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Review paper

Recent advances in alien gene transfer in wheat

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Summary

The recent advances in alien gene transfer from distantly-related species into wheat are reviewed in the present paper. The main achievements during the last ten years include the great expansion of the range of wide hybridization and development of new techniques for production and characterization of wheat-alien chromosome translocations. Updated results of wide hybridization since 1983 and comprehensive characterization of wheat-alien translocation lines in our laboratory are compiled. The future outlook for alien gene transfer in wheat is also discussed.

Introduction

As in most other crops, the genetic variation of cultivated wheat has been greatly eroded under modern agricultural systems. Genetic erosion not only limits the further improvement of yield and quality but also makes wheat increasingly vulnerable to biological and environmental stresses. A large amount of genetic variation exists in the wild relatives of cultivated wheat. The introduction of genetic variation from alien species has been a valuable method for increasing the amount of genetic diversity available to wheat breeders. In this paper, we review the most recent advances of gene transfers, especially those from distantly related alien species, in wheat.

Classification of gene pools

The wild relatives of wheat can be classified on the basis of their genomic constitutions into primary, secondary, and tertiary gene pools.

The primary gene pool of common wheat (*Triticum aestivum,* 2n = 6x = 42, AABBDD) consists of hexaploid landraces, cultivated tetraploid *Triticum turgidum* (2n = 4x = 28, AABB) and its wild form *Triticum dicoccoides,* A genome donor *Triticum monococcum* (including var. *boeoticum* and var. *urartu*), and D genome donor *Aegilops squarrosa* (syn. *Triticum tauschii*). Genes from the primary pool can be transferred by direct hybridization, homologous chromosome recombination, backcrossing and selection (Gill & Raupp, 1987; Cox, 1991). No special cytogenetic manipulation except embryo rescue in certain cases is necesaary to produce the F_1 hybrids.

The secondary gene pool consists of closely related, mostly polyploid *Triticum* and *Aegilops* species that share one genome in common with wheat. However, the diploid *Aegilops* species in the Sitopsis section which are related to the B genome of wheat are also included in the secondary pool because of the reduced chromosome pairing and difficulties in achieving gene transfer. Genes from the secondary pool can also be transferred by direct crosses and selection if they are located in an homologous genome. However, if they are present in a non-homologous genome, special cytogenetic manipulations are required as for genes from tertiary pools (see below).

The tertiary gene pool includes the diploid and polyploid species containing genomes that are non-homologous to those of wheat. Thus, genetic transfers cannot be made by homologous recombination. However, the genomes of species in the tertiary pool are genetically related (homoeologous) to the genomes of

wheat, and successful transfer can be made using special cytogenetic techniques or by inducing chromosome translocations using ionizing radiation or tissue culture. Even though such transfers may include an entire chromosome arm or part of an arm, they have been successfully bred into commercial wheat cultivars because the alien chromosome arm or segment genetically compensates for the missing wheat chromatin.

In this paper, we will focus on the recent advances in gene transfer from the tertiary gene pool. We will also discuss the state-of-the-art molecular cytogenetic techniques that are now used to characterize alien gene introgressions.

Potential of wide crosses

An F_1 hybrid between wheat and an alien species is the first prerequisite for transferring a targeted alien gene into wheat. Wide hybridization in wheat has become very successful since the application of embryo rescue techniques. Sharma & Gill (1983a) summarized the successful crosses made before 1983. A supplemental list of species successfully crossed with wheat since 1983 is given in Table 1. The published cross combinations involve almost all of the basic genomes in the Triticeae, including the P genome from *Agropyron,* N genome from *Psathyrostachys,* S, H, Y, and W genomes from *Elymus,* X genome from *Leymus,* J and E genomes from *Thinopyron,* and I genome from *Hordeum.*

The success of wide hybridization also depends on the crossability of the wheat genotype that is used. Extensive genetic variation in crossability of wheat varieties and wild relatives has been reported (Mujeeb-Kazi et al., 1987, 1989; Zeven, 1987; Farooq et al., 1990; Luo et al., 1992). At least four crossability (designated *kr*) genes have been identified. 'Chinese Spring' wheat contains recessive genes *kr1, kr2,* and *kr3* that are located on the homoeologous group 5 chromosomes (Riley & Chapman, 1967; Snape et al., 1979; Falk & Kasha 1983), and was considered as the best crossable wheat genotype by many scientists. Yen et al. (1986) and Luo et al. (1992) found new highly crossable wheat genotypes in Sichuan, China from where 'Chinese Spring' originated (Sears & Miller, 1985; Yen et al., 1988). These new genotypes are local landraces and have an additional *kr4* gene on chromosome 1A (Zheng et al., 1992). The presence of *kr4* makes these genotypes more crossable with rye than 'Chinese Spring'.

The potential range of wide hybridization of cereals, including wheat, barley (*Hordeum vulgare*), and rye (*Secale cereale*), was investigated by Zenkteler & Nitzsche (1984). Hexaploid wheat was pollinated with 13 different species from the *Poaceae* and *Panicoideae.* Although a limited numbers of ears of wheat were pollinated, embryo formation was observed in crosses involving very distantly related species, including *Alopecurus pratensis, Dactylis glomerata,* and maize (*Zea mays*).

The production of wheat × maize hybrid embryos was cytologically confirmed by Laurie & Bennett (1986). Zygotes with one complete haploid chromosome set from both wheat and maize were observed. Maize chromosomes were eliminated during the first three cell-division cycles in most embryos (Laurie & Bennett, 1989). This system has been successfully used for wheat haploid production (Comeau et al., 1988b; Laurie & Bennett, 1988). Similar results were also obtained from the crosses of wheat × sorghum (*Sorghum bicolor*) and wheat × pearl millet (*Pennisetum glaucum*) (Laurie & Bennett, 1987; Laurie, 1989).

If alien gene transfer is the goal of wide hybridization, then the spontaneous elimination of alien chromosomes in wheat wide-hybrids is a barrier to gene transfer. One possible way to overcome this barrier is to exploit genetic variation for chromosome elimination. In wheat × *Hordeum bulbosum* hybrids, chromosomes of *H. bulbosum* were elimated during early stages of embryo development, resulting in wheat haploid progenies (Barclay, 1975). But crosses involving some *H. bulbosum* strains resulted in partial or complete wheat-*H. bulbosum* hybrids (Wang et al., 1982; Blanco et al., 1986). Wheat-*H. bulbosum* chromosome addition lines derived from these crosses were reported (Wang et al., 1986). The influence of parental genotypes on chromosome elimination was also observed in barley × *H. bulbosum* crosses (Simpson et al., 1980; Pickering, 1983). Although the chromosomes of maize, sorghum and pearl millet were usually rapidly eliminated in the hybrid zygotes from the crosses with wheat, Comeau et al. (1988b) reported the presence of maize chromosomes in plants recovered from wheat × maize crosses. A similar example of chromosome retention was reported from oat (*Avena sativa*) × maize crosses (Riera-Lizarazu et al., 1992). Therefore, crosses using a wide range of genotypes of wheat and alien species may help in obtaining lines which retain one or more alien chromosomes.

Table 1. Alien species successfully crossed with hexaploid wheat (*Triticum aestivum*) since 1983*

Species	2n	Genomes**	Authors
Agropyron cristatum	14	PP	1)
Agropyron desertorum	28	$P_1P_1P_2P_2$	1), 2)
Agropyron michnoi	28	$P_1P_1P_2P_2$	2), 3)
Agropyron cristatum	28	$P_1P_1P_2P_2$	4)
Psathyrostachys juncea	14	NN	5)
Hordeum marinum	14	XaXa	6)
Hordeum californicum	14	HH	7)
Hordeum depressum	28	$H_1H_1H_2H_2$	6)
Hordeum jubatum	28	$H_1H_1H_2H_2$	8)
Hordeum geniculatum	28	$H_1H_1H_2H_2$	9)
Hordeum bulbosum	28	IIII	10)
Pseudoroegneria stipifolia	28	SSSS	11), 12)
Pseudoroegneria geniculata ssp. *scythica*	28	EESS	11), 12)
Elymus fibrosus	28	SSHH	13)
Elymus canadensis	28	SSHH	13), 14), 15)
Elymus caninus	28	SSHH	16), 17)
Elymus shandongensis	28	SSYY	18)
Elymus pendulinus	28	SSYY	19)
Elymus altissimus	28	SSYY	19)
Elymus anthosachnoides	28	SSYY	19)
Elymus dolichatherus	28	SSYY	19)
Elymus parviglumis	28	SSYY	19)
Elymus semicostatus	28	SSYY	19)
Elymus tibeticus	28	SSYY	19)
Elymus caucasicus	28	SSYY	19)
Elymus tschimganicus	42	$S_1S_1S_2S_2YY$	19)
Elymus dahuricus	42	SSHHYY	13), 14)
Elymus tsukushiensis	42	SSHHYY	20), 21)
Elymus scabrus	42	SSYYWW	22)
Elymus rectisetus	42	SSYYWW	23)
Leymus cinereus	28	NNXX	11), 24)
Leymus triticoides	28	NNXX	11), 24)
Leymus innovatus	28	NNXX	25)
Leymus multicaulis	28	NNXX	26)
Leymus angustus	56	NNNNXXXX	27)
Leymus angustus	84	NNNNNNXXXXXX	11), 24), 27)
Thinopyron curvifolium	28	$J_1J_1J_2J_2$	11), 12)
Thinopyron sartorii	28	JJEE	11), 12)
Thinopyron junceiforme	28	JJEE	11), 24)
Thinopyron junceum	42	$J_1J_1J_2J_2EE$	11), 24), 28)
Thinopyron gentryi	42	$E_1E_1E_2E_2SS$	11), 12)
Elytrigia varnense	42	———	11), 12)
Elytrigia acutum	42	———	11), 24)

Table 1. continued

Elytrigia pungens	56	————	11), 24)
Elytrigia repens	42	$S_1S_1S_2S_2XX$	11), 24), 29), 30)
El. repens/A. desertorum	70	$S_1S_1S_2S_2XXP_1P_1P_2P_2$	11), 24)

[1]) Limin & Fowler (1990), 2) Chen et al. (1990),
[3]) Li & Dong (1991), [4]) Chen et al. (1989),
[5]) Plourde et al. (1990), [6]) Jiang & Liu (1987),
[7]) Gupta & Fedak (1985), [8]) Comeau et al. (1988a),
[9]) Pershina et al. (1988), [10]) Wang et al. (1982),
[11]) Mujeeb-Kazi et al. (1984), [12]) Mujeeb-Kazi et al. (1987),
[13]) Mujeeb-Kazi & Bernard (1982), [14]) Yen & Liu (1987),
[15]) Mujeeb-Kazi & Bernard (1985), [16]) Sharma & Baenziger (1986),
[17]) Claesson et al. (1990), [18]) Lu & Bothmer (1989),
[19]) Lu & Bothmer (1991), [20]) Muramatsu et al. (1983),
[21]) Liu et al. (1990), [22]) Ahmad & Comeau (1991),
[23]) Wang et al. (1993), [24]) Mujeeb-Kazi et al. (1989),
[25]) Plourde et al. (1989a), [26]) Plourde et al. (1989b),
[27]) Plourde et al. (1992), [28]) Charpentier et al. (1986),
[29]) Comeau et al. (1985), [30]) Fedak et al. (1986).
* Crosses include reciprocals.
Crosses in which hybrids died before reaching maturity are not included.
Crosses made before 1983 were summarized by Sharma & Gill (1983a).
** X is unknown; the X in *Elytrigia repens*
might be different from the X in *Leymus* species.
Y is a genome whose origin is not yet identified.
Xa is a tentative designation proposed by the Committee
on Genome Designation, International Triticeae Symposium
(R. Wang, personal communication).

Production of amphiploids

If the production of F_1 hybrids is the first step, then the production of a true breeding, and stable amphiploid is an important second step for a successful gene transfer. First of all, it allows more reliable evaluation of the genetic value of the alien genes in the genetic background of wheat. For quantitative, physiological or other traits that are difficult to assay, the genetic expression can be more reliably measured in the amphiploid than in the wild species. Moreover, an amphiploid is a permanent resource for detection and transfer of new traits and is a suitable control for basic and applied research. If the F_1 hybrid is highly sterile, the production of an amphiploid is mandatory to restore fertility. Even if backcross progenies can be produced, it is generally difficult to isolate a complete set of alien addition lines from the original cross. Because it may take several years to establish the relationships of a set of addition lines, it is likely that the original F_1 hybrid will no longer be available. If the original F_1 hybrid is alloplasmic, it may be impossible to produce a complete set of alien addition lines because of nucleo-cytoplasmic incompatibility in later generations (Sharma & Gill, 1983b; Jiang et al., 1992, 1993b). In such cases, it will be advantageous to produce an amphidiploid and use it as a male parent in the first backcross to wheat in order to derive alien additions in an euplasmic background. Finally, homoeologous chromosome pairing is not common in F_1 hybrids, but when it occurs, it may impair the integrity of the alien chromosomes in the isolated addition lines. This type of loss of chromosome integrity is unlikely to happen in an amphiploid.

Although amphiploid production is a highly desirable goal, successful amphiploids are difficult to produce and they are often genetically unstable. Many protocols, primarily employing colchicine, have been successfully used to produce amphiploids (see review

by Kaltsikes, 1974). However, if an F_1 hybrid cannot be chromosomally doubled, the lack of success may not only be due to the technique, but also the genetic nature of the F_1 hybrid. In an attempt to obtain an amphiploid from the *Elymus trachycaulus* × Chinese Spring hybrid, we used different techniques and treated several hundred hybrid plants. However, we failed to find a doubled plant sector. It appears that F_1 hybrids involving certain wheat varieties or certain strains of an alien species may be more amenable for amphiploid production than other varieties. The genotypes of wheat and the alien species may also affect the stability of the resulting amphiploids. For example, an amphiploid, produced from an *Elymus ciliaris* × Chinese Spring hybrid in our laboratory, is highly unstable. Howver, Japanese scientists were able to produce an amphiploid from a hybrid of *E. ciliaris* × Japanese wheat cultivar 'Inayama-komugi' and the amphiploid is highly stable and fully fertile (Muramatsu et al., 1983).

Production of wheat-alien chromosome addition and substitution lines

Production of a complete set of addition lines from an alien species is a formidable task and few sets are available in wheat (see review by Shepherd & Islam, 1988). The reasons for this are many. Some alien chromosomes, such as barley chromosome 5, cause sterility when they are added to wheat (Islam et al., 1978). Other reasons include gametocidal genes on alien chromosomes or other hybrid dysgenic phenomena (Endo, 1990) and preferential transmission or elimination of certain chromosomes (Jiang et al., 1993b). The univalent alien chromosomes often undergo centric breakage-fusion, leading to translocation chromosomes. Even if most monosomic addition lines can be obtained, the isolation of disomic addition lines is sometimes very difficult due to poor pollen transmission of the alien chromosomes. Several strategies for isolation of disomic addition lines have been proposed (O'Mara, 1940; Islam et al., 1978). The efficiencies of three different methods for producing disomic additions were compared and the backcross of a partially fertile heptaploid to an octaploid amphiploid appears to be the most efficient one (Lukaszewski, 1988).

Until recently, the production of substitution lines was a difficult, time-consuming but necessary task for the determination of genetic relationships between alien chromosomes and individual wheat chromosomes (Johnson, 1966; Sears, 1968; Dvořák, 1980). With widespread use of genetic markers, it is now relatively easy to determine the homoeology of the alien chromosomes added to wheat (Hart, 1987; Sharp et al., 1989). Once the homoeology of the alien chromosomes in the wheat-alien addition lines is determined, appropriate wheat monosomic lines can be selected and crossed to the addition line as an initial step in obtaining the substitution line. The substitution line can be recovered in the selfed progeny of the double monosomic line (Islam & Shepherd, 1992).

Wheat-alien substitution lines can be produced without using addition lines as an intermediary step. The technique described by Kota & Dvořák (1985) involves production of a nullisomic amphiploid from a cross between a monotelosomic and a diploid alien species and subsequent backcrosses of the nullisomic amphiploid as male to the monotelosomic. The wheat chromosome missing in the nullisomic amphiploid will be substituted by its homoeologous partner from the diploid alien species. However, for each substitution line, a separate amphiploid must be produced and this may be difficult. Moreover, the amphiploid may be unstable or sterile. In particular, the monotelosomic/amphiploid F_1, which must be crossed as a male with the monotelosomic female parent, may be male sterile.

The technique of Zhang et al. (1992) involves a cross between a wheat-alien amphiploid and a fertile wheat nullisomic line and backcrosses of the hybrids with the nullisomic line as male parent. The missing wheat chromosome in the nullisomic line will be substituted by its homoeologous alien chromosome in the amphiploid. This technique will be dependent on the availability of both an amphiploid and a fertile nullisomic line.

Production of wheat-alien chromosome translocations

Wheat-alien chromosome addition or substitution lines are usually used as bridge materials to generate wheat-alien chromosome translocations. Strategies for producing wheat-alien chromosome translocations based on wheat-alien additions or substitutions were reviewed by several authors (Sears, 1972, 1981; Gale & Miller, 1987; Feldman, 1988), and it is not necessary to describe the details of each technique here.

The first group of methods of inducing wheat-alien translocations is by exploiting homoeologous chromo-

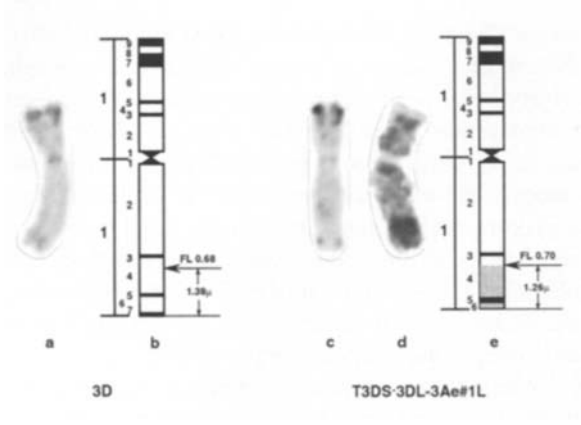

Fig. 1. Estimation of the size of the added alien chromosome segment and the missing wheat chromosome segment in wheat-alien translocation lines. Wheat-*Agropyron elongatum* translocation chromosome T3DS·3DL-3Ae#1L from wheat cultivar 'Agent' is used as an example. (a) C-banding pattern of normal chromosome 3D; (b) Idiogram of normal 3D; (c) C-banding pattern of T3DS·3DL-3Ae#1L; (d) Genomic *in situ* hybridization (GISH) pattern of T3DS·3DL-3Ae#1L; (e) Idiogram of T3DS·3DL-3Ae#1L. The chromosome segment from *A. elongatum* is shaded. The size of the chromosome segment from 3Ae#1L and the location of breakpoint can be directly measured on the translocation chromosome T3DS·3DL-3Ae#1L after GISH. The size of the missing segment on 3DL = the length of normal 3DL – (the lenght of the translocation arm 3DL-3Ae#1L – the length of the 3Ae#1L segment).

some pairing. Homoeologous pairing can be achieved by eliminating chromosome 5B (Sears, 1972), using the *ph1b* mutant (Sears, 1981; Koebner & Shepherd, 1985), or suppressing the effect of the *Ph1* gene (Riley et al., 1968). One recent accomplishment is the development of wheat lines carrying the Ph^I gene derived from *Aegilops speltoides* (Chen et al., 1994). The Ph^I gene which is also called the 'high pairing gene' suppresses the effect of the *Ph1* gene and permits homoeologous chromosome pairing (Riley et al., 1958; Dvořák, 1972; Kimber & Athwal, 1972). Because of the dominance of the Ph^I gene, wheat-alien translocations can be induced by the presence of a single dose of the Ph^I gene without a need to remove *Ph1*. The disadvantage of this technique is that the efficiency of inducing homoeologous pairing by the Ph^I gene is not as high as those obtained by eliminating 5B or using the *ph1b* mutant (Chen et al., 1994).

Spontaneous wheat-alien chromosome translocations occur frequently and form a second group of methods for homoeologous gene transfers. Wheat-alien chromosome recombination may occur in the derivatives of wheat-alien hybrids, albeit at a very low frequency. For example, 'Agent', a spontaneous wheat-*Agropyron elongatum* translocation line which carries a leaf rust resistance gene, *Lr24*, from *A. elongatum* may have arisen from a rare recombination because it involves exchange of similar-size fragments between homoeologous chromosome 3Ae#1 and 3D (Smith et al., 1968, see also Fig. 1). Another class of spontaneous wheat-alien translocations arises from centromeric-breakage and reunion and involves misdivision of univalent chromosome centromeres and reunion of the wheat and alien telocentric chromosome arms at the centromeres (Sears, 1972). The widely used wheat-rye T1BL.1RS translocation arose by this mechanism (Mettin et al., 1973; Zeller, 1973). The centromeric-breakage and reunion translocation can be directed by creating univalents involving specific wheat and alien chromosomes (Sears, 1972). Spontaneous wheat-alien translocations with non-centromeric breakpoints also occur. In our laboratory, spontaneous wheat-*E. trachycaulus* translocations with either centromeric or non-centromeric breakpoints were recovered in the alloplasmic derivatives of the *E. trachycaulus* × wheat hybrids. The translocations involved the *Elymus* chromosomes carrying fertility restoration (*Rf*) gene(s) (Jiang et al, 1993a). Plants with *Rf* carrier chromosomes or translocation chromosomes involving the *Rf* gene(s) selectively survived. Therefore, if a useful alien gene is present on a chromosome arm with a *Rf* gene, the target gene can be transferred to a wheat chromosome by selecting spontaneous wheat-alien translocations in the alloplasmic derivatives.

The third group of methods of inducing random wheat-alien translocations is either by ionizing irradiation (Sears, 1956), or tissue culture (Lapitan et al., 1984). A new technique is the application of gametocidal genes (Endo, 1988; Tsujimoto & Noda, 1988). Gametocidal genes from several *Aegilops* species can cause random chromosome breakage and then induce chromosome aberrations (see review by Endo, 1990). When the gametocidal genes are introduced into a wheat-alien addition or substitution line, random wheat-alien chromosome translocations can be recovered in the selfed progenies (Endo, 1988).

Although a large number of wheat-alien translocations carrying useful alien genes were produced, very few have been successfully incorporated into wheat cultivars. Most of the alien segments in the translocations either do not compensate well for the loss of wheat chromatin or contain undesirable genes. Wheat-rye translocations, especially T1BL.1RS and T1AL.1RS translocations, are the most successful wheat-alien translocations that have been used for

wheat improvement worldwide. The short arm of rye chromosome 1R confers resistance to several foliar diseases, including genes for resistance to powdery mildew (*Pm8* and *Pm17*), yellow rust (*Yr9*), stem rust (*Sr31*), and leaf rust (*Lr26*) (McIntosh, 1988; Heun et al., 1990) along with genetic factors for wide adaptation and high yield performance (Rajaram et al., 1983). However the presence of this rye chromosome arm in wheat also leads to poor dough quality of the flour and is unacceptable for bread making in some countries.

To break the linkage between useful and undesirable alien genes or to reduce the amount of alien chromatin in the wheat backgrounds, further wheat-alien recombinants can be produced by inducing homoeologous pairing (Sears, 1983; Koebner & Shepherd, 1985, 1986; Rogowsky et al., 1991). Wheat-rye recombinant lines isolated from a primary T1DL.1RS translocation have improved dough quality characteristics compared to the original translocation line (Koebner & Shepherd, 1988).

Characterization of wheat-alien chromosome translocations

Chracterization of a wheat-alien chromosome translocation includes the identification of the translocated chromosome, localization of the breakpoint, and estimation of the amount of transferred alien chromatin. Although conventional techniques, such as chromosome pairing analysis, may provide important information, they are inadequate to characterize wheat-alien translocations, especially interstitial translocations and those with non-centromeric breakpoints.

The development of chromosome banding techniques (see review by Gill et al., 1991) makes chromosome identification fast, reliable, and economical. The C-banding technique has been very effective to detect wheat-alien translocations, especially wheat-rye translocations because of the diagnostic terminal bands of rye chromosomes (Lukaszewski & Gustafson, 1983; Lapitan et al., 1984; Friebe & Larter, 1988). However, chromosome banding techniques are uninformative if the alien chromosome segments lack diagnostic bands. Banding polymorphism in different wheat genotypes sometimes also confuses the identification of the alien chromosome segments.

Dispersed species-specific repetitive DNA sequences have been used to characterize wheat-alien translocations (Appels & Moran, 1984; Zhang & Dvořák, 1989, 1990; Guidet et al., 1991; Rogowsky et al., 1991). The relative amounts of alien chromatin of different wheat-alien recombinant chromosomes can be determined from the number and intensity of bands in Southern blots (Zhang & Dvořák, 1989, 1990) or the intensity of the band on dot blots (Rogowsky et al., 1991). However, molecular analysis with species-specific repetitive DNA sequences does not identify the translocation chromosomes and the exact location of the breakpoints. The estimation of the relative amounts of alien chromatin also assumes uniform distribution of the repetitive DNA sequences in alien chromosomes which may or may not be the case. Unfortunately, it is difficult and time-consuming to isolate highly dispersed and uniformly-distributed species-specific repetitive DNA sequences.

Lapitan et al. (1986) were the first to successfully detect the breakpoints of wheat-rye translocations by *in situ* hybridization using a dispersed rye repetitive DNA sequence as a probe. Le et al. (1989) developed the technique of 'genomic *in situ* hybridization' (GISH). This technique uses genomic DNA from the alien species as probes in combination with an excess of unlabelled wheat DNA in the hybridization solution to block cross hybridizations. Wheat-alien translocations and their breakpoints can be clearly identified by GISH (Le et al., 1989; Heslop-Harrison et al., 1990; Mukai & Gill, 1991). However, GISH only allows the detection of alien chromosome segments. Chromosome banding analysis is required to identify the wheat chromosomes involved in the translocations (Friebe et al., 1992; Friebe et al., 1993; Mukai et al., 1993; Jiang et al., 1993c). The combination of chromosome banding and GISH techniques also allows the estimation of the size of the added alien chromosome segment and the missing wheat chromosome segment (Friebe et al., 1992; Friebe et al., 1993; Jiang et al., 1993c, see also Fig. 1).

Recently, a sequential chromosome banding and GISH technique was developed in our laboratory (Jiang & Gill, 1993). Using this techniques, the size, position, breakage and reunion point of the alien chromosome segment along with the identity of the wheat and alien chromosomes involved in the translocation can be determined in a single experiment.

Staff at the Wheat Genetics Resource Center have characterized a number of wheat germplasm lines by chromosome banding and *in situ* hybridization analysis (Table 2). Table 2 also includes previously unpublished work on analysis of three near-isogenic germplasm lines (K3304, K2056, and K2046, provided by Dr. D.R. Knott) carrying rust resistance genes *Sr24/Lr24*,

Table 2. Wheat germplasm lines characterized in Wheat Genetics Resource Center at Kansas State Univerity

Germplasm	Alien species	Alien target gene(s)	Wheat-alien translocation	Size of alien segment	Size of missing wheat segment	References
Amigo	*Secale cereale*	Resistance to powdery mildew (*Pm17*)	T1AL·1RS	1RS	1AS	Lapitan et al. 1986
88HF16	*Secale cereale*	Resistance to Hessian fly (*H25*)	T6BS·6BL-6RL	6.95 μ	–[2]	Mukai et al. 1993
88HF79	*Secale cereale*	Resistance to Hessian fly (*H25*)	T4BS·4BL-6RL	3.88 μ	–[2]	Mukai et al. 1993
88HF89	*Secale cereale*	Resistance to Hessian fly (*H25*)	T4AS·4AL-6RL-4AL	0.70 μ	none	Mukai et al. 1993
KS85HF011-5	*Secale cereale*	Resistance to Hessian fly (*H25*)	T2BS·2RL	2RL	2BL	Friebe et al. 1990
CI17766	*Agropyron intermedium*	Resistance to WSM[1] (*Wsm1*)	T4AL·4Ai#2S	4Ai#2S	4AS	Friebe et al. 1991b
W49	*Agropyron intermedium*	Resistance to leaf rust (*Lr38*)	T2AS·2AL-7Ai#2L	2.4 μ	1.4 μ of 2AL	Friebe et al. 1992
T4	*Agropyron intermedium*	Resistance to leaf rust (*Lr38*)	T3DL·3DS-7Ai#2L	2.78 μ	0.67 μ of 3DS	Friebe et al.1993
T7	*Agropyron intermedium*	Resistance to leaf rust (*Lr38*)	T6DS·6DL-7Ai#2L	4.19 μ	1.45 μ of 6DL	Friebe et al. 1993
T24	*Agropyron intermedium*	Resistance to leaf rust (*Lr38*)	T5AL·5AS-7Ai#2L	4.20 μ	0.88 μ of 5AS	Friebe et al. 1993
T25	*Agropyron intermedium*	Resistance to leaf rust (*Lr38*)	T1DS·1DL-7Ai#2L	2.55 μ	0.82 μ of 1DL	Friebe et al.1993
CI15322	*Agropyron elongatum*	Resistance to WSM[1]	T4DS·4DL-1Ae#1L	1.31 μ	0.73 μ of 4DL	Jiang et al.1993c
K3304	*Agropyron elongatum*	Resistance to stem rust (*Sr24*) and leaf rust (*Lr24*)	T3DS·3DL-3Ae#1L	1.26 μ	1.38 μ of 3DL	Present study
K2056	*Agropyron elongatum*	Resistance to stem rust (*Sr25*) and leaf rust (*Lr19*)	T7DL·7DS-7Ae#1L	2.55 μ	2.62 μ of 7DS	Present study
K2046	*Agropyron elongatum*	Resistance to stem rust (*Sr26*)	T6AS·6AL-6Ae#1L	2.73 μ	3.63 μ of 6AL	Present study

[1] WSM, wheat streak mosaic. [2] No data available.

Sr25/Lr19, and *Sr26* from *A. elongatum.* These genes have been incorporated into a number of wheat cultivars (McIntosh, 1988). K3304 was derived from 'Agent', a wheat variety containing a spontaneous wheat-*A. elongatum* translocation (Smith et al., 1968). C-banding and GISH analysis revealed that an *A. elongatum* chromosome segment from 3Ae#1L with a size of 1.26 μ replaced a 1.38 μ wheat chromosome segment at the distal part of 3D long arm (Table 2, Fig. 1). The translocated chromosome can be described as T3DS·3DL-3Ae#1L. The original wheat-*A. elongatum* translocations T7DS·7DL-7Ae#1L in K2056 and T6AS·6AL-6Ae#1L in K2046 were both derived from x-ray treatments (Knott, 1968). The breakpoints of these two translocations are close to the centromeres.

The size of the *Agropyron* chromosome segments are 2.55 μ and 2.73 μ, respectively.

Evaluation of different strategies for producing wheat-alien chromosome translocations

Wheat-alien translocations induced by homoeologous pairing

The main advantage of inducing translocations by homoeologous pairing is the directional manipulation for the translocations to occur between the alien chromosome and a specific wheat chromosome. A specific wheat chromosome can be selected to recombine preferentially with a specific alien chromosome. In an experiment attempting to induce translocations between *A. elongatum* chromosome 3Ae and wheat chromosome 3D (Sears, 1972), 17 of the 20 resulting translocations involved 3D, the other three involved 3B (Sears, 1978, 1981). Because the translocations involved homoeologous chromosomes of wheat and the alien species, the transferred alien chromosome segment will genetically compensate for the missing wheat segment.

Careful consideration should be given to the recipient chromosome to be selected as a 'home' for the alien segment. Structurally modified chromosomes, such as 4A, 5A, and 7B which are involved in a cyclical translocation (Naranjo et al., 1987; Liu et al., 1992), should be avoided as candidates for alien chromosome translocations. Similarly, chromosome arms containing pivotal genes involved in the diploidization of the wheat genome, i.e. 2AS, 4BS and 6BS with fertility genes and 3DS and 5BL with *Ph* (pairing homoeologous) genes cannot be replaced with alien segments because it may have severe effects in genomic stability and fertility.

Apart from these considerations, the major disadvantage of this strategy is that the efficiency is limited by the potential of chromosome pairing and recombination between the alien chromosome and its wheat homoeologues. The recombination rate of alien chromosomes from some species, such as rye, with those of wheat is very low (Koebner & Shepherd, 1986). If the alien chromosomes are structurally modified as in the case with most rye chromosomes (Devos et al., 1993), their chances of recombining with wheat chromosomes are extremely low. Even if they do recombine in a proximal region, most recombinant chromosomes will be non-compensating and agronomically undesirable.

Recent genetic analysis has shown that genetic recombinations occur more frequently near the telomeric area than in the proximal half of a chromosome arm (Curtis & Lukaszewski, 1991; Werner et al., 1992). We have analyzed the 3D-3Ae recombinant chromosomes produced by induced homoeologous pairing by Dr. E.R. Sears (1972). The breakpoints of most of the translocations are located at the middle of the chromosomes (our unpublished data). Therefore, if the target alien gene is located near the centromeric or subcentromeric area, the efficiency of inducing useful translocations by homoeologous pairing will be further reduced.

Wheat-alien translocations induced by irradiation

Ionizing radiation breaks chromosomes at random. It has several advantages for the induction of wheat-alien translocations. First, the efficiency will not be affected by the location of the target gene on the alien chromosome and the pairing potential between the alien and wheat chromosomes. Second, the alien chromosome segment containing the target gene could be theoretically inserted into a wheat chromosome without losing any wheat chromatin. However, in such a case the size of the transferred alien segment must be small to minimize deleterious effects arising from gene duplication.

The biggest disadvantage of the irradiation technique is the genetic imbalance associated with most of the translocations. The translocations between the alien chromosome and nonhomoeologous wheat chromosomes most likely result in deficiencies for a wheat segment and duplication of genes carried by the alien segment and its wheat homoeologues. In the first such experiment conducted by Dr. E.R. Sears (1956), only one of the 17 wheat-*Aegilops umbellulata* translocations was not deleterious. Knott (1968) pointed out that translocation involving homoeologous chromosomes occur preferentially. However, the relatively high frequency of recovery of compensating translocations probably resulted from the agronomic selection pressure during subsequent germplasm development. The recent characterization of rust-resistant wheat-*Agropyron intermedium* translocations showed that the x-ray-induced wheat-*Agropyron* translocations occurred randomly and none of them involved homoeologous chromosomes (Friebe et al., 1992, 1993).

Because of the genetic imbalance, only a few wheat-alien translocations produced by irradiation have been exploited in wheat breeding. The most successful example is the wheat-*A. elongatum* translo-

cation T6AS·6AL-6Ae#1L that carries the stem rust resistance gene *Sr26*. This translocation was incorporated into a number of Australian wheat cultivars and resistance conferred by gene *Sr26* has been durable (Knott, 1987). The results of cytological characterization of this translocation showed that T6AS·6AL-6Ae#1L probably involved a replacement of a nearly complete 6AL with a nearly complete 6Ae#1L. The deficiency of wheat chromatin of 6AL and/or the duplication of genes carried by 6Ae#1L in this translocation is probably small, resulting in a well-compensated translocation. However, wheat cultivars with this translocaion may still suffer a yield penalty of 9% (The et al., 1988).

Radiation treatment not only induces wheat-alien translocations, but also causes other chromosome aberrations in the wheat genomes. The stabilization of a useful translocation may need several generations of backcrossing. These types of chromosome aberrations were detected in some of the released wheat germplasm derived from irradiation treatment. For example, in addition to the T1AL.1RS translocation, several chromosomes of 'Amigo' wheat, a wheat-rye translocation line selected for greenbug resistance, were modified (Heun et al., 1990). In addition to a wheat-*A. elongatum* translocation, the wheat streak mosaic virus resistant germplasm line, CI15322, also has a 2A-2D reciprocal translocation (Jiang et al., 1993c), and a modified 4A chromosome (our unpublished data).

A most desirable type of wheat-alien translocation would be the insertion of a small alien segment carrying the target gene into a wheat chromosome without loss of wheat chromatin. Although this type of translocation can be theoretically produced by irradiation treatment, the chance of the recovery of such a translocation is very low because it requires two chromosome breaks near the target gene, one break in a wheat chromosome, and the reunion of these chromosome segments. A strongly favorable selection pressure is also important for detecting such translocations. So far only one irradiation induced intercalary wheat-alien translocation (Ti4AS·4AL-6AL-4AL) was cytologically characterized, where a gene for resistance to Hessian fly derived from rye chromosome arm 6RL was inserted in the long arm of wheat chromosome 4A (Friebe et al., 1991a; Mukai et al., 1993).

Wheat-alien translocations induced by tissue culture

Plants regenerated from tissue culture exhibited various chromosome aberrations, including chromosome translocations (see review by Larkin & Scowcroft, 1981). Thus, this technique could be used to induce wheat-alien translocations in hybrids and wheat-alien addition or substitution lines. Although the mechanism of the generation of translocations is not known, limited results from cytological analyses of tissue culture-derived plants showed that the translocations involved non-homoeologous chromosomes (Lapitan et al., 1984). Therefore, this technique may introduce similar problems to the irradiation method, i.e. the genetic imbalance of the resulting translocations.

Because of the limited use of this technique, the tissue culture-derived wheat germplasm lines with alien genes have not been adequately assessed. Recently, introgression of a cereal cyst nematode resistance gene(s) from rye chromosome 6R and a barley yellow dwarf virus resistance gene(s) from *A. intermedium* chromosome L1 into wheat was reported (Larkin et al, 1990). However, the resistant lines have not been cytologically characterized. Therefore, the potential and efficiency of introducing alien genes into wheat through tissue culture still needs to be proved.

Concluding remarks

Considerable progress has been made in alien gene transfer from distantly-related species into wheat since an earlier review by Sharma & Gill (1983a). The range of wide hybridization of wheat has been greatly expanded with the improvement of crossing techniques. Crosses between wheat and any of the several hundred species in the Triticeae and beyond, including species such as maize, sorghum, and pearl millet, are possible. However, posthybridization barriers such as chromosome elimination, preferential transmission of certain alien chromosomes, and adverse genetic interactions leading to hybrid dysgenesis, chromosome breakage, and sterility impede further progress in alien transfer. Diverse selection of host and donor genotypes in the initial hybridization can often overcome some of these barriers.

The production of true breeding amphiploids and alien addition lines are necessary steps for a successful gene transfer. They allow thorough evaluation and expression of target traits and a permanent genetic repository for further evaluation and transfer of additional traits from the target species. Alien addition lines are also valuable for gene synteny analysis. The information on gene synteny of the alien chromosome is critical for choosing a method to induce wheat-alien

translocations. If the gene synteny is conserved and the target gene has a distal chromosomal location, then induced homoeologous pairing is the method of choice. However, if gene synteny is disrupted or the gene has a proximal location, then ionizing irradiation is the method of choice.

The need for sensitive methods of chromosome, chromatin or gene detection for a successful alien gene transfer cannot be overemphasized. The methods of chromosome banding, *in situ* hybridization, and RFLP probes have greatly expedited the production of alien addition and translocation lines. However, some of these methods of analysis are highly technical and expensive; national and international networking and collaborative research in wide hybridization is recommended (see Raupp et al., 1993 for an example of networking in wheat germplasm enhancement research).

Finally, a question that begs discussion is whether transgenic technology (Vasil et al, 1992; Weeks et al., 1993) means the end of wide hybridization research as we know it now? The answer is an emphatic 'no!'. Transgenic techniques merely add another tool for manipulation of single gene traits in the arsenal of a breeder to improve a crop plant. The following arguments support the continued need and future acceleration of wide hybridization research: i) Land races and wild species will continue to be inexhaustible reservoirs of genetic diversity (i.e. heterotic vigor, resistance to environmental and biological stresses, physiological and quality traits) and wide hybridization is the best means to utilize this variation. ii) Wide hybridization, production of addition and translocation lines are necessary steps for genetic characterization of the alien phenotypic traits. iii) In a polyploid crop like wheat, transfer of adaptive linkage blocks may be more desirable than single gene transfers. An outstanding example is the rye chromosome arm 1RS which when substituting for 1BS (or 1AS) of wheat, adds significant yield potential to wheat worldwide (Rajaram et al., 1983). It is our hope that this review of current work and future outlook will stimulate further research in wide hybridization of crop plants.

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