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FLUORESCENT IN SITU HYBRIDIZATION AS A GENETIC TECHNOLOGY TO ANALYZING CHROMOSOMAL ORGANIZATION OF ALIEN WHEAT RECOMBINANT LINES

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Abstract

Fluorescent in situ hybridization is a valuable method for physical mapping of DNA sequence to chromosomes and genomes and to analyzing their organization, diversity, evolution and function. Using genomic DNA the origin of chromatin in hybrids and alien introgression lines can be identified and followed through breeding programmes. We have applied this technology to study the chromosome composition of new recombinants and genomes derived from spontaneous and induced translocations in particular involving rye and the goat grass *Thinoyrum intermedium* that transfer disease and stress resistance to wheat. We have established flow diagrammes for easy identification of the alien chromosome material.

1. INTRODUCTION

Plant breeders have been remarkably successful in developing new varieties of most major crops, with a significant acceleration in the last 50 years [1,2]. Modern crop varieties deliver higher yields and better quality than their predecessors and this has been achieved despite the continuing emergence of new diseases and new races of diseases (biotic stress), changing cultivation practices, and movements in areas where crops are grown, with exposure to new environmental (abiotic) stresses. The potential yield increase and the resistances exclusively arise from improvements in the genetic constitution of the plants; a study of cereals in the UK shows that 90% of the recent increase in yield can be attributed to improved varieties [3].

It is important that the plant breeders continue with their success, meeting the challenges of continuing to increase yields, diseases adapting to current varieties, changing climates including water and salinity, and new market requirements for varieties suitable for storage and processing. A remarkably similar range of challenges face crops whether they are grown by subsistence farmers or smallholders, or large commercial operators, and whether in developing or industrialized countries. It is also essential that the successful genetic improvement strategies applied in the major crops are available widely for minor, niche and high-value species.

New genes, gene regulation and combinations of genes are needed to meet the new challenges faced by crops. Most crop species have a genetic bottleneck in their ancestry associated with a small number of genes critical to domestication see Ref. [2] Vaughan *et al.,* 2007 and 'Crop Domestication' Annals of Botany Special Issue 100/5; 2007), so the full range of variation present in the wild genepool is not available. It is also possible that intensive crossing, selection and improvement over recent decades has eliminated not only undesirable genetic alleles but also reduced the useful variation for generation of new varieties, although the evidence for this is equivocal: [4] found no reduction in variation in bread wheat and genetic diversity of wheat has remained at least constant for 70 years [5] while only some reduction in variation in durum wheat was reported by [6].

New gene alleles can be generated by induced mutation, and procedure that can be very beneficial in correcting well-defined weaknesses in existing varieties. The 2000 mutant varieties produced by direct use of induced mutations (IAEA Mutant Variety and Genetic Stocks Database, http://mvgs.iaea.org) all have important new characters but maintain other quality and agronomic attributes of their progenitor.

The genepool contained in wild species is also an important source for new variation to introduce new characters into the gene pool. This can be accessed by sexual crossing of cultivated and wild relatives of crops. Within the cereals, the Triticeae tribe in the grasses includes wheat, barley, rye and some forage grasses. The whole tribe can be intercrossed using appropriate techniques (including crossing intermediates or bridge-crosses, chromosome doubling and tissue culture rescue of embryos), and thus forms a single pool with enormous variation available at each genetic locus. To exploit the variation, characterization of the chromosomal constitution of the parents, design of crossing and backcrossing strategies, and analysis of chromosomes and chromosome segments that are transferred is essential. In this chapter, we show methods that allow detailed analysis of the introgressed alien (wild) chromatin into crop species, an approach which can accelerate and improve success rates in practical breeding.

A key method is in situ hybridization, allowing alien chromatin to be identified in chromosome preparations of alien-cross derived plants. Because of the evolutionary separation of the progenitor species, total genomic DNA, with a high content of genome specific repeats, can be used as probe for in situ hybridization to identify chromosomes and chromosome segments of different origin [7,8]. It has therefore become the method of choice when interspecific crosses and derived introgressed lines are analysed to reveal alien chromosomes and translocations [9-17].

For in situ hybridization, genomic DNA can be combined easily with cloned probes to give further information about the specificity of the chromosome involved in the introgression or detect rearrangements. In the Triticeae several repetitive probes are cloned and can be used for identification of chromosomes and often are specific for certain genomes [18-22]. Physical mapping of differentially labelled repetitive DNA sequences simultaneously in one experiment and in combination with total genomic DNA allows to detect the presence of introgressed chromosomes or chromosome segments from alien species in wheat lines, identifying the chromosomes involved and determining the nature and organization of any chromosome rearrangements [10,13-15,23-26].

In this report, we aim to show two examples of the analysis using in situ hybridization of wild wheat germplasm and the introgression of alien wheat chromosomes or chromosome segments in material with novel genetic variation which is of value to plant geneticists and breeders.

2. MATERIAL AND METHODS

2.1. Lines

The following wheat lines and varieties were used: *Triticum aestivum* hexaploid wheat 'Chinese Spring', and 'Chinese Spring' line carrying a 1DL.1RS translocation form rye 'Imperial' and variety 'Beaver' carrying a 1BL.1RS translocation.

Fixed triticale x wheat derivatives (RL lines) from CIMMYT material were selected after continuous selfing and growing in the remote regions of Trans- Himalayas.

Wheat breeding lines with *Wsm-1* from the materials evaluated in the study of Ref. [27] including the line subsequently named '*Mace*' [28].

2.2. Chromosome and probe preparation

Seeds were germinated on Petri dishes in the dark and 1-2cm long emerging roots were fixed in alcohol:acetic acid 3:1 after synchronization with 24h ice treatment. Roots were digested with 3% (w/v) pectinase (Sigma, 450units ml^{-1}), 1.8% (w/v) cellulase (Calbiochem, 4000units g^{-1}) and 0.2% (w/v) cellulase (Onozuka RS, 5000units g^{-1}) and chromosome preparations made on glass slides by squashing in 45% and 60% acetic acid as described by [29].

The following probes were used:

pTa71 contains a 9 kb *Eco*RI fragment of the repeat unit of 25S-5.8S-18S rDNA isolated from *T. aestivum* [30] recloned in pUC19.

pSc119.2 or CS13 both contain a 120 bp tandem repeated DNA sequence isolated from *Secale cereale* [31, 20]

dpTa1 contains a tandem repeat with a monomeric length of 390 bp isolated from *T. aestivum* and subcloned by [32] and homologous to pAs1 [33] and the 340bp Afa-repeat sequences [21].

Total genomic DNA from rye 'Petkus' wheat 'Chinese Spring' and *Thinopyrum intermedium* 'Haymaker' was sheared, to 5-8kb pieces by autoclaving.

pTa71 was linearised with *Hind*III before labelling. CS13 and dpTa1 inserts were amplified by PCR using the universal M13 sequencing primers and the products of respective lengths were cut and cleaned out after running on 1.2% agarose gels. For labelling of cloned and gnomic DNA, biotin-16-dUTP and digoxigenin-16-dUTP (Roche Diagnostics) were incorporated in separate reactions using the Bioprime Biotin or CGH kits (Invitrogen) respectively.

2.3. In situ hybridization

DNA:DNA in situ hybridization followed the method described by [29] with minor modification. The probe mixture contained 50% (v/v) formamide, 20% (w/v) dextran sulphate, 2 x SSC, 25-100ng probe, 20 µg of salmon sperm DNA and 0.3% SDS (sodium dodecyl sulfate) as well as 0.12mM EDTA (ethylene-diamine-tetraacetic acid) and autoclaved total genomic DNA from wheat 'Chinese Spring' as blocking DNA at 4-20 x probe concentration. Probe and chromosomal DNA was denatured together on a Hybaid Omniblock for 6-10mins at 72-78°C and slowly cooled to the hybridization temperature of 37°C. Washes were carried out with 20% (v/v) formamide and 0.1 x SSC at 42°C at an equivalent to 85% stringency. Hybridization sites were detected with 2.0µg/ml streptavidin conjugated to Alexa594 (Molecular Probes) and 4µg/ml antidigoxigenin conjugated to FITC (flourescein isothiocyanate) (Roche Diagnostics). Chromosomes were counterstained with 0.2µg/ml DAPI (4′,6-diamidino-2-phenylindole) diluted in McIlvaines buffer pH7 and mounted in antifade solution (Citiflour). Preparations were analysed on a Zeiss epifluorescence microscope single band pass filters equipped with a CCD camera (Optronics, model S97790) and overlayed using Adobe Photoshop CS2 or 3.

3. RESULTS AND DISCUSSION

3.1. Wheat rye translocation lines

The rye (*Secale cereale* L.) genome has shown potential for improvement of bread wheat (*Triticum aestivum* L.), where wheat-rye substitutions and translocations have been and are frequently used in resistance breeding [34] and the 1BL.1RS wheat-rye translocation is present in the highest yielding cultivars currently grown in Europe and Canada [35,36]. However, the 1BL.1RS varieties have lost high molecular glutenins of 1BS that are responsible for good bread making quality and are hence used for feed and biscuit wheat only [37]. Several breeding programmes have hence tried to exchange either the 1AS or 1DS arm for the 1R arm as well as aiming to introgress other rye arms into wheat [38,34,17].

In order to analyse such lines, chromosome numbers should be established first using phase contrast, Feulgen or DAPI staining and genomic in situ hybridization using rye DNA will identify whether whole chromosomes, whole arms or segments have been transferred as addition, substitution, or translocation lines (See Fig. 1). 45S rDNA have been mapped to the large nucleolus organising regions (NORS) half way up the short arm of wheat chromosome 6B and 1B, 6B generally being

stronger than 1B [39] see Fig. 3B. Chromosome 5D carries a medium 45S rDNA site on the end of the short arm, while the small site at the end of the small arm of 1A is visible in most, but not all FISH experiments. Additionally, very small sites sub-telomerically on the long arm of the equal armed chromosome 7D and variable sites on other wheat chromosomes.

Combining genomic in situ hybridization with the 45S rDNA probe and counting major and minor sites, allows identification of the presence of 1RS and whether it is substituting the 1BS, 1AS or 1DS arm (Fig. 2). In Fig. 3A, a root tip metaphase of a 1B.1R translocation line is shown. The genomic rye DNA has labelled the two rye 1RS arms that are characterized by a large 45S rDNA site. Further 45S sites are on chromosome 6B, 5D 1A and 7D, while chromosome arm 1B is missing. In the 1DL.1RS translocation line (Fig. 3A) six major 45rDNA sites are visible on 1R, 6B and 1B.

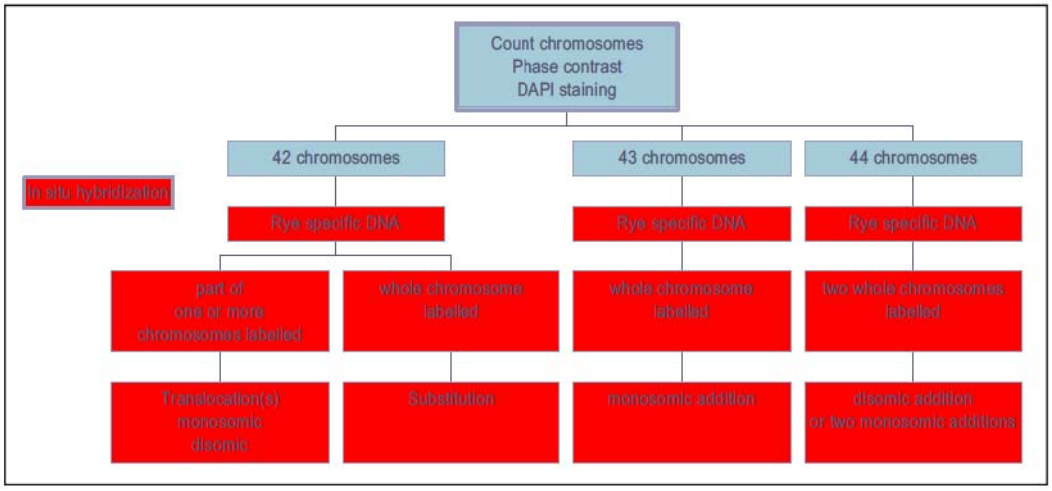

Fig. 1. Flow diagram to determining the presence of rye-wheat additions, substitutions or translocations.

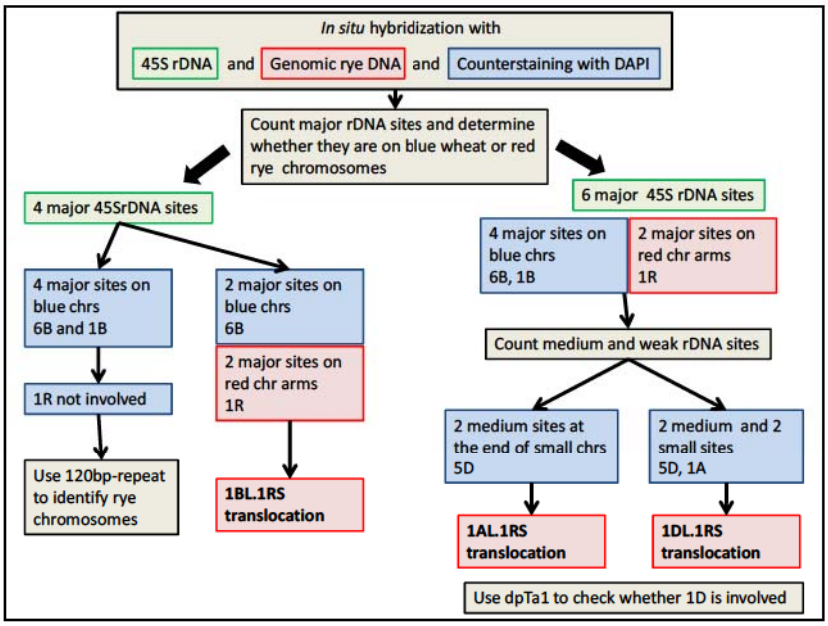

Fig. 2. Flow diagram for the identifying rye and wheat chromosomes involved in translocations. Fluorescent in situ *hybridization is assumed with green detection of the 45S rDNA and red detection of rye chromatin while the wheat chromosomes are blue with DAPI (see Fig.3A and B).*

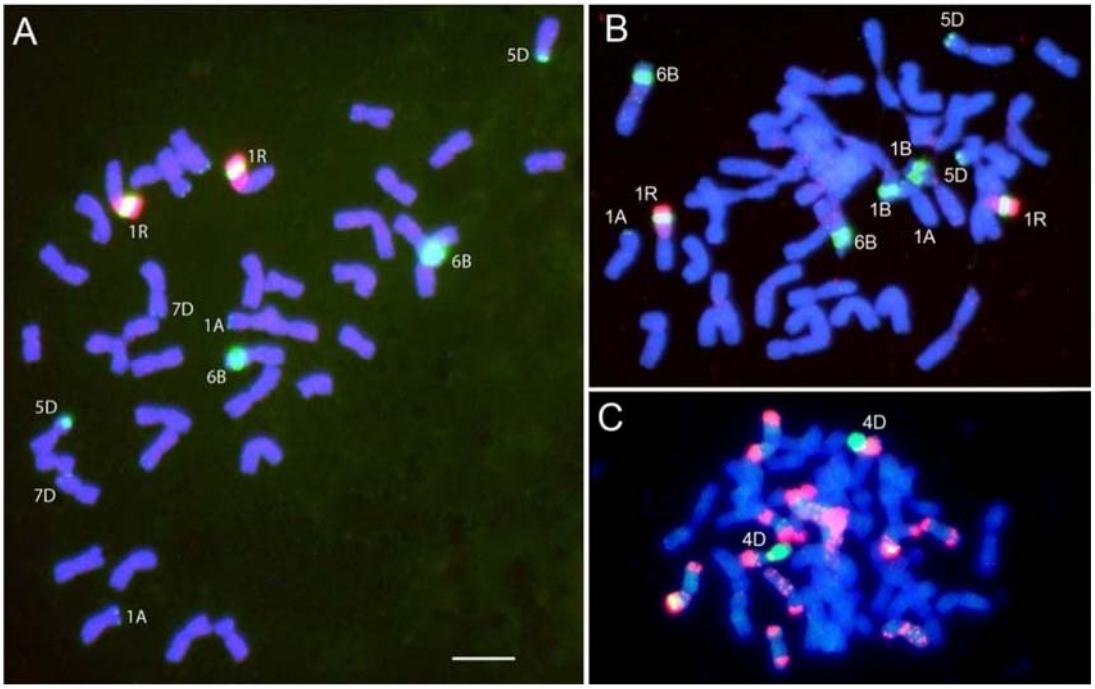

Fig. 3. Root tip metaphases of alien-to-wheat chromosomal translocation lines after FISH with genomic DNA from rye (shown in red; A: 1BL.1RS 'Beaver' and B: 1DL.1RS) and Thinopyrum intermedium (green in C; line giving rise to 'Mace'). Wheat chromosomes are shown in blue with DAPI fluorescence and repetitive probes are used to identify chromosomes. The 45S rDNA (green in A and B) identifies the major NOR sites on chromosomes 1R, 6B, 1B as well as minor sites on chromosomes 5D, A and 7D). In C, the Th. intermedium chromosome arm is translocated to chromosome 4D identified by the D-genomic specific sequences dpTa1. Bar equals 10µm.

In an example of a project to examine derivatives of wheat-rye breeding programme, sixteen spring-type bread wheat lines derived through wheat-rye introgression following hexaploid triticale x wheat hybridization were used. The programme involved further reshuffling of the genes by making crosses amongst the reconstituted lines involving various north-west Himalayan landraces, and these lines were screened for the detection and characterization of the rye chromatin substitutions/translocations by fluorescent in situ hybridization. Genomic in situ hybridization with rye genomic DNA detected the presence of one arm of a rye chromosome translocated into the 10 reconstituted wheat lines out of 16, and total number of the somatic chromosomes was unchanged. The presence of this part of the chromosome was further confirmed as short arm of 1R chromosome of rye through fluorescent in situ hybridization (FISH) using the 45S rDNA probe recognizing the NOR region on the sub-terminal end of this translocated chromosome's arm similar to the variety 'Beaver' shown in Fig. 3A. Additional karyotyping and was carried out using the B genome specific 120pb repeat sequence (not shown) and confirmed the involvement of the 1BL arm in the rye translocation. The remaining six lines were observed to be exhibiting neither any substitution/addition/translocation nor deletion of any wheat chromosome.

3.2. Wheat-Thinopyrum lines

Wheat Streak Mosaic Virus (WSMV) is an important disease, limiting winter wheat production in the US. The disease is seed born and spread via curl mite (WCM) *Aceria tosichella Kiefer* [40]. There is no known effective WSMV resistance within the genetic pool of wheat, and if present is non-effective above 18°C [41]. The Triticeae species *Thinopyrum intermedium* (2n=6x=42) has a vast reservoir of useful agronomic traits and shows good resistance to WSMV. It has been used to increase the genetic diversity in wheat. *Th.intermedium has* the resistant gene Wsm1, which limit infection and WCM colonization [16,42,27]. Hybrids and back crosses were developed with the aim to transfer WSM resistance gene, Wsm1 to wheat from *T. intermedium* [27], and these authors obtained six populations, each comprised of resistant and susceptible lines. Breeding lines with potential WSMV resistance can

be screened and selected at seedling stage without difficult pathological tests, and if analysed by PCR using the primers named STSJ15L &STSJ15R [43] giving a 420bp product, a marker linked to resistance. A new cultivar, Mace, incorporating WSMV resistance from the wild species has been released [28]. Genomic in situ hybridization with total genomic DNA from *T. intermedium* (Fig. 3C) is able to identify the introgresses translocated chromosome segment as a 4D.4Ag chromosome.

Currently, this programme is being extended to identify breakpoints and for further characterization of a family of wheat-intermediate wheatgrass (*Triticum aestivum – Thinopyrum intermedium*) recombinant lines spanning the wheat streak mosaic virus resistance locus; methods including both SSR markers and FISH are being applied.

4. CONCLUSIONS

The examples above show how molecular cytogenetics with genomic DNA and cloned DNA probes can be used to identify chromosome introgression in wheat breeding material. These crosses are valuable to increase the range of variation present in bread wheat, in some situations bringing in gene alleles that are entirely absent from current varieties. The cytogenetics is also valuable to identify ploidy of lines (in parents and in the hybrids themselves), to check for aneuploidy, and to track marker chromosomes through breeding programmes.

Parallel approaches are valuable in other crops, and relatively little background information is required about the material, at least compared to the extensive marker development or recombinant DNA libraries needed is some programmes. Thus, molecular cytogenetics and wide hybridization can be applied to minor crops, and enables more directed germplasm exploitation and improvement to be associated with breeding programmes.

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to improved varieties [3].

It is important that the plant breeders continue with their success, meeting the challenges of continuing
to increase yields, diseases adapting to current varieties, changing climates including water and salinity, and new market requirements for varieties suitable for storage and processing. A remarkably similar range of challenges face crops whether they are grown by subsistence farmers or smallholders, or large commercial operators, and whether in developing or industrialized countries. It is also essential that the successful genetic improvement strategies applied in the major crops are available widely for minor, niche and high-value species.

New genes, gene regulation and combinations of genes are needed to meet the new challenges faced by crops. Most crop species have a genetic bottleneck in their ancestry associated with a small number of genes critical to domestication see Ref. [2] Vaughan *et al.,* 2007 and 'Crop Domestication' Annals of Botany Special Issue 100/5; 2007), so the full range of variation present in the wild genepool is not available. It is also possible that intensive crossing, selection and improvement over recent decades has eliminated not only undesirable genetic alleles but also reduced the useful variation for generation of new varieties, although the evidence for this is equivocal: [4] found no reduction in variation in bread wheat and genetic diversity of wheat has remained at least constant for 70 years [5] while only some reduction in variation in durum wheat was reported by [6].

New gene alleles can be generated by induced mutation, and procedure that can be very beneficial in correcting well-defined weaknesses in existing varieties. The 2000 mutant varieties produced by direct
use of induced mutations (IAEA Mutant Variety and Genetic Stocks Database, http://mvgs.iaea.org) all have important new characters but maintain other quality and agronomic attributes of their progenitor.

The genepool contained in wild species is also an important source for new variation to introduce new characters into the gene pool. This can be accessed by sexual crossing of cultivated and wild relatives of crops. Within the cereals, the Triticeae tribe in the grasses includes wheat, barley, rye and some forage grasses. The whole tribe can be intercrossed using appropriate techniques (including crossing intermediates or bridge-crosses, chromosome doubling and tissue culture rescue of embryos), and thus

forms a single pool with enormous variation available at each genetic locus. To exploit the variation, characterization of the chromosomal constitution of the parents, design of crossing and backcrossing strategies, and analysis of chromosomes and chromosome segments that are transferred is essential. In this chapter, we show methods that allow detailed analysis of the introgressed alien (wild) chromatin into crop species, an approach which can accelerate and improve success rates in practical breeding. A key method is in situ hybridization, allowing alien chromatin to be identified in chromosome preparations of alien-cross derived plants. Because of the evolutionary separation of the progenitor species, total genomic DNA, with a high content of genome specific repeats, can be used as probe for in situ hybridization to identify chromosomes and chromosome segments of different origin [7,8]. It has therefore become the method of choice when interspecific crosses and derived introgressed lines are analysed to reveal alien chromosomes and translocations [9-17].

For in situ hybridization, genomic DNA can be combined easily with cloned probes to give further information about the specificity of the chromosome involved in the introgression or detect rearrangements. In the Triticeae several repetitive probes are cloned and can be used for identification of chromosomes and often are specific for certain genomes [18-22]. Physical mapping of differentially

labelled repetitive DNA sequences simultaneously in one experiment and in combination with total genomic DNA allows to detect the presence of introgressed chromosomes or chromosome segments from alien species in wheat lines, identifying the chromosomes involved and determining the nature and organization of any chromosome rearrangements [10,13-15,23-26].

In this report, we aim to show two examples of the analysis using in situ hybridization of wild wheat germplasm and the introgression of alien wheat chromosomes or chromosome segments in material with novel genetic variation which is of value to plant geneticists and breeders.

2. MATERIAL AND METHODS

2.1. Lines

The following wheat lines and varieties were used: *Triticum aestivum* hexaploid wheat 'Chinese Spring', and 'Chinese Spring' line carrying a 1DL.1RS translocation form rye 'Imperial' and variety 'Beaver' carrying a 1BL.1RS translocation.

Fixed triticale x wheat derivatives (RL lines) from CIMMYT material were selected after continuous selfing and growing in the remote regions of Trans- Himalayas.

Wheat breeding lines with *Wsm-1* from the materials evaluated in the study of Ref. [27] including the line subsequently named '*Mace*' [28].

2.2. Chromosome and probe preparation

Seeds were germinated on Petri dishes in the dark and 1-2cm long emerging roots were fixed in alcohol:acetic acid 3:1 after synchronization with 24h ice treatment. Roots were digested with 3% (w/v) pectinase (Sigma, 450units ml^{-1}), 1.8% (w/v) cellulase (Calbiochem, 4000units g^{-1}) and 0.2% (w/v) cellulase (Onozuka RS, 5000units g^{-1}) and chromosome preparations made on glass slides by squashing in 45% and 60% acetic acid as described by [29].

123

The following probes were used:

pTa71 contains a 9 kb *Eco*RI fragment of the repeat unit of 25S-5.8S-18S rDNA isolated from *T. aestivum* [30] recloned in pUC19.

pSc119.2 or CS13 both contain a 120 bp tandem repeated DNA sequence isolated from *Secale cereale*

[31, 20]

dpTa1 contains a tandem repeat with a monomeric length of 390 bp isolated from *T. aestivum* and subcloned by [32] and homologous to pAs1 [33] and the 340bp Afa-repeat sequences [21].

Total genomic DNA from rye 'Petkus' wheat 'Chinese Spring' and *Thinopyrum intermedium* 'Haymaker' was sheared, to 5-8kb pieces by autoclaving.

pTa71 was linearised with *Hind*III before labelling. CS13 and dpTa1 inserts were amplified by PCR using the universal M13 sequencing primers and the products of respective lengths were cut and cleaned out after running on 1.2% agarose gels. For labelling of cloned and gnomic DNA, biotin-16-dUTP and digoxigenin-16-dUTP (Roche Diagnostics) were incorporated in separate reactions using the Bioprime Biotin or CGH kits (Invitrogen) respectively.

2.3. In situ hybridization

DNA:DNA in situ hybridization followed the method described by [29] with minor modification. The probe mixture contained 50% (v/v) formamide, 20% (w/v) dextran sulphate, 2 x SSC, 25-100ng probe, 20 μg of salmon sperm DNA and 0.3% SDS (sodium dodecyl sulfate) as well as 0.12mM EDTA (ethylene-diamine-tetraacetic acid) and autoclaved total genomic DNA from wheat 'Chinese Spring' as blocking DNA at 4-20 x probe concentration. Probe and chromosomal DNA was denatured together
on a Hybaid Omniblock for 6-10mins at 72-78°C and slowly cooled to the hybridization temperature of 37°C. Washes were carried out with 20% (v/v) formamide and 0.1 x SSC at 42°C at an equivalent to 85% stringency. Hybridization sites were detected with 2.0μg/ml streptavidin conjugated to Alexa594 (Molecular Probes) and 4μg/ml antidigoxigenin conjugated to FITC (flourescein isothiocyanate) (Roche Diagnostics). Chromosomes were counterstained with 0.2μg/ml DAPI (4′,6-diamidino-2-phenylindole) diluted in McIlvaines buffer pH7 and mounted in antifade solution (Citiflour). Preparations were analysed on a Zeiss epifluorescence microscope single band pass filters equipped with a CCD camera (Optronics, model S97790) and overlayed using Adobe Photoshop CS2 or 3.

3. RESULTS AND DISCUSSION

3.1. Wheat rye translocation lines

The rye (*Secale cereale* L.) genome has shown potential for improvement of bread wheat (*Triticum aestivum* L.), where wheat-rye substitutions and translocations have been and are frequently used in resistance breeding [34] and the 1BL.1RS wheat-rye translocation is present in the highest yielding cultivars currently grown in Europe and Canada [35,36]. However, the 1BL.1RS varieties have lost high molecular glutenins of 1BS that are responsible for good bread making quality and are hence used for feed and biscuit wheat only [37]. Several breeding programmes have hence tried to exchange either the 1AS or 1DS arm for the 1R arm as well as aiming to introgress other rye arms into wheat [38,34,17].

In order to analyse such lines, chromosome numbers should be established first using phase contrast, Feulgen or DAPI staining and genomic in situ hybridization using rye DNA will identify whether whole chromosomes, whole arms or segments have been transferred as addition, substitution, or translocation lines (See Fig. 1). 45S rDNA have been mapped to the large nucleolus organising regions (NORS) half way up the short arm of wheat chromosome 6B and 1B, 6B generally being
124
stronger than 1B [39] see Fig. 3B. Chromosome 5D carries a medium 45S rDNA site on the end of the short arm, while the small site at the end of the small arm of 1A is visible in most, but not all FISH experiments. Additionally, very small sites sub-telomerically on the long arm of the equal armed chromosome 7D and variable sites on other wheat chromosomes.

Combining genomic in situ hybridization with the 45S rDNA probe and counting major and minor sites, allows identification of the presence of 1RS and whether it is substituting the 1BS, 1AS or 1DS arm (Fig. 2). In Fig. 3A, a root tip metaphase of a 1B.1R translocation line is shown. The genomic rye DNA has labelled the two rye 1RS arms that are characterized by a large 45S rDNA site. Further 45S sites are on chromosome 6B, 5D 1A and 7D, while chromosome arm 1B is missing. In the 1DL.1RS translocation line (Fig. 3A) six major 45rDNA sites are visible on 1R, 6B and 1B.

Fig. 1. Flow diagram to determining the presence of rye-wheat additions, substitutions or translocations.

Count major rDNA sites and determine whether they are on blue wheat or red rye chromosomes
4 major 45SrDNA sites
6 major 45S rDNA sites
4 major sites on blue chrs 6B and 1B
2 major sites on blue chrs 6B
2 major sites on red chr arms 1R
1BL.1RS translocation
1R not involved

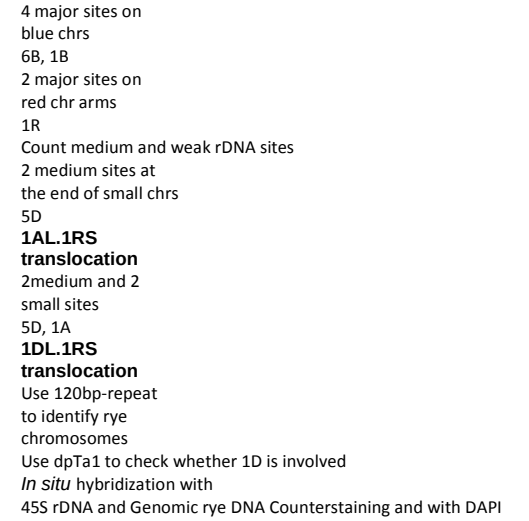

Fig. 2. Flow diagram for the identifying rye and wheat chromosomes involved in translocations. Fluorescent in situ *hybridization is assumed with green detection of the 45S rDNA and red detection of rye chromatin while the wheat chromosomes are blue with DAPI (see Fig.3A and B).*

In situ hybridization Translocation(s) Monosomic disomic part of one or more
chromosomes labelled Substitution whole chromosome labelled Rye specific DNA 42 chromosomes monosomic addition whole chromosome labelled Rye specific DNA 43 chromosomes disomic addition or two monosomic additions two whole chromosomes labelled Rye specific DNA 44 chromosomes Count chromosomes Phase contrast DAPI staining

125

Fig. 3. Root tip metaphases of alien-to-wheat chromosomal translocation lines after FISH with genomic DNA from rye (shown in red; A: 1BL.1RS 'Beaver' and B: 1DL.1RS) and Thinopyrum intermedium (green in C; line giving rise to 'Mace'). Wheat chromosomes are shown in blue with DAPI fluorescence and repetitive probes are used to identify chromosomes. The 45S rDNA (green in A and B) identifies the major NOR sites on chromosomes 1R, 6B, 1B as well as minor sites on chromosomes 5D, A and 7D). In C, the Th. intermedium chromosome arm is translocated to chromosome 4D identified by the D-genomic specific sequences dpTa1. Bar equals 10μm.

In an example of a project to examine derivatives of wheat-rye breeding programme, sixteen springtype
bread wheat lines derived through wheat-rye introgression following hexaploid triticale x wheat hybridization were used. The programme involved further reshuffling of the genes by making crosses amongst the reconstituted lines involving various north-west Himalayan landraces, and these lines were screened for the detection and characterization of the rye chromatin substitutions/translocations by fluorescent in situ hybridization. Genomic in situ hybridization with rye genomic DNA detected the
presence of one arm of a rye chromosome translocated into the 10 reconstituted wheat lines out of 16, and total number of the somatic chromosomes was unchanged. The presence of this part of the chromosome was further confirmed as short arm of 1R chromosome of rye through fluorescent in situ hybridization (FISH) using the 45S rDNA probe recognizing the NOR region on the sub-terminal end of this translocated chromosome's arm similar to the variety 'Beaver' shown in Fig. 3A. Additional karyotyping and was carried out using the B genome specific 120pb repeat sequence (not shown) and confirmed the involvement of the 1BL arm in the rye translocation. The remaining six lines were observed to be exhibiting neither any substitution/addition/translocation nor deletion of any wheat chromosome.

3.2. Wheat-Thinopyrum lines

Wheat Streak Mosaic Virus (WSMV) is an important disease, limiting winter wheat production in the US. The disease is seed born and spread via curl mite (WCM) *Aceria tosichella Kiefer* [40]. There is no known effective WSMV resistance within the genetic pool of wheat, and if present is non-effective above 18°C [41]. The Triticeae species *Thinopyrum intermedium* (2n=6x=42) has a vast reservoir of useful agronomic traits and shows good resistance to WSMV. It has been used to increase the genetic diversity in wheat. *Th.intermedium has* the resistant gene Wsm1, which limit infection and WCM colonization [16,42,27]. Hybrids and back crosses were developed with the aim to transfer WSM resistance gene, Wsm1 to wheat from *T. intermedium* [27], and these authors obtained six populations, each comprised of resistant and susceptible lines. Breeding lines with potential WSMV resistance can

126

be screened and selected at seedling stage without difficult pathological tests, and if analysed by PCR using the primers named STSJ15L &STSJ15R [43] giving a 420bp product, a marker linked to resistance. A new cultivar, Mace, incorporating WSMV resistance from the wild species has been released [28]. Genomic in situ hybridization with total genomic DNA from *T. intermedium* (Fig. 3C) is
able to identify the introgresses translocated chromosome segment as a 4D.4Ag chromosome. Currently, this programme is being extended to identify breakpoints and for further characterization of a family of wheat-intermediate wheatgrass (*Triticum aestivum – Thinopyrum intermedium*) recombinant lines spanning the wheat streak mosaic virus resistance locus; methods including both SSR markers and FISH are being applied.

4. CONCLUSIONS

The examples above show how molecular cytogenetics with genomic DNA and cloned DNA probes can be used to identify chromosome introgression in wheat breeding material. These crosses are valuable to increase the range of variation present in bread wheat, in some situations bringing in gene alleles that are entirely absent from current varieties. The cytogenetics is also valuable to identify ploidy of lines (in parents and in the hybrids themselves), to check for aneuploidy, and to track marker chromosomes through breeding programmes.

Parallel approaches are valuable in other crops, and relatively little background information is required
about the material, at least compared to the extensive marker development or recombinant DNA libraries needed is some programmes. Thus, molecular cytogenetics and wide hybridization can be applied to minor crops, and enables more directed germplasm exploitation and improvement to be associated with breeding programmes.

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