of cereals. Barley β-glucans are beneficial to human health, as they are a major source of soluble dietary fibre and have been recognized both as potential cholesterol-lowering polysaccharides (Kerckhoffs et al. 2003) and as non-specific immune-activators (Allendorf et al. 2005). The grain of barley is one of the most important β-glucan sources, having a β-glucan content ten times higher than that of wheat. The cellulose synthase-like F6 (*CslF6*) gene, encoding a putative β-glucan synthase, has been assigned to the 7H chromosome (Burton et al. 2008). The presence of the *HvCslF6* gene, responsible for β-glucan production, was revealed in the centromeric region of 7HL using the 4BS.7HL Asakaze-Manas translocation line (Cseh et al. 2011). An increased β-glucan level was also detected in the translocation line, demonstrating that the *HvCslF6* gene is of potential relevance for the manipulation of wheat β-glucan levels. The Mv9kr1-Igri 1HS ditelosomic and Mv9kr1-Igri 7H disomic wheat/barley addition lines carrying the *HvCslF9* and *HvCslF6* barley genes, respectively, were used to investigate the additive effect of barley cellulose synthase-like genes on the wheat β-glucan content (Cseh et al. 2013). A significantly higher β-glucan level was detected in the leaves and grains of the wheat/barley 1HS and 7H addition lines compared to the control wheat line. The expression of the *HvCslF9* and *HvCslF6* genes in the genetic background of wheat was also determined by quantitative RT-PCR, and the *HvCslF9* gene was mapped to the short arm of the 1H chromosome (Cseh et al. 2013). The *HvGlb1* barley gene, encoding β-glucan endohydrolase isoenzyme EI, is possibly involved

in the regulation of the β-glucan level during grain development. Previously this was also mapped to the barley 1H chromosome, and this study made it clear that it was located on the 1HL chromosome arm. Zou et al. (2012) recently identified wheat–barley 2HL chromosometranslocation lines derived from crosses between CS-Betzes 2H disomic substitution lines and Chinese wheat varieties. These translocations carry the *Isa* gene encoding the barley bifunctional α-amylase/subtilisin inhibitor (BASI). Because BASI is more effective in inhibiting wheat AMY2 than the α-amylase inhibitors of other cereals (Henry et al. 1992), the introduction of the barley *Isa* gene into wheat may regulate endogenous α-amylase activity during starch granule synthesis in the developing grain and reduce the level of preharvest sprouting damage.

The drought tolerance of a spontaneous 4H(4D) substitution line was studied under laboratory and field conditions (Molnár et al. 2007; Hoffmann et al. 2009) to investigate the ability of the barley 4H chromosome to compensate for wheat 4D in response to mild drought stress (15 % PEG) in barley and in the 4H(4D) substitution line. Mild osmotic stress induced intensive stomatal closure, resulting in reduced water loss through transpiration and unchanged relative water content in the leaves. The water use efficiency under mild osmotic stress increased greatly in these lines (Molnár et al. 2007). The drought tolerance of the 4H(4D) substitution line and of wheat/barley addition and translocation lines was studied under a rain shelter in the field in Keszthely. The difference in water supply between the control and stress treatment was 180 mm (Hoffmann et al. 2009). The largest root/shoot ratio was observed in the 4H(4D) substitution line. Large root biomass could contribute to better drought tolerance. The grain yield of the genotypes was also analysed and no yield loss was observed in the 4H(4D) substitution line during drought treatment. This was confirmed by observations in the next vegetative season (Hoffmann et al. 2010). However, the grain yield of the 4H(4D) substitution was much lower than that of the 3HS.3BL or the 5HS-7DS.7DL translocation lines.

The aluminium tolerance of wheat/barley disomic addition, substitution and translocation lines carry chromosomes from three different barley cultivars (Manas, Igri, Betzes) was evaluated by comparing the root growth in a solution containing 75 μM $AlCl_3$ at pH 4.0 to that of known Al-tolerant and sensitive wheat genotypes (Darkó et al. 2012). The wheat Asakaze komugi, the barley Manas cultivar and their hybrid derivatives were found to have high levels of Al tolerance, while the wheat line Mv9kr1, the barley cultivar Igri and their hybrid progenies were sensitive to Al. In most cases, the Al tolerance of the wheat/barley introgression lines derived from Al-sensitive wheat Mv9kr1 and barley Betzes, which has moderate Al tolerance, was similar to that of the wheat parents, but the 2DS.2DL-1HS translocation line of Mv9kr1/Betzes exhibited more intensive root growth, while accumulating less Al than the parental lines. This indicates that either the lack of the distal part of chromosome 2DL or the presence of the distal part of 1HS improved the Al tolerance level (Darkó et al. 2012).

Salt responses were studied by Darkó et al. (2015) during germination and in young plants of the wheat–barley disomic addition lines 2H, 3H, 4H, 6H and 7H, in

ditelosomic addition lines 3HS and 7HL, developed from the Asakaze×Manas hybrid (Molnár-Láng et al. 2000b, 2012), and in the parental genotypes. Two other wheat genotypes, namely Mv9kr1, a winter wheat line originating from Martonvásár and used as a parental genotype in other addition lines and Chinese Spring (CS), the wheat parental genotype of addition lines previously tested for salt tolerance (Colmer et al. 2006) were also used for comparison. Asakaze possesses relatively high salt tolerance, as indicated by the less pronounced reduction in germination % and in root and shooth growth and the retention of high leaf water content and photosynthetic activity, as compared to CS and Mv9kr1. The barley cv Manas showed better salt tolerance than wheat cv Asakaze, although Manas accumulated more Na in the root, its transport to the shoots was restricted. Among the addition lines tested, the disomic addition line 7H and the ditelosomic addition line 7HL exhibited higher salt tolerance both during germination and in the early developmental stages than the wheat parent, which may be related to the elevated osmotic adjustment capacity of these addition lines, similar to that found for barley cv Manas (Darkó et al. 2015).

A spontaneous 3HS.3BL Robertsonian translocation was obtained from the progenies of a Chinese Spring×Betzes wheat–barley hybrid produced in Martonvásár (Molnár-Láng and Sutka 1994). The hybrid was backcrossed with wheat line Mv9kr1, after which it was transferred into the modern Martonvásár wheat variety Mv Bodri. The translocation was detected by means of GISH (Molnár-Láng et al. 2000a). Fluorescence in situ hybridization (FISH) using the barley telomere- and centromere-specific repetitive DNA probes (HvT01 and (AGGGAG)n confirmed that the complete barley chromosome arm was involved in the Robertsonian translocation. Wheat-specific repetitive DNA probes identified the presence of the whole wheat genome, except for the short arm of the 3B chromosome (Fig. 12.7). Genotypes homozygous for the 3HS.3BL translocation were selected, after which morphological analysis was performed on the plants and the yield components were measured in the field during two consecutive vegetative seasons. The introgression of the 3HS.3BL translocation into the modern wheat cultivar Mv Bodri significantly reduced the plant height of the initial translocation line due to the incorporation of the dwarfing allele *RhtD1b* from Mv Bodri and increased its productivity (seeds/spike). The presence of the 3HS.3BL translocation in the Mv9kr1 and MvBodri wheat background improved tillering and seeds/plant productivity in field experiments carried out in Martonvásár and Keszthely, Hungary (Türkösi et al. 2014a, b).

Infection with fungal biotrophic pathogens causing powdery mildew diseases was studied on wheat–barley addition and translocation lines by Aranyi et al. (2014b). Most powdery mildew species are strictly host-specific, colonizing only a narrow range of species or one particular host species. *Blumeria graminis* f. sp. *tritici* isolate 14 (HM484334) was identified on the wheat parent and all the wheat/barley inrogression lines, while *B.graminis f.* sp. *hordei* isolate MUMH1723 (AB 273556) was identified on the barley parent, so the added barley chromosomes did not result in host range expansion for barley powdery mildew.

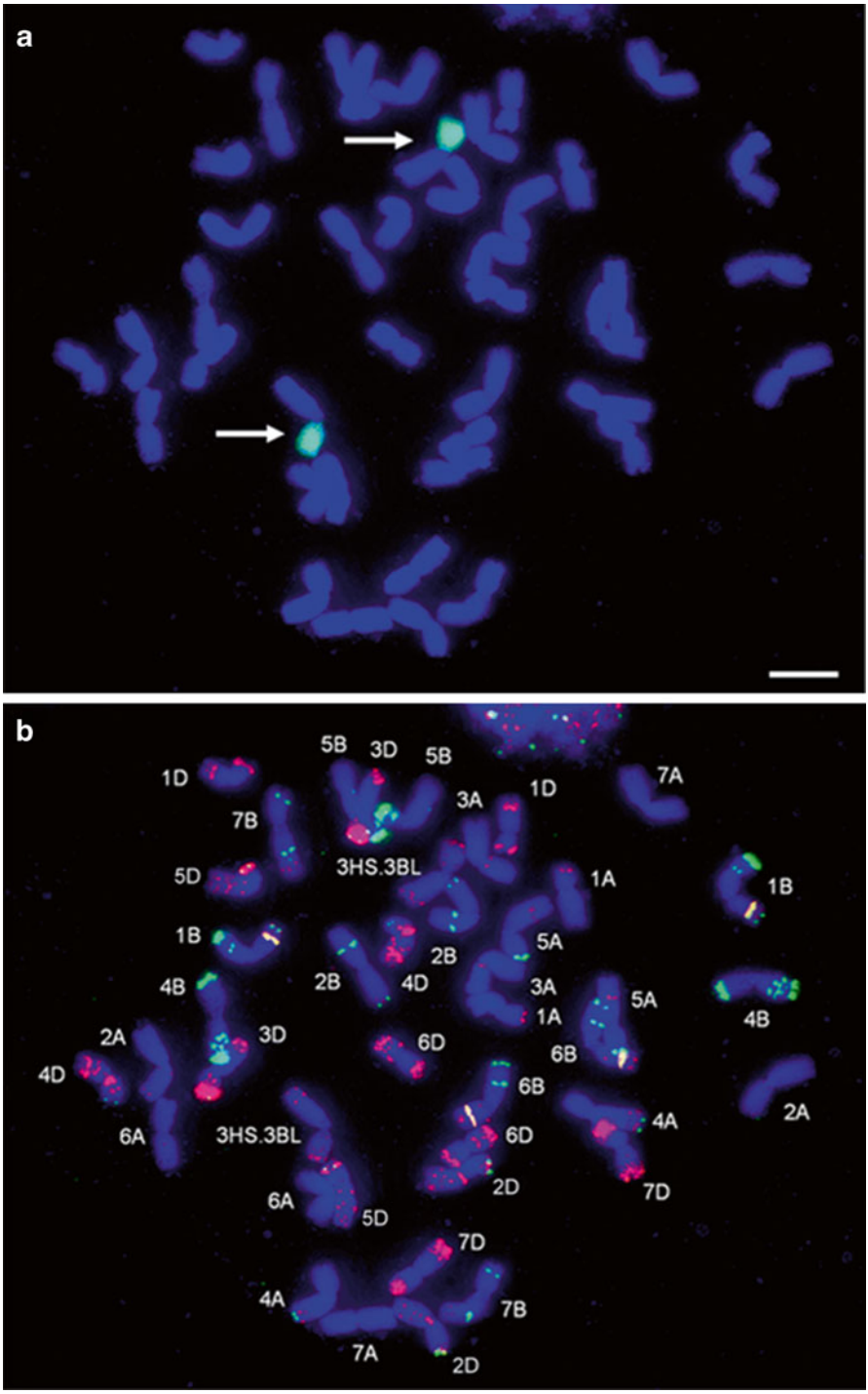

Fig. 12.7 Sequential GISH-FISH analysis on mitotic chromosomes of 3HS.3BL wheat–barley Robertsonian translocation line. (**a**) Labelled barley genomic DNA was used as probe and barley chromosome arm 3HS is highlighted in *green*. Wheat chromosomes were counterstained with DAPI. (**b**) Chromosomes were identified by means of fluorescence in situ hybridization using DNA repetitive probes: Afa family (*red*), pSc119.2 (*green*), and pTa71 (*yellow*)

12.7 Molecular Genetic Studies on Wheat–Barley Introgression Lines

Wheat–barley chromosome addition lines are useful genetic resources for studying the transcript accumulation patterns of barley in a wheat genetic background and for the large-scale physical mapping of genes. In a study performed by Cho et al. (2006), CS-Betzes addition lines were examined with the Barley1 Affymetrix GeneChip probe array and a total of 1787 barley transcripts were detected and physically mapped to barley chromosomes and to the long and short arms of chromosome 6H. The same method and plant materials were used to physically map barley genes to their respective chromosome arm locations by Bilgic et al. (2007), who mapped 1257 barley genes to chromosome arms 1HS, 2HS, 2HL, 3HS, 3HL, 4HS, 4HL, 5HS, 5HL, 7HS and 7HL. The number of genes assigned to individual chromosome arms ranged from 24 to 197. Flow sorting can be effective for isolating large samples of alien chromosomes from metaphase suspensions if the flow karyograms of sorted additions demonstrate distinct peaks not present in those of the parental species (Doležel et al. 2005). The telocentric chromosomes of Betzes barley were isolated from CS-Betzes ditelosomic addition lines, thus allowing the barley genome to be dissected into fractions each representing only about 6–12 % of the total genome (Suchánková et al. 2006). The DNA of flow-sorted chromosomes can be used for the isolation of molecular markers, for physical mapping using PCR and FISH, for the integration of genetic and physical maps and for the construction of chromosome-specific DNA libraries, including sequences cloned in bacterial artificial chromosome vectors. The first barley chromosome to be isolated by flow sorting and shotgun sequencing was 1H (Mayer et al. 2009). As there is no significant difference in the size of the barley chromosomes, the other six chromosomes could only be sorted from wheat/barley ditelosomic addition lines, although some barley chromosomes are identifiable based on morphology. Twelve barley chromosome arms (2HS to 7HL) were purified separately by flow cytometry (Suchánková et al. 2006), after which the DNA was amplified by multiple displacement amplification (MDA) and then shotgun sequenced (Mayer et al. 2011). Using this procedure, between 2261 and 3616 genes were tentatively positioned along each of the individual barley chromosomes, representing a cumulative set of 21,766 genes across the entire barley genome. An additional set of 5815 genes could not be integrated into the genome zippers based on conserved synteny models, but were associated with individual chromosomes/chromosome arms. Overall, it was possible to tentatively position 27,581 barley genes, or 86 % of the estimated 32,000 gene repertoire of the barley genome, into chromosomal regions (Mayer et al. 2011). Among the 21,766 genes anchored to the genome zipper, 3125 genes (14 %) were allocated to the genetic centromeres. Based on the 454 sequence and array-based gene assignments to chromosome arms, all but nine of these 3125 genes were distributed to specific arms of chromosomes 1H to 7H. Not much later, using the whole genome

approach, a deep physical map of 4.98 Gb was developed and more than 3.90 Gb was anchored to a high-resolution genetic map (The International Barley Genome Sequencing Consortium 2012). By combining the physical map with a complementary short-read whole-genome assembly and with high-coverage RNAseq data, approximately 80 % of the barley genome could be delineated, including more than 90 % of the expressed genes. These chromosome- or whole-genome-derived genomic resources provide an essential platform to advance gene discovery and genome-assisted crop improvement.

12.8 Conclusions

From the practical point of view new wheat–barley hybrids need to be produced using a wider range of barley genotypes carrying genes responsible for useful agronomic traits (e.g. drought tolerance, high β-glucan content, salt tolerance, earliness). The major limitation for successful gene transfer from barley into wheat is the low crossability between these species. The efficiency with which wheat × barley hybrids can be produced should be increased by hormone treatment at pollination, and by improving the yield of embryo culture. Among the various methods available for producing translocations from wheat × barley hybrids and additions, the gametocidal system is currently the most promising. Although Tritordeum already exists as the product of the wheat × *H. chilense* combination, and fertile amphiploids have been produced with *H. marinum* and *H. californicum*, fertile *T. aestivum* × *H. vulgare* amphiploids have not yet been developed. Unfortunately, chromosome 1H of *H. vulgare* carries a gene (*Shw*) that causes sterility in the wheat background (Taketa et al. 2002), thus preventing the production of a fertile amphiploid. The development of small barley introgressions in the wheat genetic background has been started, but a much larger number of translocations carrying genes responsible for useful traits is needed. Wheat/barley translocations are ideal material for the physical mapping of wheat and barley chromosomes, as the two genomes can be clearly identified using GISH, and the physical landmarks can be used in genome mapping. The efficient manipulation of alien chromatin and the selection of proper recipient genotypes play a central role in the success of alien introgression. The physical, genetic and functional assembly of the barley genome (Mayer et al. 2011; The International Barley Genome Sequencing Consortium 2012) has made an important contribution to the targeted introgression of barleygenes into the wheat genome and the exact identification of wheat–barley introgression lines.

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