African Journal of Biotechnology Vol. 9 (54), pp. 9142-9147, 29 December, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Review

Historical perspective of *in situ* hybridization for the analysis of genomic constitution of plants

Fida M Abbasi*, M. Tariq Khan, Farzana Perveen, Rabia Masood, Inamullah, Uzma Khan, Sadia Tabassum and Muqarrab Shah

Department of Genetics, Hazara University, Mansehra, Pakistan.

Accepted 17 December, 2010

***In situ* hybridization involves hybridization of DNA or RNA probes to the cytological preparations. The technique originally used auto-radiographic labeling to map both repetitive and low copy DNA sequences. The problem associated with this technique was its short half life, lack of safety and long exposure time which hindered its widespread use in DNA hybridization. To overcome these problems, non isotopic *in situ* hybridization was developed for use in animal and plant species. In the last decade, the development of haptens and fluorochromes enabled simultaneous multicolored detection of differentially labeled probes. Characterization of parental genomes in interspecific hybrids, restructured chromosomes, gene mapping, detecting nature of chromosome pairing, establishing phylogenetic relationship among the species and localizing introgressed segment have been successfully achieved by fluorescence *in situ* hybridization.**

Key words: *In situ* hybridization, phylogenetic relationship, homoeologous pairing.

INTRODUCTION

In situ hybridization technique was developed by Gall and Pardue (1969) and John et al. (1969). The technique originally used auto-radiographic labeling to map both repetitive and low copy DNA sequences. Although, it was very sensitive but problems associated with this technique include short half life, safety problem and long exposure time which hindered its widespread use in DNA hybridization. To overcome these problems, non isotopic *in situ* hybridization was developed but its use was limited to animal system. The non isotopic *in situ* hybridization using biotin labeled DNA probes was first introduced in plant species by Rayburn and Gill (1985). Biotin labeled repetitive DNA sequences (120bp) of rye were used as probe to mitotic chromosomes of *Triticum aestivum* and double ditelosomic lines of B and D genome chromosomes and 4A of Chinese spring. The hybridization sites occurred as brown bands on blue chromosomes. Based on this color difference, the hybridization sites were discriminated from the non-hybridization and Giemsa staining bands. The hybridization pattern revealed that Chinese spring has 7B genome chromosomes and chromosomes 4A, 2D and 5D are from the ditelosomic lines. The technique was modified by using genomic *in situ* hybridization (Durnam et al., 1985). Further progress in this field, lead to the development of fluorescence *in situ* hybridization (FISH).

In the last decade, FISH techniques have been refined, especially with the development of haptens and fluorochromes that enabled simultaneous multicolored detection of differentially labeled probes. Many probes labeling in fluorescent reagent had been developed and are available for simultaneously detecting multiple target sequences in different colors. Both total genomic DNA and repetitive DNA sequences are being used as probes in FISH. The repetitive DNA sequences had been used as markers for the chromosomes and had been used for the analysis of parental genomes, alien addition lines and translocations. This method was mainly developed for the area of human molecular cytogenetics in 1990.

The first application of FISH to plant cytogenetics was the work of Leitch et al. (1991) on the simultaneous detection and localization of repetitive DNA sequences in rye chromosome. The two color FISH technique had been applied to map repetitive DNA sequences on the

*Corresponding author. E-mail: muhammaddr@yahoo.com.

chromosomes of wheat (Leitch et al., 1991), tobacco (Kenton et al., (1993), barley (Leitch and Harrison, 1993), sugar beet (Schmidt et al., 1994), triticale (Cuadrado and Jouve, 1994) and aegilops (Yamamoto and Mukai, 1995).

DETECTION OF HOMOEOLOGOUS PAIRING

Introduction of alien gene requires hybridization followed by meiotic pairing and recombination between the chromosomes of cultivated and wild species. Successful gene transfer could be greatly facilitated, firstly by knowledge of occurrence and frequency of chromosome pairing (Miller et al., 1995), and secondly, through the identification of the presence of alien chromatin in the recipient progenies. The conventional technique for pairing analysis may underestimate or overestimate the homologous pairing. It is difficult to distinguish intergenomic and intragenomic chromosomes pairing by conventional meiotic analysis (Begona et al., 1995). *In situ* hybridization using genomic probe has been demonstrated to be an effective technique for the detection of meiotic pairing. Le and Armstrong (1991) showed that labeled chromosomes of rye and unlabeled chromosomes of wheat can be observed throughout all meiotic stages in the triticale. They used 1:1 ratio of biotin-labeled rye to unlabeled wheat DNA as a blocking in the probe mixture. Begona et al. (1995) analyzed triticale x tetraploid rye hybrid by genomic *in situ* hybridization (GISH) and C banding. Total genomic DNA of rye was used as labeled probe and DNA from wheat was used as blocking DNA. The results obtained revealed considerable difference between these techniques with regard to their efficiency in detecting chromosome pairing. The percentage of pollen mother cell containing wheat/rye homologous association determined by C banding and GISH was 2.5 and 9.2, respectively. This indicated that a certain proportion of wheat/rye association was not identified by C banding. By contrast, three types had been determined precisely by GISH. Abbasi et al. (2009) reported for the first time the detection of nature of chromosome pairing in *Oryza* by GISH.

IDENTIFICATION OF PARENTAL GENOMES IN WIDE-CROSS DERIVATIVES

Characterization of parental genomes in interspecific hybrids and restructured chromosomes is important for precise transfer of alien chromatin to cultivated species. In the analysis of parental genomes and to establish relationship between species, both genomic DNA as a probe or cloned repetitive DNA sequences were used. Schwarzacher et al. (1989) was the first to demonstrate the applicability of GISH in the identification of parental genomes in an interspecific hybrid between *Hordeum chilense* and *Secale affricanum*. Since then, this technique has been used extensively for the characterization of parental chromosomes in interspecific hybrids. It is being used for the elucidation of the ancestry of the hybrid and the polyploidy. The technique involved the label genomic DNA from the suggested ancestor as probe to the cytological preparations from the species under investigation. Based on the positive or negative hybridization and hybridization strength, relationship was established. GISH has been used for discriminating autopolyploid from allopolyploid species (Bennett et al., 1992), discriminating closely related genomes (Ananthawatt-Jonsson et al., 1990), establishing the parental genomes of natural amphiploids (Parokonny et al., 1992) and studying the genomic organization and evolution of polyploidy species (Chen and Armstrong, 1994; Orgaard and Heslop-Harrison, 1994).

Mukai and Gill (1991) reported the detection of barley chromatin added to wheat using 1:2 ratio of biotin labeled genomic DNA of barley to block (unlabeled sheared) DNA of wheat. The barley chromatin was shown as brownish domain against bluish background in metaphase, prophase and interphase. Three genomes had been successfully identified in hexaploid wheat (Mukai et al., 1993). The DNA from the progenitor of the A genome (*Triticul urartu*) was labeled with biotin; while digoxigenin labeled total genomic DNA of the diploid D genome progenitor (*Aegilops squarrosa*) was used as probe. Non labeled total genomic DNA of B genome progenitor (*A. speltoides*) was used as blocking DNA in *in situ* hybridization to metaphase chromosome spread of *T. aestivum*; Fluorescein and rhodamin were used for detection. Three genomes A, B and D were simultaneously detected by their yellow, brown and orange fluorescence, respectively. It was also indicated that 32% of distilled part of chromosome 4A was derived from B genome chromosome. Similarly, *Thinopyrum bessarabicum* chromosome was identified in the hybrid *T. aestivum* x *T. basserbicum* (Rayburn et al., 1993). Mujeeb et al. (1995) reported the identification of alien chromosomes in several hybrids and backcross derivatives such as *Thinopyrum scirpeum* chromosomes in the *T. scirpeum* x *Secale cereale* (F1) and their BC1. Similarly, using total genomic DNA as a probe in *in situ* hybridization, parental chromosomes have been identified in several hybrids. Some examples include identification of *T. bessarabicum* chromosomes in *T. aestivum* x *T. bessarabicum* (F1) and in *T. aestivum* x *T. bessarabicum* x *S. cereale;* Variabilis chromosomes in *T. aestivum* x *Anabaena variabilis* (F1) and in *T. aestivum x A. varibilis x T. aestivum* (BC1); *Curvifolium* chromosome in *T. aestivum* x *Thinopyrum curvifolium* x *T. aestivum* (BC1); and *Thinopyrum elongatum* chromosomes in *T. elongatum* x *T. aestivum* (F1).

GISH has been used to identify the parental chromosomes in rice. The D genome chromosomes were discriminated from C genome in *Oryza latifolia*. Similarly, the C genome chromosomes were discriminated from B genomes in *Oryza minuta* (Fukui et al. 1997). GISH is a powerful l method for identifying alien addition and

substitution lines. Restriction fragment length polymorphism (RFLP) analysis can identify the presence of particular individual chromosome but whether they are present as a pair or as a single chromosome cannot be determined. Such a situation was confirmed by Jacobson et al. (1995). Parental genomes were successfully discriminated in F1 hybrid of *Lolium multiflorum* and *Festuca arundina* using total genomic DNA as probe (Mizukami et al., 1998).

DETERMINING SPECIES RELATIONSHIP

In situ hybridization using genomic DNA as probe to related species gives information about the similarities of the DNA of two different species. Such hybridization also provides information about the physical distribution of sequences that are common or different between the two species. An integrated account of such data can be used to support and develop theories about phylogenies, hybridization and diversification of plant species. The closer the two species, the greater would be the cross hybridization. Genomic affinity was assessed between *Oryza sativa* and *Oryza brachyantha* by using three strategies: GISH, chromosome pairing and pollen/spikelet sterility (Abbasi et al., 2010). No cross hybridization was examined between these two genomes leading to the conclusion that *O. brachyantha* and *O. sativa* are far related. GISH was used to assess the genomic relationship between *O. sativa* and *Oryza australiensis* (Abbasi et al., 2010). No cross hybridization was examined between the labeled genomic DNA of *O. australiensis* and the chromosomes of *O. sativa*. Genomic divergence has been investigated in *Gibasis* using GISH (Parakonny et al., 1992). Despite the similarity of karyotype and close taxanomic affinity of *Gibasis karwinksyana* and *Gibasis consobrina*, probing with genomic DNA distinguished the chromosomes and only the region proximal to each nucleolus organizer was strongly conserved between the chromosome sets. Similarly, the studies performed on *Avena* (Katsiotis et al., 1997) revealed that there was no apparent difference in the A and B genomes of this species as thought earlier and suggested for their clubbing together as A and A'. The difference between genomes could be due to the difference in repetitive DNA sequences. Almost half of the DNA in a genome may be repetitive. The repetitive sequences may be dinucleotides, tri, tetra, hexa or as many as 180 to 10,000 base pairs (bp) or more like 18s and 5.8s rRNA gene families (Lavania, 1998). The distribution of these sequences may be chromosome or species specific and these repetitive sequences show variation between species. The repetitive DNA sequences may be tandem repeats, that serve as blocker and many copies could be dispersed throughout the genome.

These repetitive DNA sequences have been used as probe *in situ* hybridization to understand plant genome organization, chromosome structure (Moor et al., 1993), physical position of genes (Mukai et al., 1991) and relative order of DNA sequences along the chromosome arm (Leitch et al., 1991). The physical distribution of highly conserved 5S, 5.8S and 25S rRNA gene loci on the chromosomes are useful markers to examine genome evolution and have been examined in several species. Using rRNA sequences as a probe *in situ* hybridization on the *Thinopyrum distichum* chromosomes, the *Distichum* chromosomes were identified in 7 whole chromosome addition lines and on telosomic addition lines (Fominaya et al., 1997). Using these repeats as probe, it was suggested that *Saccharum officinarum* and *Saccharum robustum* have one locus and 10 chromosomes, while *Saccharum spontaneum* has 8 chromosomes (D' hont et al., 1998).

There are many tandemly repetitive sequences that may be genus specific or even unique to a particular species. The physical distribution of such families, their location, and copy number may help in differentiation of various linkage groups of the chromosomes complement. The tandemly repetitive sequences PSC119.2 (McIntyre et al., 1990) isolated from rye produces specific and district banding pattern thus facilitating identification of all the seven chromosome pairs.

The centromere plays an important role in the proper segregation of chromosome during mitosis and meiosis for equal distribution of genetic material to the next generation. Many plants have cluster of highly repetitive sequences at or near their centromere. Two different repetitive sequences approximately the length of one nucleosome have been characterized in the tetraploid species *Brassica napus* and its diploid ancestral species *Brassica compestris* and *Brassica oleracea* (Herrison and Heslop-Harrison, 1995). A 745 bp repetitive DNA clone, PSau3A9, has been isolated from sorghum (*Sorghum bicolor*). This DNA element was located in the centromeric region of all the chromosomes and homologous to this element which is present in other cereal including rice, maize, wheat, barley, rye and oat as demonstrated by FISH and the absence of such DNA sequences homologous to PSau3A9 in dicot species suggested rapid divergence of centromere related sequences when compared with telomere related sequences in plant (Jiang et al., 1996). Such sequences could be used as important chromosome markers and also to understand the pattern of changes during speciation.

The true telomere of angiosperm consists of a short sequence TTTAGGG which was added to the ends of chromosome by an enzyme telomerase. When this sequence was used as probe in cereals, the probe hybridized to the end of all the chromosomes (Schwarzacher and Heslop-Harrison, 1991). Other repetitive DNA sequences were located at sub-telomeric position and used as markers for chromosomes. The FISH method was applied to localize rice repeated sequences adjacent to the telomeric region of chromosomes (Ohmido and Fukui,

1995).

Similarly, highly repetitive B chromosome specific sequences had been isolated from carrier plant in certain species such as *Secale cereale* and *Brachycome dichromosomatica* (John et al., 1991; Leach et al., 1995) and had been used in FISH based investigation to understand the molecular organization of B chromosome and their special arrangement during interphase.

LOCATION OF ALIEN INTROGRESSED SEGMENTS ON CHROMOSOMES

Recent development in using FISH followed by signal amplification have significantly enhanced the sensitivity of the detection procedure allowing the detection of unique DNA sequences in human and animal system. However, these techniques have only been used to a limited extent in genetic studies in plants and recent reports have either used genomic DNA (Mukai et al., 1993) or repetitive DNA sequences (Nkongolo et al., 1993). The ability to detect smaller (around 2 kb) single or low copy number DNA sequences is very important for physical mapping of plant genomes. A great effort was made in several laboratories to improve the sensitivity of the *in situ* hybridization techniques for mapping low or single copy sequence such as B. hordein locus to chromosome 5 of barley (Melody and Karp, 1989) and chromosome 14 (Lehfer et al., 1993). Alpha amylase 2 genes in barley were reported by Leitch and Heslop-Harrison (1993). Location of 2.0 kb single low copy DNA sequence have been reported by Haishui and Quick (1995) on wheat and rye, and legumine gene in pea (Simpson et al., 1988). The instability and non expression of the transgene is being studied by *in situ* hybridization which identifies its physical location within the genome. Such reports included localization of introduced gene on the chromosomes of transgenic barley, wheat and triticale (Pederson et al., 1997), tobacco (Moscon et al., 1996) and rice (Abbasi et al., 2010).

CHARACTERIZATION OF TRANSLOCATION

Characterization of translocation includes the identification of translocated chromosome, localization of break point and estimation of the amount of the transferred alien chromatin. Conventional techniques such as pairing analysis may provide important information but are inadequate to characterize translocation. Banding techniques are uninformative if alien chromosome segment lacks diagnostic bands (Jiang et al., 1994). Lapitan et al. (1986) were the first to successfully detect the break point of wheat rye translocation by *in situ* hybridization using dispersed rye repetitive DNA sequences as probe. Wheat alien translocation and their break points were clearly identified by GISH (Le et al., 1989; Mukai and Gill, 1991). Dispersed species specific repetitive DNA sequences have been used to characterize wheat alien translocation (Rogosky et al., 1991). Heslop-Harrison et al. (1990) characterized translocation in aneuploid wheat. When labeled total genomic DNA as a probe was hybridized to cytological preparation of wheat lines having translocated segment, all the break points were found at or near the centromere. The translocations were identified at all the stages of the cell cycle.

Radiation induced wheat-rye chromosomes translocation lines resistance to Hessian fly were analyzed by *in situ* hybridization, using total genomic and highly repetitive DNA probes. The bacterial artificial chromosomes (BAC) with Sh2 gene from maize was used as probe in FISH and localized the Sh2 gene on the chromosome 3 of sorghum (Gomez et al., 1997). Similarly, Tang et al. (1997) identified introgressed segment in tetrageneric hybrid between *Triticum, Avena*, *Thinopyrum* and *Secale* which conferred high resistance to leaf rust, stem rust, stripe rust, powdery mildew and root rot of wheat. Genomic *in situ* hybridization showed that introgressed segment has originated from three wild related genera. The centromeric chromosomal segment have been identified in the interphase nuclei of Arabidopsis by using different probe combination including repeats of centromeric, telemetric, YACs and Cosmid (Fransz et al., 1999). As reviewed earlier, *in situ* hybridization can precisely establish relationship between species and identify introgressed segment, and alien chromosomes in addition lines, detect translocation and precisely locate break point under a situation where RFLP cannot detect such chromosomal aberration.

REFERENCES

Abbasi FM, Ahmad H, Perveen F, Inamullah, Sajid M, Brar DS (2010). Assesment of genomic relationship between *Oryza sativa* and *Oryza australiensis*. Afr. J. Biotechnol. 9: 1312-1316

Abbasi FM, Shah AH, Perveen F, Afzal M, Sajid M, Masood R, Nawaz F (2010). Genomic affinity between *Oryza sativa* and *Oryza brachyantha* as revealed by in situ hybridization and chromosome pairing. Afr. J. Biotechnol. 9: 3068-3072

Abbasi FM, Shah SH, Gul A, Majid A, Afzal M, Mujaddad R, Shah AH, Masood R, Shafqat N, Bukhari I, Ali A (2010). Localizing introgression on the chromosome of rice by genomic *in situ* hybridization (GISH). Afr. J. Biotechnol. 9(30): 4651-4654

Abbasi FM, Ahmad H, Sajid M, Inamullah, Brar DS (2009). Detecting nature of chromosome pairing in A and E genomes of *Oryza*. Cytologia, 74: 229-233

Anamthawat-Jonsson K, Schawarazacher T, Leitch AR, Bennett MD, Heslop-Harrison JS (1990). Discrimination between closely related *Triticeae* species using genomic DNA as probe. Theo. Appl. Genet. 79: 721-728.

Begona FC, Benavente E, Orellana J (1995). Meiotic pairing in whet-rye derivatives detected by genomic *in situ* hybridization and C-banding. A comparative analysis. Chromosoma, 103: 554-558.

Bennett ST, Kenton AY, Bennett MD (1992). Genomic *in situ* hybridization reveals the allopolyploidy nature of *Milium montianum* (Gramineae). Chromosoma, 101: 420-424.

Chen Q, Armstrong K (1994). Genomic *in situ* hybridization in *Avena sativa*. Genome, 37: 607-612.

Cuadrado A, Jouve N (1994). Mapping and organization of highly repeated DNA sequences by means of simultaneously and sequential FISH and C-banding 6x Triticale. Chromosome Res. 2: 331-338.

D' hont A, Ison D, Alix K, Roux C, Glaszmann JC (1998). Determination of basic chromosome numbers in the genus Saccharum by physical mapping of ribosomal RNA genes. Genome, 41: 221-225.

Durnam DM, Gelinas R, Myerson D (1985).Detection of species specific chromosomes in somatic cell hybrids. Somatic cell Mol. Genet. 11: 571-577.

Fominaya A, Molnar S, Kim NS, Chen O, Fedak G, Armstrong K (1997). Characterization of *Thinopyrum distichum* chromosomes using double fluorescence *in situ* hybridization, RFLP analysis of 5S and 26S rRNA and C banding of parents and addition lines. Genome, 40: 689-696.

Fransz P, Hans De J, Zabel P (1999). Compartmentalization of distant chromosomal sub regions in interphase nuclei of *Arabidopsis thaliana*. Plant and Animal Genome VII. San diego, California. p. 82.

Fukui K, Shishido R, Kinoshita T (1997). Identification of the rice D-genome chromosomes by genomic *in situ* hybridization. Theo. Appl. Genet. 95: 1239-1245.

Gall JG, Pardue ML (1969). Formation and detection of RNA-DNA hybrid molecules in cytological preparations. Proc. Natl. Acad. USA. 63: 378-383.

Gomez MI, Islam-Faridi MN, Woo SS, Schertz KF, Czeschin DJ, Zwick MS, Wing RA, Stelly DM, Price HJ (1997). FISH of a maize SH2 selected sorghum BAC to chromosomes of *Sorghum bicolor*. Genome, 40: 475-478.

Haishui D, Quick JS (1995). Detecting of 2.6 kb sigle/low copy DNA sequence on chromosomes of wheat (*Triticum aestivum*) and rye (*Secale cereal*) by fluorescence *in situ* hybridization. Genome, 38: 246-249.

Heslop-Harrison JS, Harrison GE (1995). Centromeric repetitive DNA sequences in the genus brassica. Theor. Appl. Genet. 90: 157-165

Heslop-Harrison JS, Leitch AR, Schawarzacher T, Anamthawat-Jonsson K (1990). Detection and characterization of 1B/1R translocations in hexaploid wheat. Heredity, 65: 385-392.

Jacobsen E, Jong DE, Kamstra JH, Van Den Berg SA, Ramanna MS (1995). Genomic *in situ* hybridization (GISH) and RFLP analysis for the identification of alien chromosomes in the backcross progeny of potato (+) tomato hybrids. Heredity, 74: 250-257.

Jiang J, Friebe B, Gill BS (1994). Recent advances in alien gene transfer in wheat. Euphytica, 73: 199-212.

Jiang J, Nasuda S, Dong F, Scherrer CW, Woo SS, Wing RD, Gill BS, Ward DC (1996). A conserved repetitive DNA element located in the centromeres of cereal chromosomes. Proc.Natl. Acad. Sci. USA. 93: 14210-14213.

John HA, Birnstile ML, John KW (1969). RNA-DNA hybrids at the cytological level. Nature, 223: 582-587.

John UP, Leach CR, Timmis JN (1991). A sequence specific to B chromosome of *Brachycome dichromosomatica*. Genome 34: 739-744

Katsiotis A, Hagidimitrious M, Heslop-Harrison JS (1997). The close relationship between the A and B genome in Avena L. determined by molecular cytogenetic analysis of total genomic tendemly and dispersed repetitive DNA sequences. Ann. Bot. 79: 103-109.

Kenton A, Parokonny AS, Gleba YY, Bennet MD (1993). Characterization of the *Nicotiana tabaccum* L. genome by molecular cytogenetics. Mol. Gen. Genet. 240: 159-169.

Lavania UC (1998). Fluorescence *in situ* hybridization in genome, chromosome and gene identification in plants. Curr. Sci. 74: 126-133.

Le HT, Armstrong KC, Miki B (1989). Detection of rye DNA in Wheat-rye hybrids and wheat translocation stocks using total genomic DNA as a probe. Plant Mol. Biol. Rep. 7: 150-158.

Le HT, Armstrong KC (1991). *In situ* hybridization, a rapid mean to asses meiotic pairing and detection of alien DNA transfer in interphase of wide crosses involving wheat and rye. Mol. Gen. Genet. 225: 33-37.

Leach CR, Donald TM, Franks TK, Spiniello SS, Hanrahan CF, Timmis JN (1995). Organization and origin of B chromosome centromeric sequence from *Brachycome dichromosomatica*. Chromosoma, 103: 709-714.

Lehfer H, Busch W, Martin R, Herrmann RG (1993). Localization of the B-hordein locus on barley chromosomes using fluorescence *in situ* hybridization. Chromosoma, 102: 428-432.

Leitch IJ, Leitch AR, Heslop-Harrison JS (1991). Physical mapping of plant DNA sequences by simultaneous *in situ* hybridization of two differently labeled fluorescent probes. Genome, 34: 329-333.

Leitch IJ, Heslop-Harrison JS (1993). Physical mapping of four sites of 5S rDNA sequences and sites of the a-amylase 2 gene in balrley (*Hordeum vulgare*). Genome, 34: 517-523.

McIntyre CL, Pereira S, Moran LB, Appls R (1990). New *Secale cearale* (rye) DNA derivatives for the detection of rye chromosome segments in Wheat. Genome, 33: 635-640.

Melody C, Karp A (1989). Physical mapping of β–hordein loci on barley chromosome 5, by *in situ* hybridization. Genome, 32: 925-929.

Miller TE, Reader SM, Pordie KA, Abbo S, Donford RP, King IJ (1995). Fluorescent *in situ* hybridization as an aid to introducing alien genetic variation into Wheat. Euphytica, 85: 275-279.

Mizukami Y, Sugita SI, Ohmido N, Fukui K (1998). Agronomic and cytological characterization of F1 hybrids between *Lolium multiflorum* Lam and *Festuka arundinaceae* var. glaucescens Boiss. Grassland Sci. 44: 14-21.

Moor G, Cheung W, Foote T, Bale ME, Koebner R, Leitch AR, Leitch IJ, Money T, Stancombe P, Yano M, Flavell R (1993). Key feature of cereal genome organization as revealed by the use of cytosine methylation sensitive restriction endonuclease. Genomics, 15: 472-482.

Moscone EA, Matzke MA, Matzke AJM (1996). The use of combined FISH/GISH in conjuction with DAPI counterstaining to identify chromosomes containing transgene inserts in amplidiploid tobacco. Chromosoma, 105: 231-236.

Mujeeb KA, Islam-Faridi MN, Cortes A (1995). Genome identification in some wheat and alien Triticeae species intergeneric hybrids by fluorescence *in situ* hybridization. Cytologia. 61: 307-315.

Mukai Y, Gill BS (1991). Detection of barley chromatin added to wheat by genomic *in situ* hybridization. Genome, 34: 448-452.

Mukai Y, Friebe J, Hatchett H, Yamamoto M, Gill BS (1993). Molecular cytogenetic analysis of radiation induced wheat-rye terminal and intercalary chromosomal translocation and the detection of rye chromatin specifying resistance to Hessian fly. Chromosoma, 102: 88-95.

Nkongolo KK, Lapitan NLV, Quick JS, Muhalamann MD (1993). An optimized fluorescence *in situ* hybridization procedure for detecting rye chromosomes in wheat. Genome, 36: 701-705.

Ohmido N, Fukui K (1995). Physical mapping of rice DNAs by an improved FISH method. JARQ, 29: 83-88.

Orgaard M, Heslop-Harrison JS (1994). Investigation of genome relationships between *Leymus*, *Psathyrostachys*, and *Hordeum* inferred by genomic DNA: DNA *in situ* hybridization. Ann Bot. 73: 195-203.

Parakonny AS, Kenton AY, Meredith L, Owens SJ, Bennett MD (1992). Genomic divergence of allopatric sibling species studies by molecular cytogenetics of their F1 hybrids. Plant J. 2: 695-704.

Pederson C, Zimny J, Becker D, Jahne-gartner A, Lorz H (1997). Localization of introduced genes on the chromosomes of transgenic barley, wheat and triticale by fluorescence *in situ* hybridization. Theor. Appl. Genet. 94: 749-757.

Rayburn AL, Gill BS (1985). Use of of biotin-labeled probes to map specific DNA sequences on wheat chromosomes. J. Heredity, 76: 78-81.

Rayburn AL, William HM, Mujeeb Kazi A (1993). Visualization of *Thinopyrum bessarabicum* chromosomes in a Triticum aestivum/*Th. Bessarabicum* amphiloid by genomic *in situ* hybridization. J. Genet. Breed. 47: 245-248.

Rogowsky PM, Guidet FLY, Langridge P, Shepherd KW, Koebner RMD (1991). Isolation and characterization of wheat rye recombinants involving chromosome arm 1DS of wheat. Theor. Appl. Genet. 82: 537-544.

Schawarzacher T, Leitch AR, Bennett MD, Heslop-Harrison JS (1989). *In situ* localization of parental genome in a wide-hybrid. Ann. Bot. 64: 315-324.

Schawarzacher T, Heslop-Harrison JS (1991). *In situ* hybridization to plant telomeres using synthetic oligomer. Genome, 34: 317-323.

Schmidt T, Schwarzacher T, Heslop-Harrison JS (1994). Physical mapping of rRNA genes by fluorescence *in situ* hybridization and structural analysis of 5S rRNA genes and intergenic spacer sequences in Sugar beet (*Beta vulgaris*) Theor. Appl. Genet. 88: 629-

636.
Simpson PR, Newnan MA, Davies DR (1988). Detection of legumin gene DNA sequences in Pea by *in situ* hybridization. Chromosoma, 96: 454-458.
Tang SJ, Zhuang WY, Li H, Xu J (1997). Identification of introgressed segments conferring disease resistance in tetrageneric hybrid of *Triticum, Sccale, Thinopyrum* and *Avena.* Genome, 40: 99-103.
Yamamoto M, Mukai Y (1995). Physical mapping of ribosomal RNA genes in *Aegilops* and *Triticum*. In. Z.S. and Xin Z.Y. (Eds) Proc. 8th Int.Wheat Genet Symp. Beijing China. pp. 807-811.

.