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Detection of homoeologous chiasma formation in *Triticum durum* x *Thinopyrum bessarabicum* hybrids using genomic *in situ* hybridization

I. P. KING, K. A. PURDIE, S. E. ORFORD, S. M. READER & T. E. MILLER
Cambridge Laboratory, JI Centre, Colney Lane, Norwich NR4 7UJ, U.K.

Genomic *in situ* hybridization (GISH) was used to study the nature of homoeologous chiasma formation in crosses between *Triticum durum* cv. Creso, homozygous for the *ph1c* mutation and *Thinopyrum bessarabicum*. The relative frequencies of wheat/wheat and wheat/*Th. bessarabicum* chiasma formation were determined. Pairing between apparently non-homologous *Th. bessarabicum* chromosomes was also observed. The potential of GISH as a tool for analysing homoeologous chiasma formation in wheat/alien hybrids is discussed.

Keywords: genomic *in situ* hybridization, homoeologous recombination, *Thinopyrum bessarabicum, Triticum durum.*

Introduction

Wheat, which belongs to the tribe Triticeae, can be sexually hybridized with many of its wild relatives. Although the genomes of wheat and the alien species in such hybrids are related, with a few notable exceptions such as wheat x *Aegilops speltoides*, very little chiasma formation between wheat/wheat and wheat/alien homoeologous chromosomes occurs (Riley *et al.*, 1959). This is the result of the action of pairing control genes, located on wheat chromosomes, which restrict chiasma formation to homologous chromosomes (for review see Gale & Miller, 1987). The strongest of the pairing control genes, *Ph1*, is located on the long arm of wheat chromosome 5B (Okamoto, 1957; Riley & Chapman, 1958). In hybrids which lack *Ph1*, obtained by crossing alien species with lines of wheat which either lack chromosome 5B or in which *Ph1* has been mutated (Sears, 1977; Giorgi, 1983), homoeologous pairing can occur.

The study of the relative frequency of homoeologous chiasma formation between wheat chromosomes and alien chromosomes in high pairing hybrids is important for two reasons. First, alien species have been found to carry agronomically useful genes. The introduction of these genes to wheat can be achieved by recurrently backcrossing high pairing wheat x alien hybrids with the wheat parent and selecting for the presence of the target gene at each generation. The ultimate aim of this process is to introduce into wheat a small segment of alien chromatin containing the target gene (for review see Gale & Miller, 1987). A knowledge of the relative frequency of wheat/alien chiasma formation in wheat/alien hybrids is of interest as it gives an indication of the likelihood of transferring alien genes to wheat. Secondly, the relative frequency of wheat/alien and wheat/wheat homoeologous chiasma formation in high pairing wheat x alien hybrids is important as it provides a measure of the closeness of the relationship between the genomes of wheat and the genome(s) of the wild species.

The determination of the frequency of homoeologous chiasma formation in wheat x alien hybrids requires that the chromosomes of each parent can be identified in pollen mother cells (PMCs). One way in which this can be determined is to highlight differentially either the wheat or the alien chromosomes using genomic *in situ* hybridization (GISH) (Schwarzacher *et al.*, 1992). The work in this paper describes the use of GISH to determine the nature and frequency of homoeologous chiasma formation in a hybrid between the macaroni wheat *Triticum durum* cv. Creso, homozygous for the *ph1c* mutation, and the sand couch grass *Thinopyrum bessarabicum* which has been shown to

Correspondence: I. P. King.

carry a gene(s) conferring salt tolerance (Forster *et al.*, 1987, 1988).

Materials and methods

The plant material used consisted of the *T. durum* Desf. cv. Creso homozygous for the *ph1c* mutation ($2n = 4x = 28$, AABB) and *Th. bessarabicum* (Savul. and Rayss) Löve ($2n = 2x = 14$, E^bE^b).

Creso *phlc* x *Th. bessarabicum* hybrids were produced as follows: emasculated spikes of Creso *ph1c* were pollinated with *Th. bessarabicum*; the following day the internodes and the florets of the pollinated spikes were treated with 10 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) as described by Laurie & Reymondie (1991) with the modification that 10 mg l^{-1} of $AgNo_3$ was added to the 2,4-D solution (Laurie & O'Donoughue, 1993). Embryo rescue was performed as described by Laurie & Reymondie (1991) with the exception that Gamborg B5 medium was used instead of Difco Orchid agar.

DNA was extracted from leaves of Creso *ph1c* and *Th. bessarabicum* by the method described by Sharp *et al.* (1988). Probe preparation and *in situ* hybridization were performed as described by Schwarzacher *et al.* (1992) with the following modifications. Both *Th. bessarabicum*, used as probe, and Creso *ph1c*, used as unlabelled blocking DNA, were mechanically sheared using a Decon Fs minor sonicating water bath. Creso *ph1c* was used as unlabelled blocking DNA at concentrations between 60 and 80 times greater than that of the *Th. bessarabicum* probe DNA (70 ng per slide). Anthers from Creso *phlc* x *Th. bessarabicum* hybrids containing PMCs at metaphase I of meiosis were placed in 45 per cent acetic acid for 10 min prior to slide preparation. Slides were analysed by epifluorescence microscopy using a Nikon Microphot-SA microscope. Photographs were taken using Kodak Ekta 1000 ASA print film.

Results

Three Creso *phlc* x *Th. bessarabicum* hybrid embryos were obtained from six pollinated spikes. Each of the three embryos rescued gave rise to a mature 21-chromosome plant. The majority of PMCs examined contained dense cytoplasm. It was not possible to score these PMCs for the frequency of wheat/wheat and wheat/*Th. bessarabicum* chromosome pairing as both the wheat and *Th. bessarabicum* chromosomes appeared to fluoresce with equal intensity. However, in 66 PMCs, which appeared to contain considerably less cytoplasm, the wheat and *Th. bessarabicum* chromosomes could be clearly distinguished.

In total, 79 chiasmata resulting in the formation of 75 chromosome associations (74 bivalents and one trivalent), were observed (Table 1). In no case were paired chromosome arms with more than one chiasma observed. Rod bivalents were by far the most frequent type of configuration observed, (94.6 per cent). Only three ring bivalents (4 per cent) and a single trivalent (1.3 per cent) were seen. Of the 79 chiasma scored 65 (82.3 per cent) occurred between wheat chromosomes presumably A and B genome homoeologues (Fig. 1a), 10 (12.7 per cent) between wheat chromosomes and the E^b genome chromosomes of *Th. bessarabicum* (Fig. 1b) and four (5.1 per cent) were between E^b genome chromosomes (Fig. 1c). These latter four must be the result of non-homologous *Th. bessarabicum*/*Th. bessarabicum* chromosome pairing. The number of wheat/wheat bivalents in the 66 PMCs examined ranged from 0 to 3 per PMC as compared with a range of 0 to 1 for the wheat/*Th. bessarabicum* and the *Th. bessarabicum*/*Th. bessarabicum* bivalents.

Overall, chiasma formation between a wheat and *Th. bessarabicum* chromosome was observed in nine out of 66 (13.6 per cent) of the PMCs examined.

Table 1 The number of wheat/wheat, wheat/*Th. bessarabicum* and *Th. bessarabicum*/*Th. bessarabicum* homoeologous chromosome pairing configurations in 66 PMCs from three Creso *phlc* x *Th. bessarabicum* hybrids

W/W		W/*Th*		W/W/*Th*	*Th*/*Th*	
Rod Bivalent	Ring Bivalent	Rod Bivalent	Ring Bivalent	Trivalent	Rod Bivalent	Ring Bivalent
60	2	7	1	1	4	0

W = wheat; *Th* = *Th. bessarabicum*.

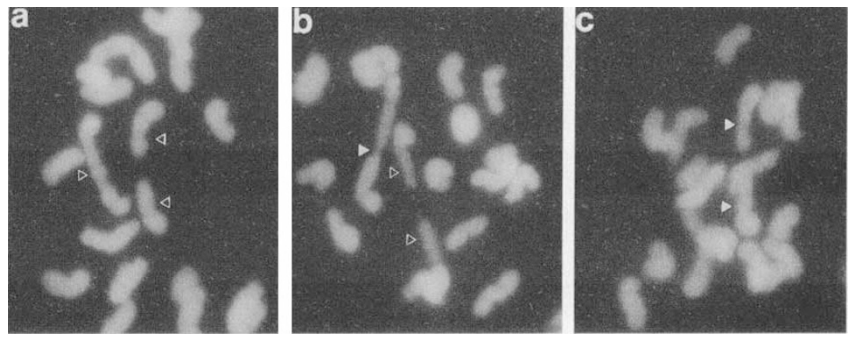

Fig. 1 PMCs showing (a) two wheat bivalents (open triangles), (b) a wheat/*Th. bessarabicum* bivalent (closed triangle) and a wheat/wheat bivalent (open triangles) and (c) a single *Th. bessarabicum*/*Th. bessarabicum* bivalent (closed triangles).

Discussion

Using GISH it was possible to determine the relative frequency of wheat/wheat (82.3 per cent), wheat/*Th. bessarabicum* (12.7 per cent) and *Th. bessarabicum*/*Th. bessarabicum* (5.1 per cent) recombination. The frequency of wheat/wheat homoeologous pairing was considerably higher than wheat/*Th. bessarabicum* homoeologous pairing, demonstrating that the A and B genomes of wheat are more closely related to each other than to the E^b genome of *Th. bessarabicum*. A study of the relative frequencies of wheat/wheat and wheat/alien recombination provides a means by which genomic relationships can be analysed. It is expected that an alien species more closely related to wheat than *Th. bessarabicum* would have a higher frequency of wheat/alien recombination while a more distantly related species would have a lower frequency of recombination. However, such information should be treated with caution because if a wild species carries extensive translocation differences relative to wheat the frequency of wheat/alien recombination may be significantly reduced. Moreover, alien species may also carry genes which promote or restrict chromosome pairing.

Chiasmata have been demonstrated to be the result of exchange events between chromatids of different chromosomes (Tease & Jones, 1978). Thus the study of chiasma formation provides a valuable method of estimating the frequency of recombination in hybrids (Naranjo *et al.*, 1989). The frequency of chiasma formation between wheat and alien chromosomes is of interest as it gives an indication of the ease or the difficulty with which a chromatin segment from a wild species can be introgressed into wheat. *Th. bessarabicum*, the wild species used in this study, is of interest as it carries genes conferring salt tolerance (Forster *et al.*, 1987, 1988). Soils that contain a high concentration of salt are proving to be a major world problem which is increasing due to the extensive use of irrigation. In this work wheat/*Th. bessarabicum* chiasmata were shown to occur in 13.6 per cent of the PMCs examine, indicating that it should be possible to transfer a gene conferring salt tolerance from *Th. bessarabicum* to wheat.

Four *Th. bessarabicum*/*Th. bessarabicum* rod bivalents were observed in four different PMCs. The rationale for pairing between apparently unrelated chromosomes is unknown. However, similar pairing has previously been observed between non-homologous rye chromosomes in wheat x rye hybrids (Hutchinson *et al.*, 1983). One possibility is that in the absence of a pairing partner chiasma formation occasionally occurs between homologous repetitive DNA sequences which are distributed throughout the genome resulting in bivalent formation between otherwise unrelated chromosomes.

It would not have been possible to determine the frequency of homoeologous chiasma formation nor the presence of *Th. bessarabicum*/*Th. bessarabicum* bivalents using Feulgen or carmine stained preparations since these techniques would not distinguish wheat from *Th. bessarabicum* chromosomes. Although multivalents, which have previously been assumed to indicate wheat/alien chiasma formation, could have been identified using these techniques, in this work considerably more wheat/*Th. bessarabicum* bivalents (eight) were observed than wheat/*Th. bessarabicum* multivalents (one). This indicates that the frequency of multivalent formation cannot be used as a reliable estimate of the relative frequency of wheat/*Th. bessarabicum* chiasma formation.

To date the only other method of accurately studying homoeologous pairing in wheat/alien F_1 hybrids is by using C-banding (Hutchinson *et al.*, 1983; Jouve & Giorgi, 1986). However, to determine the frequencies of homoeologous chiasma formation accurately it is necessary that each of the alien chromosomes has a pattern distinct from those of the wheat chromosomes which may not always be the case. This is not a problem using GISH where alien chromosomes can be clearly distinguished providing that the wild species is sufficiently diverged from wheat.

In this work total genomic *Th. bessarabicum* DNA was used as a probe allowing the E^b genome chromo-

somes to be distinguished from the A and B genomes of wheat and hence it was possible to determine the relative frequency of wheat/wheat and wheat/*Th. bessarabicum* chiasma formation. However, it was not possible to determine if the frequency of pairing of the A and B genome chromosomes with the E^b genome chromosomes was the same. A difference in the frequency of chiasma formation would indicate that one of the wheat genomes might be more closely related to the E^b genome than the other. It is therefore desirable that techniques are developed such that all the chromosomes of each genome in a hybrid can be identified. In the case of the *T. durum* x *Th. bessarabicum* hybrids, used in this work, this might be achieved by using two total genomic probes, i.e. *Th. bessarabicum* and *T. urartu* (A-genome), labelled with different fluorochromes, and unlabelled blocking DNA from a third genome, i.e. *Ae. speltoides* which is thought to be closely related to the B genome donor.

One drawback with using GISH to study the frequency and nature of wheat/alien chiasma formation in the Creso x *Th. bessarabicum* hybrids was that the frequency of scorable PMCs was low. This appeared to be related to the variable amount of cytoplasm retained by the PMCs in the preparations, i.e. the *in situ* technique failed to work in cells with dense cytoplasm but worked in cells which appeared to contain less cytoplasm. Recent work (manuscript in preparation), however, has revealed that the frequency of scorable PMCs can be considerably increased by treating slides with proteinase K prior to the addition of the probe mix.

In our hands GISH proved to be a robust and valuable technique for studying meiosis in a wheat x alien hybrid.

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References

FORSTER, B. F., GORHAM, J. AND MILLER, T. E. 1987. Salt tolerance of an amphidiploid between *Triticum aestivum* and *Agropyron junceum*. *Plant Breeding*, **98**, 1–8.

FORSTER, B. F., MILLER, T. E. AND LAW. C. N. 1988. Salt tolerance of two wheat-*Agropyron junceum* disomic addition lines. *Genome*, **30**, 559–564.

GALE, M. D. AND MILLER, T. E. 1987. The introduction of alien genetic variation in wheat. In: F.G.H. Lupton (ed.) *Wheat Breeding: its scientific basis*, pp. 173–210.

GIORGI, B. 1983. Origin, behaviour and utilization of a *Ph1* mutant of *durum* wheat, *Triticum turgidum* (L.) var. *durum*. In: Sakamoto, S. (ed.) *Proc. 6th Int. Wheat Genet. Symp. Kyoto, Japan*, pp. 1033–1040.

HUTCHINSON, J., MILLER, T. E. AND READER, S. M. 1983. C-banding at meiosis as a means of assessing chromosome affinities in the triticeae. *Can. J. Genet. Cytol.*, **25**, 319–323.

JOUVE, N. AND GIORGI, B. 1986. Analysis of induced homoeologous pairing in hybrids between 6x triticale *ph1* mutant and *Triticum aestivum* L. *Can. J. Genet. Cytol.*, **28**, 696–700.

LAURIE, D. A. AND REYMONDIE, S. 1991. High frequencies of fertilization and haploid seedling production in crosses between commercial hexaploid wheat varieties and maize. *Plant Breeding*, **106**, 182–189.

LAURIE, D. A. AND O'DONOUGHUE, L. S. 1993. Wheat x maize crosses for the production of wheat haploids. *Agriculture and Forestry*, **25**, in press.

NARANJO, T., FERNANDEZ-RUEDA, P., GOICOECHEA, P. G., ROCA, A. AND GIRALDEZ, R. 1989. Homoeologous pairing and recombination between the long arms of group 1 chromosomes in wheat x rye hybrids. *Genome*, **32**, 293–301.

OKAMOTO, M. 1957. Asynaptic effect of chromosome V. *Wheat Inf. Serv.*, **5**, 6.

RILEY, R. AND CHAPMAN, V. 1958. Genetic control of the cytologically diploid behaviour of hexaploid wheat, *Nature*, **182**, 713–715.

RILEY, R., CHAPMAN, V. AND KIMBER, G. 1959. Genetic control of chromosome pairing in intergeneric hybrids with wheat. *Nature*, **183**, 1244–1246.

SCHWARZACHER, T., ANAMTHAWAT-JONSSON, K., HARRISON, G. E., ISLAM, A. K. M. R., JIA, J. Z., KING, I. P., LEITCH, A. R., MILLER, T. E., READER, S. M., ROGERS, W. J., SHI, M. AND HESLOP-HARRISON, J. S. 1992. Genomic *in situ* hybridization to identify alien chromosome segments in wheat. *Theor. Appl. Genet.*, **84**, 778–786.

SEARS, E. R. 1977. An induced mutant with homoeologous pairing in common wheat. *Can. J. Genet. Cytol.*, **19**, 585–593.

SHARP, P. J., KRIES, M., SHEWRY, P. R. AND GALE, M. D. 1988. Location of β-amylase sequences in wheat and its relatives. *Theor. Appl. Genet.*, **75**, 286–290.

TEASE, C. AND JONES, G. H. 1978. Analysis of exchanges in differentially stained meiotic chromosomes of *Locusta migratoria* after BrdU-substitution and FPG staining. 1. Crossover exchanges in monochiasmate bivalents. *Chromosoma*, **69**, 163–178.