Molecular markers, cytogenetics and epigenetics to characterize wheat-*Thinopyrum* hybrid lines conferring *Wheat streak mosaic virus* resistance

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Niaz Ali
Department of Biology
University of Leicester

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Abstract

Molecular markers, cytogenetics and epigenetics to characterize wheat-*Thinopyrum* hybrid lines conferring *Wheat streak mosaic virus* resistance

Niaz Ali

Genetic resistance to *Wheat streak mosaic virus* (WSMV) offers the most attractive and environmentally safe strategy for disease control. While effective resistance in hexaploid bread wheat (*Triticum aestivum*) has recently been described in only one case, the *Wsm2* gene, more successful resistance has been introgressed from the related hexaploid wheatgrass, *Thinopyrum intermedium*, as the *Wsm1* and *Wsm3* genes. In the current study, fluorescent *in situ* hybridization (FISH) with genomic DNA from *Th. intermedium*, *Aegilops tauschii* and repetitive DNA probes was applied to four breeding populations of wheat-*Th. intermedium*, previously tested for WSMV-resistance. Three different wheat-*Th. intermedium* recombinant chromosomes, the well-known 4Ai#2S.4DL and two novel, 1B and 3D, were identified to be associated with WSMV-resistance. These novel introgressed genes from *Th. intermedium* were designated as *Wsm4* and *Wsm5* respectively. The *Wsm4* gene was pinpointed to a 6% interstitial map region on the 1BS flanked by *Xgwm4144* and *Xgwm1100* markers. Six new PCR-markers, five linked to *Wsm1* and one to *Wsm4* were identified. Molecular markers now provide a good coverage of the 4Ai#2S arm for effective marker assisted selection and the new genes increase our arsenal to combat the disease.

Two highly repetitive satellite DNA families, Afa and pSc119.2, were isolated for the first time from *Th. intermedium* and their diversity in respect to copies from other *Triticeae* species were investigated. They showed contrasting evolutionary dynamics leading to time dependent or independent homogenization of Afa and pSc119.2 sequences. Both repeats are excellent cytological markers and characterized the 4Ai#2S chromosomal arm, in the alien wheat lines and the *Th. intermedium* genome. Southern hybridization, with methylation sensitive and insensitive restriction enzymes and immunostaining with anti-5-methyl-cytosine antibodies were employed to assess DNA methylation. Overall, no massive changes were evident in the wheat genome, however the alien arm showed reduced cytosine methylation which is characteristic for actively transcribing chromatin.

Declaration

I hereby declare that no part of this thesis has been previously submitted to this or any other university as part of the requirements for a higher degree. The content of this thesis is the result of my own work unless otherwise acknowledged in the text or by reference.

The work was conducted in the Department of Biology at the University of Leicester, UK during the period April 2008 to April 2012.

Signed……

Niaz Ali

THIS THESIS IS DEDICATED
TO MY TEACHERS

(Teaching is not a lost art, but the regard for it is a lost tradition)

Jacques Barzun

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Abbreviations

%	Percentage
5-MeC	5-methyl cytosine
BAC	Bacterial artificial chromosome
BCIP	5-bromo-4-chloro-3-indolyl-phosphate
bp	Base pairs
BLAST	Basic local alignment search tool
BYDV	*Barley yellow dwarf virus*
cM	CentiMorgans
CS	Chinese spring
CTAB	Cetyltrimethylammonium bromide
DAPI	4´,6-diamidino-2-phenylindole
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytosine triphosphate
dGTP	Deoxyguanosine triphosphate
dUTP	Deoxyuridine triphosphate
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
dTTP	Deoxythymidine triphosphate
dUTP	Deoxyuridine triphosphate
EDTA	Ethylenediamine tetra-acetic acid
FISH	Fluorescent *in situ* hybridization
g	Gram
GISH	Genomic *in situ* hybridization
HCl	Hydrochloric acid
HPV	*High plains virus*
hr	Hour(s)
Indels	Insertions-deletions
INT	2-(4-iodophenyl)-5-(4-nitrophenyl)-3-phenyltetrazolium chloride
IPTG	Isopropyl-β-Δ-thiogalactopyranoside
IRAP	Inter-retrotransposon amplified polymorphism
LTRs	Long terminal repeats
M	Molar
M&M	Materials and Methods
Mg	Milligram(s)
Min	Minute
ml	Millilitre(s)
mM	Millimolar
mm	Millimetre
MYA	Million year ago
NOR	Nucleolar organizer region
NT	Nullisomic tetrasomic
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
Pu	Purine
PVP	Polyvinylpyrrolidone
R	Resistant
RAPD	Random amplified polymorphic DNA
rDNA	Ribosomal DNA
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RNAse	Ribonuclease
rpm	Rotations per minute
RT	Room temperature
S	Susceptible
SDS	Sodium dodecyl sulfate
Sec	Seconds
SSC	Saline sodium citrate

SSRs	Simple sequence repeats
STS	Sequence tagged site
TE	Tris-EDTA
TEs	Transposable elements
Tm	Melting temperature
TMV	*Triticum mosaic virus*
U	Unit
v/v	Volume added to volume
w/v	Weight added to volume
µl	Microlitre
µM	Micromolar

CHAPTER I: GENERAL INTRODUCTION

1.1 Importance of wheat

With over 650 million tons of annual production from 240 million hectares, wheat (*Triticum* spp.) has become one of the most important and extensively cultivated food crops. It is the staple food in more than 40 countries and over 35% of the global population (Curtis *et al.,* 2002, Matsuoka, 2011, Peng *et al.,* 2004, Williams, 1993). Wheat is adapted to a wide range of environmental conditions and is grown on an area larger than assigned to any other crop and its trade exceeds that of all other combined cereal crops (Feldman and Sears, 1981, Gustafson *et al.,* 2009). Common bread wheat (*T. aestivum* L., 2n=6x = 42, AABBDD) and durum wheat (*T. durum* Desf., 4x = 28, AABB) are the two main cultivated species, bread wheat accounts for about 95% of the world's wheat crop, while durum and other wheats such as einkorn (*T. monococcum* L., 2x = 14, AA), emmer (*T. turgidum* L., 4x = 28, AABB) and spelt wheat (*T. spelta* L., 6x = 42, AABBDD) are crops of minor economic importance and make up to 5% of the world wheat today (Curtis *et al.,* 2002, Dubcovsky and Dvorak, 2007, http://www.fao.org).

Human population is expected to reach 9.4 billion by 2050. The growing needs of worldwide food production will require greater yields from the existing cropland without horizontal expansion or proportionate increases in the use of water or fertilizer (Bao *et al.,* 2009, Foulkes *et al.,* 2011, http://www.fao.org). Environmental hazards, urban expansion and conversion of croplands into non cropping areas are undermining our ability to bring more land into wheat cultivation and by year 2050 will reduce the global cropping area by 8-20% (Young, 1999, Nellemann *et al.,* 2009). The situation can become worse, and affect another 25% of the world's cereal production if climate changes and melt waters of Himalayan glaciers alter the monsoon, flooding and drought regimes in Asia (Chakraborty and Newton, 2011).

Despite global combat of food shortages, in 2003 over 800 million people suffered daily hunger and under nutrition, while in 2009, the highest ever level of world hunger was recorded and 1.02 billion people were estimated to have gone hungry (http://www.fao.org). To meet the growing demands of world's hunger and projected population of 2050, global food production must increase by 50% (Chakraborty and Newton, 2011). Wheat being a universal cereal and a foremost crop plant after maize

and rice, supplies one fifth of all human calories. Among the crop plants, wheat is a cheap and rich source of energy and proteins for the world population (Feldman *et al.*, 1995, http://www.fao.org, Kumar and Sharma, 2011, Zohary and Hopf, 2000). Domestication of wheat was responsible for the increase in human population and the emergence of human civilization (Heun *et al.*, 1997, Sakuma *et al.*, 2011).

Future food security is a major challenge for mankind, and studies for the improvement of bread wheat as a high quality food are paramount. Since the origin of agriculture, crop improvement has been a continuous process driven by the needs for improved quality, yield, disease resistance and adaptation to new and changing climates. Once the evolutionary mechanism, involved in the formation and stabilization of wheat is unfolded, we can design better programs that will enable us to look for more efficient ways to capitalize on traits that may play a significant role in wheat improvement, and in feeding the ever-increasing human population (Matsuoka, 2011, Peng *et al.*, 2011, Purugganan and Fuller, 2009).

1.2 Early history and domestication of wheat

Perhaps some 10,000 years ago, when human population became too large, the transition from hunting and gathering to agrarian lifestyles started, that set off the road to human civilization (Eckardt, 2010, Feldman *et al.*, 1995, Heun *et al.*, 1997, Sakuma *et al.*, 2011). Humans turned to invest their labour in selected plant species for food and consciously or unconsciously started the complex process of genetic selection i.e. domestication of wild plants and animals (Dvorak *et al.*, 2011, Parra *et al.*, 2010, Peleg *et al.*, 2011, Purugganan and Fuller, 2009, Purugganan and Fuller, 2011).

Domestication is a gigantic evolutionary experiment of adaptation and speciation performed by humans during the last 10,000 years (Darwin, 1905, Feldman and Kislev, 2007). It has given rise to increased adaptation of both plants and animals and made all the cultivars, including wheat, human-dependent only, capable of surviving under human agricultural niches (Brown, 2010, Diamond, 2002, Matsuoka, 2011). Domestication not only gave birth of agriculture, but also around the same time, people adopted a sedentary lifestyle and started living in villages (Gepts and Papa, 2002). *Triticum* spp. (diploid, tetraploid and hexaploid) were the earliest domesticated species that marked the beginning of agriculture (Ozkan *et al.*, 2005, Purugganan and Fuller, 2009). Archaeological and phylogenetic studies suggest that south eastern Turkey–

northern Syria is the most likely site of cereal domestication (Heun *et al.,* 1997, Lev-Yadun *et al.,* 2000). Even today the wild progenitors of wheat, barley and rye grow there (Salamini *et al.,* 2002) and seeds of the both wild and cultivated einkorn and emmer have been excavated in early archaeological studies of these sites (Lev-Yadun *et al.,* 2000, Zohary and Hopf, 2000). As the hexaploid wheat is not directly derived from a wild progenitor through domestication selection but from *T. turgidum spp. dicoccon* x *Aegilops tauschii*, after the wild einkorn and emmer wheats were subjected to domestication selection (Dvorak *et al.,* 2011).

Domestication resulted in both genetic and phenotypic changes, which are beneficial to crop plants for domestication and were selected by the early farmers. These changes also differentiate domesticated taxa from their wild ancestors, and are grouped together as domestication syndromes (Heslop-Harrison and Schwarzacher, 2011b, Matsuoka, 2011). In wheat like other cereals, all these adaptations are for two main reasons, including those for successful germination and to facilitate threshing. In wheat like other cereals, important traits involved in the domestication syndrome were, loss of spike shattering and tough glumes, minimization of seed dormancy, increase in both seed size and number, reduced number of tillers, larger inflorescences, synchronized seed maturation, and more erect growth (Peng *et al.,* 2011, Ross-Ibarra *et al.,* 2007, Vaughan *et al.,* 2007, Zohary and Hopf, 2000). Seeds of free-threshing wheat appeared some 8500 years ago, when a natural mutation changed the ears of both emmer and spelt into a more easily threshed type, which later evolved and resulted in modern free-threshing ears of durum and bread wheat (Diamond, 2002, Dvorak *et al.,* 2006).

Despite the immense role of plant domestication in human history we still know very little about adaptation under domestication (Ross-Ibarra *et al.,* 2007). Once the genomic sequencing of diploid wild progenitors (*T. urartu* or *Ae. tauschii*) is completed, it will reveal some more details of the *Triticeae* genomics and genes or factors having roles in domestication. That will be of great importance in the improvement of wheat cultivars, exploitation of their genetic diversity and conservation of the wheat germplasm (Peng *et al.,* 2011).

1.3 Origin and evolution of wheat

Emergence of agricultural practices in the east Mediterranean area 10,000 ago, and its subsequent spread around the Mediterranean Sea were known from the earlier biogeographic studies conducted in the late 19th century. These studies also revealed specific regions for centers for the origin of cultivated plants (Charmet, 2011, Zeder, 2008). For the genus *Triticum,* the centre of origin was identified in the region of the Fertile Crescent, between the Mediterranean coast in the west and the plain of Tigris and Euphrates in the east throughout the Syrian desert (Feldman and Sears, 1981). The wild diploid and polyploid wheats are still widespread in this region and grow polymorphic or mixed populations (Eckardt, 2010, Feldman and Kislev, 2007, Feldman and Sears, 1981, Ozkan *et al.*, 2005).

Bread wheat (genome AABBDD), has evolved from two independent polyploidization events (Figure 1.1). The first event took place ~ 0.5 million years ago (MYA) when the diploid A genome donor hybridized to another species of the B genome donor resulting in tetraploid *T. turgidum*. The second spontaneous allopolyploidization event took place ~10,000 years ago between the early-domesticated tetraploid *T. turgidum ssp. dicoccum* and the diploid D genome donor *Ae. tauschii* (Dubcovsky and Dvorak, 2007, Feldman and Kislev, 2007, Salse *et al.*, 2008).

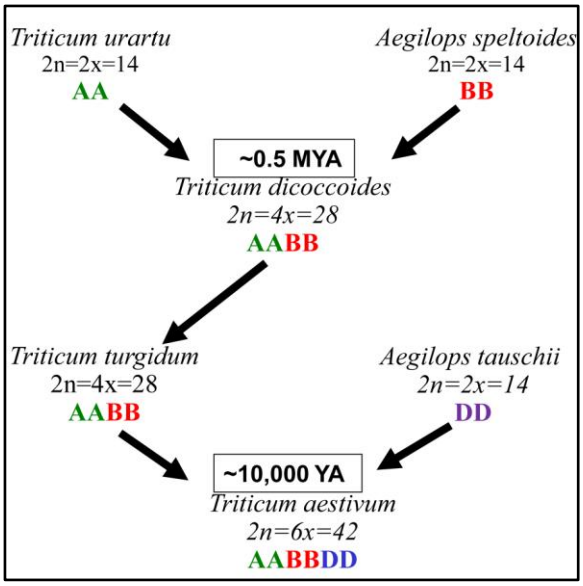


Figure 1.1: Diagrammatic representation of the evolution of hexaploid wheat. Data obtained from Feldman 2001, Feldman & Levy 2005 and Dubcovsky and Dvorak 2007.

The pioneering work of Sears and Kihara in the early 1940s revealed that the A and D genomes of hexaploid wheat are derived from the diploid *T. urartu* and *Ae. Tauschii* (Feldman and Levy, 2005b, Kihara, 1944). However, the origin of B genome is still unclear but evidences suggest, that *Ae. speltoides* (from the *Sitopsis* section) is the most likely B genome donor. The reason for this ambiguity may be, the ancestral form does not exist anymore or it has evolved rapidly in the allopolyploid condition (Feldman and Levy, 2005b, Salina *et al.,* 2006).

Polyploidization and selective pressure, exerted by man has led to a dramatic reduction in the genetic diversity of cultivated wheat (Buckler Iv *et al.,* 2001, Vaughan *et al.,* 2007, Fu and Somers, 2009, Roussel *et al.,* 2005). Lack of cross pollination and homoeologous recombination, imposed further genetic bottlenecks. Thus, ancestral species remain the primary sources of genetic diversity for wheat (Akhunov *et al.,* 2010). However, a large amount of research over the last few decades in cereals, legumes and other crops could not find any overall reduction in the genetic diversity of released varieties, suggesting that introduction of new germplasm has kept pace with the loss of diversity through inbreeding (Huang *et al.,* 2007, Van de Wouw *et al.,* 2010, Heslop-Harrison and Schwarzacher, 2011b).

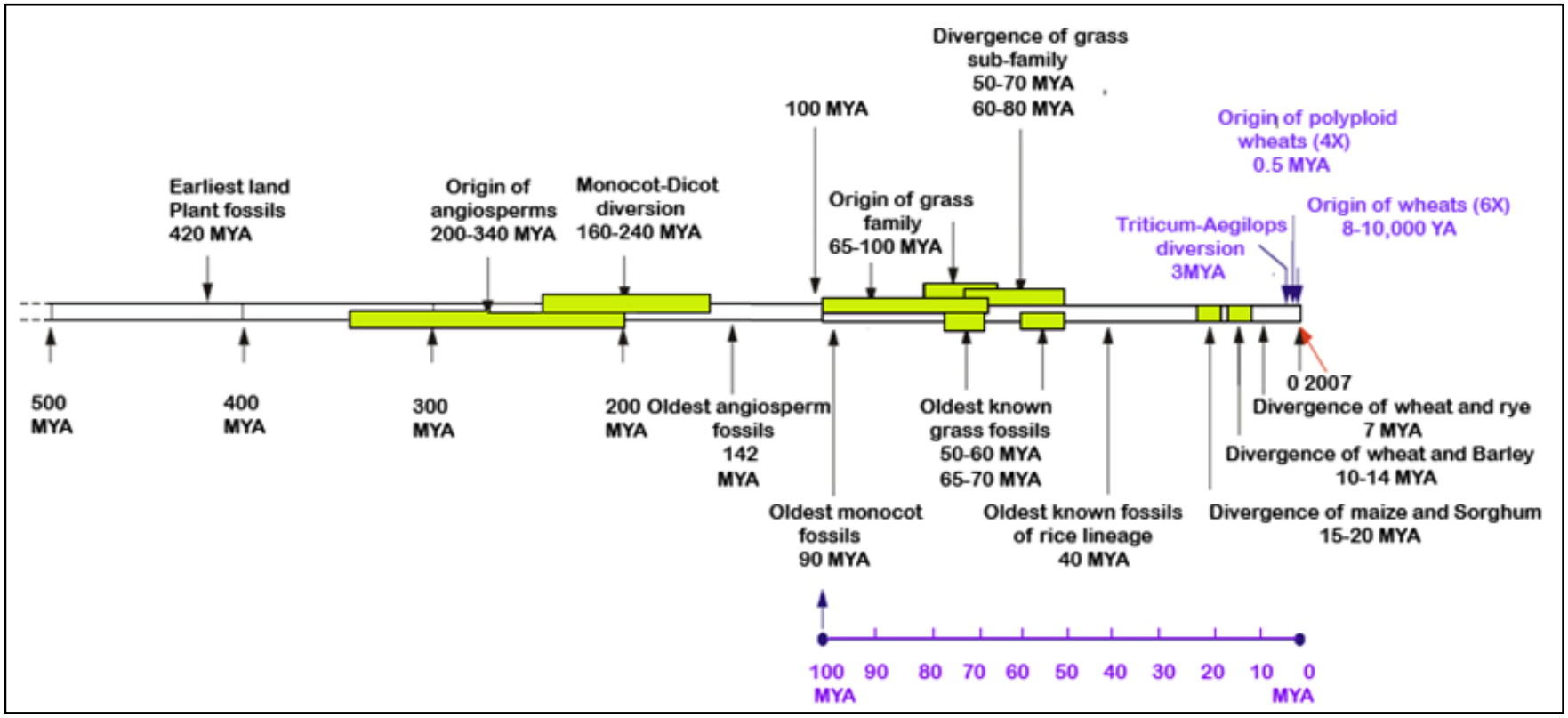


Figure 1.2: "Time line for the evolution of wheat, source of information: P. F. Byrne, Colorado state, with new data added by P. Gornicki, University of Chicago".Modified from Annual Wheat Newsletter 57-2011. http://wheat.pw.usda.gov/ggpages/awn/57/).

1.4 Taxonomy of the *Triticeae*

Taxonomy has always been a fascinating but a controversial field of biology, and the *Triticeae* is also no exception to this statement (Goncharov, 2011). All cereals are members of the grass family (*Poaceae*), the fourth largest and ecologically dominant family among the angiosperms with about 700 genera and 10,000 species (Gaut, 2002, Feuillet and Keller, 2002b). The grass family is taxonomically challenging, and most grass taxonomists recognize 6 or 7 major subfamilies within the family. The economically important tribe *Triticeae* is assigned to the subfamily Pooideae (Gaut, 2002). The name *Triticeae* was first recognized by the Belgian botanist Dumortier in 1823 (Yen and Yang, 2009).

Most early attempts of the *Triticeae* taxonomy concentrated on morphological and phytogeographic aspects (Bentham, 1882, Hackel, 1887, Melderis, 1980). However, Kihara introduced genome analysis and cytogenetic research into *Triticeae* and refined the classification and evolutionary relationships within the group (Kihara, 1930, Kihara, 1954, Kihara and Nishiyama, 1930). Lack of absolute boundaries among different genera and unavoidable arbitrariness above the species level make the classification of *Triticeae* very complex (Yen and Yang, 2009). Among the various views about the generic classification, the stands taken by Stebbins (1956) and Löve (1984, 1986) represent opposite extremes. Stebbins supports keeping all species into a single genus, while Löve splits the *Triticeae* into 39 genera (see Dizkirici *et al.*, 2010).

Nevertheless, the *Triticeae* is a heterogeneous group of some 400-500 diploid and polyploid species of varying complexity. It includes both wild and cultivated genera such as *Aegilops, Agropyron, Crithopsis, Dasypyrum, Elymus, Eremopyrum, Festucopsis, Hordelymus, Hordeum, Psathyrostachys, Secale, Taeniatherum* and *Triticum* etc. (Melderis *et al.* 1980). The genus *Triticum*, which is perhaps the most important genus in the tribe, is represented by species of various ploidy levels.

Very briefly the genus *Triticum*, comprises six species (Table 1). *T. monococcum* L., *T. urartu* Tumanian ex Gandilyan, *T. turgidum* L., *T. timopheevii* (Zhuk.) Zhuk., *T. aestivum* L. and *T. zhukovskyi* Menabde & Ericz. These species are subdivided in three sections, Monococcon, Dicoccoidea and Triticum consisting of diploid, tetraploid and hexaploid species respectively. *T. urartu* exists in wild, while *T. aestivum* and *T. zhukovskyi* exist in the cultivated form only. The remaining three species can exist in either form (see Matsuoka, 2011).

Table 1.1: Nomenclature of wild and cultivated wheats (after Van Slageren 1994) modified from Matsuoka, 2011.

Section	*Triticum* species and subspecies	Genomic constitution	Common examples
Monococum	*T. monococum* L.	AA	
	subsp. *aegilopoides* (Link) Thell.		Wild einkorn
	subsp. *monococcum*		Cultivated einkorn
	T. urartu Tumanian ex Gandilyan	AA	
Dicoccoidea	*T. turgidum* L.	AABB	
	subsp. *dicoccoides* (körn. Ex Asch. & Graebn.)		Wild emmer
	subsp. *dicoccon* (Schrank) Thell.		Cultivated emmer
	subsp. *durum* (Desf.) Husn.		Macaroni wheat
	subsp. *polonicum* (L.) Thell.		Polish wheat
	subsp. *turanicum* (jakubz) A. Löve & D. Löve		Khorasan wheat
	subsp. *turgidum*		Rivet wheat
	subsp. *carthlicum* (Nevski) A. Löve and D. Löve		Persian wheat
	subsp. *paleocolchicum* (Menabde) A. Löve and D. Löve		Georgian wheat
	T. timopheevii (Zhuk.) Zhuk	AAGG	
	subsp. *armeniacum* (Jakubz.) van Slageren		Wild timopheevii
	subsp. *timopheevii*		Cultivated timopheevii
Triticum	*T. aestivum L.*	AABBDD	Common wheat
	subsp. *aestivum*		Bread wheat
	subsp. *compactum* (Host) Mackey		Club wheat
	subsp. *sphaerococcum* (Percival) Mackey		Indian dwarf wheat
	subsp. *macha* (Dekapr. & Manabde) Mackey		
	subsp. *spelta* (L.) Thell.		Spelt
	Triticum zhukovskyi Menabde & Ericz.	AAAAGG	

1.5 Plant pests and their management

Since the onset of human civilization, plant diseases have been an everlasting constraint on food production, and will continue to cause human suffering and economic losses (Baker *et al.*, 1997). One of the major challenges for the future has been food security. Avoiding widespread hunger will require a substantial increase in yield from the existing cropland. But in their efforts to meet the demands of global production, plant breeders are finding less appropriate germplasm with desirable traits (Foulkes *et al.*, 2011, Mujeeb-Kazi and Hettel, 1995).

The rapid increase of human population and shrinkage of the land surface for agricultural practices is putting all measures of food security at risk (section 1.1). Perhaps, shielding food crops from pathogenesis is the most important factor that can substantially increase agricultural production (Baker *et al.*, 1997). Fungicides are successfully applied to control the diseases, but they are too expensive particularly for small farmers in the developing countries and are environmentally hazardous (Liu *et al.*, 2011). Utilization of high yielding varieties has significantly improved crop productivity, but in plants like wheat, which was domesticated 10,000 years ago, and is predominantly self-pollinated, this has only added to its existing genetic bottlenecks (Gustafson *et al.*, 2009, Matsuoka, 2011).

Studies indicate that climate plays a critical role in the evolution of both flora and fauna, and changes in the climate may alter the entire ecological landscape (Li and Yap, 2011, Manole and Bazgă, 2011). In the last 100 years, the earth's climate has changed in response to human activities. For example, the mean global temperature has increased by 0.74°C and atmospheric CO2 level has raised from 280ppm in 1750 to 368ppm in 2000 (see Watson, 2001, Chakraborty and Newton, 2011). These changes have impacts on the geographical distribution and growth of plant species as well as host-pathogen interactions (see Coakley *et al.* 1999, Heslop-Harrison and Schwarzacher 2011b).

Plant diseases could potentially reduce the attainable yield by as much as 82% in the case of cotton and more than 50% for other major crops (Oerke, 2006). Every year about 10–16% of the global harvest is lost to plant diseases, which is equivalent to US$220 billion. It excludes the additional postharvest losses of 6–12%, which are particularly common in the developing world (see Chakraborty and Newton, 2011). Although different cultural practices and use of pesticides have dramatically reduced plant diseases, the cost, potential environmental problems, and increased tolerance

through selective pressure are main concerns for the future (Chen, 2005, Curtis *et al.*, 2002). Since, the emergence of the Ug99 group of stem rust races that has reaffirmed the need to deploy diverse and effective resistance sources to safeguard wheat production. Ug99 races are virulent to resistance genes deployed in most wheat varieties currently under cultivation throughout the world (Singh *et al.*, 2008, Stokstad, 2007). It is known, increasing genetic diversity in host populations retards the rate at which virulence evolves. In such circumstances breeding resistant cultivars is the most practical approach to safeguard man's prime food sources like wheat on permanent basis (Li and Wang, 2009, Graybosch *et al.*, 2009, Gill *et al.*, 2011) and will be discussed below in detail.

1.6 Genetic resources for wheat improvement

When cultivated wheat was growing as a mixture of land races, there was a wide range of variation (Feldman and Sears, 1981, Gustafson *et al.*, 2009). Intense breeding and selection for greater yield potential has not only eliminated the undesirable alleles but also reduced the useful genetic variation, especially that of resistance to biotic (fungal and insect pests) and abiotic (cold, drought and salt) stresses. In the *Triticeae*, the past few decades of selection for yield alone and the failure to secure primitive cultivated varieties, has given rise to a substantial loss of total genetic variability. Such genetically uniform varieties cultivated over enormous areas are susceptible to devastating epidemics (Feldman and Sears, 1981, Fu and Somers, 2009, Heslop-Harrison, 2002, Heslop-Harrison and Schwarzacher, 2011b, Li *et al.*, 2005a).

Fortunately, wheat genetic restoration is possible, by exploiting a vast reservoir of genetic resources distributed across three gene pools (Ayala-Navarrete *et al.*, 2007, King *et al.*, 1997a, King *et al.*, 1997b, Schwarzacher *et al.*, 2011, Schwarzacher *et al.*, 1992). The primary gene pool is constituted by two species, the tetraploid *T. turgidum* and diploid *Ae. tauschii*, which hybridized and resulted in hexaploid wheat. Recombination between the primary gene pool and wheat genome takes place through direct hybridization and homologous recombination (Sehgal *et al.*, 2011, Gill and Raupp, 1987, Qi *et al.*, 2007). Diploid species of the Sitopsis section, *T. monococum* and the polyploid *Triticum-Aegilops* group, sharing one of the three genomes of wheat constitute the secondary gene pool. Gene transfer between the two genomes takes place through direct crosses and backcrosses with varying level of homologous exchange between the related genome or through special manipulation strategies (Sharma and Gill, 1983, King

et al., 1997a, Li and Wang, 2009, Mujeeb-Kazi and Hettel, 1995, Qi *et al.,* 2007). *Triticeae* species, with genomes non-homologous to wheat constitute the tertiary gene pool. These species are rich sources of wheat improvement, but because of their non-homologous genomes, gene transfer is not possible by homologous recombination and requires special techniques such as irradiation, callus culture mediated translocation or through *ph* mutants etc. (see section 1.7).

Various annuals and perennials from the tertiary gene pool, especially the *Thinopyrum* group have enormous genetic variability and have proven to be excellent sources of disease resistance. Member of the *Thinopyrum* group have been successfully used for the introgression of alien material especially against biotic and abiotic stresses in the form of small segments to entire chromosomes (Ayala-Navarrete *et al.,* 2007, Chen, 2005, Divis *et al.,* 2006, Fahim *et al.,* 2011, King *et al.,* 1997a, Li and Wang, 2009, Mujeeb-Kazi and Hettel, 1995, Qi *et al.,* 2007, Schwarzacher *et al.,* 2011, Schwarzacher *et al.,* 1992, Sharma and Gill, 1983, Wells *et al.,* 1973) and will be discussed in detail in the results chapter.

1.7 Introduction of alien material for useful traits

In order to develop new plant varieties with high yield potential and a broader genetic base, breeders need some variation to initiate with (Feldman and Sears, 1981, Gill *et al.,* 2011, Heslop-Harrison, 2002). To a greater extent, wheat breeders have overcome this challenge by the wise utilization of both the wild (often referred to as alien) and cultivated *Triticeae*, harboring agronomically important genes for the enrichment of wheat cultivars and for introducing novel variation (Schwarzacher *et al.,* 1992, King *et al.,* 1997a, Wells *et al.,* 1973, Wang *et al.,* 2002, Singh *et al.,* 2008a, Singh *et al.,* 1998, Divis *et al.,* 2006). Transfer of desirable traits derived from alien sources as chromosomal arms or segments has been a successful practice in broadening the genetic base of wheat. There are numerous examples of gene transfers between the *Triticeae* species and common wheat for varied traits such as, improved grain quality, resistance to mites, fungi, viral diseases, drought and salinity etc. that have successfully transferred new variation to the wheat germplasm (Heslop-Harrison, 2010, Mujeeb-Kazi and Hettel, 1995, Chen *et al.,* 1996, Gill *et al.,* 2011, King *et al.,* 1997b, Larkin *et al.,* 1995, Carvalho *et al.,* 2009, Ribeiro-Carvalho *et al.,* 2004, Liu *et al.,* 2011, Mutti *et al.,* 2011, Schwarzacher *et al.,* 2011).

In intergeneric or wide-crosses, wheat is used as a maternal parent with significant success (Figure 1.3). The procedure involves bud pollination, post-pollination gibberellic acid treatment, 14-18 days post-pollination excision followed by embryo culture, so that the embryo differentiates into a plantlet (Mujeeb-Kazi and Hettel, 1995). This plantlet or F_1 hybrid is usually self-sterile, however in some hybrids few pollen mother cells may undergo meiotic restitution and produce unreduced gametes. Thus rare F_1 intergeneric hybrids may be partially fertile (see Islam and Shepherd, 1980). Nevertheless, the sterile F_1 hybrid on colchicine treatment results in fertile amphidiploids (King *et al.,* 1997a). The production of amphidiploids between wheat and the desired species is followed by the production of individual alien chromosome addition lines through backcrosses. The entire alien chromosomal arms can be transferred to wheat backgrounds by exploiting the centric breakage-fusion property of univalents. Once the homoeologous relationship of the alien chromosome carrying the desired gene has been established the alien chromosome and a homoeologous wheat chromosome are isolated in monosomic condition. In double monosomic plants, both monosomes do not pair at meiotic metaphase I, and have the tendency to break at the centromeres, followed by fusion of the broken arms, giving rise to Robertsonian whole arm translocations (see Mujeeb-Kazi and Hettel, 1995, Qi *et al.,* 2007).

The efficiency of alien material is determined by its ability to substitute the homoeologous segments of wheat chromosomes. Although, linkage drag effects are less pronounced or buffered in polyploid wheat compared to the diploids, having more sensitive genomes to the genetic imbalance (King *et al.,* 1992, Friebe *et al.,* 1996a, Qi *et al.,* 2007, Gill *et al.,* 2011). Still, wheat-alien compensating translocations with minimal alien chromatin are of immense importance, as they introduce new characters and have less likelihood of a linkage drag, which can affect the essential agronomic and end-use quality attributes (Friebe *et al.,* 2009, Forsström *et al.,* 2002, Gill *et al.,* 2011).

Plant breeders have been remarkably successful in developing new varieties of all major crops, with desired traits (Borlaug, 1983). Hybrids deliver higher yields and better quality than either of the parent alone, and this has been achieved despite the rapid emergence of more aggressive and virulent races of pathogens, different cultivation practices, and in a more disturbed and changing environmental conditions. In the UK, 90% increase in the yield potential of cereals is attributed to the improved varieties released in the last 25 years having a better genetic constitution (Vaughan *et al.,* 2007, Heslop-Harrison and Schwarzacher, 2011b).

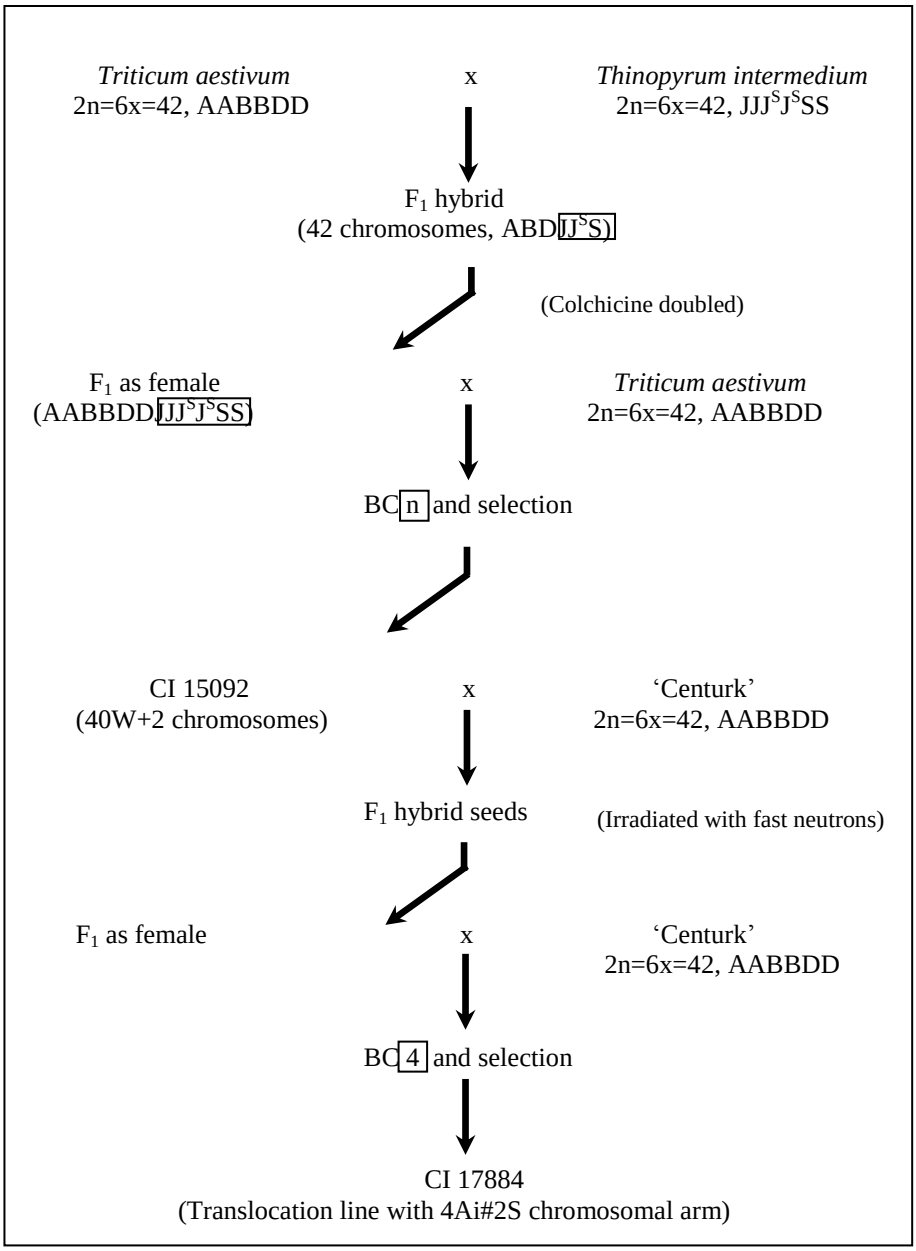


Figure 1.3: Schematic representation of wide hybridization and alien gene transfer from *Th. intermedium* (JJJ^{S}J^{S}SS genome) to *T. aestivum* (AABBDD genome). CI 17884 is a *Wheat streak mosaic virus* (WSMV) resistant line. This resistance is present on the short arm of *Th. intermedium* chromosome 4Ai#2. Experimental lines used in the current study were putative carriers of the same resistance. BC represents back cross, W represent wheat chromosomes, 'Centurk' is a wheat cultivar. *Sources:* Wells *et al.*, 1973, 1982, Divis *et al.*, 2006.

1.8 Genomic research for crop improvement

In addition to population growth, the availability of arable land, water for agriculture and global climatic changes will not only affect crop growth but will also threaten the conservation of land under cultivation (Takeda and Matsuoka, 2008). The 20th century has witnessed a tremendous increase in crop production, which primarily became possible due to the application of Mendel's principles in breeding practices (Zhang *et al.*, 2009). Conventional breeding practices allowed breeders to manipulate novel variations required for resistance and productivity (Ayala-Navarrete *et al.,* 2007, Feldman and Sears, 1981, Fedak *et al.,* 2001). Overall, there has been significant increase in the productivity of important cereal crops, but now the available traditional practices of crop improvement are no longer sufficient to meet the demands of future population or new crop uses such as bio-fuels (Heslop-Harrison and Schwarzacher, 2011b).

The remarkable progress in crop improvement over the last decades is attributed with good reason to the development of new genomic technologies like, next generation sequencing, high-throughput marker genotyping, omics and an understanding of the variation at the DNA, RNA and protein level (see Varshney and Dubey, 2009). These new insights into the plant genome have opened up an exciting era of plant molecular breeding. Unlike conventional breeding, that follows the paradigm of "cross best with the best and hope for the best", the linkage of gene for specific trait leads to more precise and predictable breeding outcomes. In the past where the increased crop productivity was based on improved agricultural practices and chemicals, future gains will rely on improved genetics (see Heslop-Harrison and Schwarzacher 2011b).

This better understanding of plant genomics has been essentially possible due to the increasing availability of genomic sequences. In some cases the whole genomic sequence is available (e.g. *Arabidopsis*, Rice, Maize and *Brachypodium* etc.) while in others the genome is partly available. These sequencing projects will enhance our knowledge about the major crops (Mochida and Shinozaki, 2010). Nevertheless, major efforts are underway to sequence the full genome of variety of organisms. The available sequence data has made it possible to develop a variety of functional molecular markers and is clearly shaping our approaches to key biological processes (Zhang *et al.,* 2009, Varshney and Dubey, 2009, Korzun, 2002, Collard and Mackill, 2008).

Genomic research is generating a variety of molecular and cytogenetic markers (see Heslop-Harrison and Schwarzacher 2011a) that has increased the efficiency of crop

species, and will allow breeders to perform their tasks with great ease and precision. Molecular markers can be used to tag genes of interest, through marker-assisted selection (MAS). In cereals MAS, has been applied to develop improved cultivars with better traits for biomass and tolerance to biotic and abiotic stresses (Reddy *et al.,* 2008, Talbert *et al.,* 1996, Ayala *et al.,* 2001, Fahim *et al.,* 2010b, Krattinger *et al.,* 2011). Genomic *in situ* hybridization (GISH), identifies plants that carry alien segments and can be used in association mapping with molecular markers linked to traits of interest, which provides a powerful system to tag genes, and allow screening of cultivars with desirable and undesirable alleles in early generations and save valuable resources (Schwarzacher *et al.,* 1992, Schwarzacher *et al.,* 1989, Castilho and Heslop-Harrison, 1995, Mukai *et al.,* 1993, Tsujimoto *et al.,* 1997).

Advances in DNA sequencing projects and analytical approaches have greatly increased our understanding of the plant genome, and there is no reason to think that we are close to maximum possible yields, but still genomic research is in its infancy and future goals of plant breeding are to be determined. Knowledge of the loci that influence tolerance, high yield and domestication like traits are still not enough and needs to be capitalize upon (Takeda and Matsuoka, 2008, Matsuoka, 2011, Stukenbrock and McDonald, 2008). Nevertheless, production of high yielding and resistant cultivars will remain the primary goal of most breeding programs, but integration of new genomic approaches with traditional breeding strategies is required to put theory into practice. This will empower breeders in their efforts to select the best available combination of traits available within species and will help them, to solve the major issue of sustainable agricultural production (Mochida and Shinozaki, 2010, Gustafson *et al.,* 2009, Varshney and Dubey, 2009, Heslop-Harrison and Schwarzacher, 2011b).

1.9 Genome organization in grasses

Recent advancements in sequencing technologies along with reduced costs, have allowed the sequencing of five important species of the grass family. This has given a direct access to the gene content and genomic architecture of grasses (Stein, 2007, Devos, 2010). Major cereals diverged from a common ancestor about 65MYA (Figure 1.2) and show considerable variation in genome size. The 1C value varies significantly among grasses i.e. 400Mb-8,000Mb for rice and wheat respectively. But, despite of the millions years of co-evolution and enormous variation in genome sizes, member of the

grass family show remarkable similarity in gene content and order (Bennetzen, 2005, Luo *et al.,* 2009, Paterson *et al.,* 2009, Flavell *et al.,* 1974). The sequenced data suggest, that gene content in related genomes are not exactly the same, mainly due to non uniform or lineage-specific evolution of genes, and frequent chromosomal rearrangements like deletions, duplications and inversions (Devos and Gale, 2000, Ilic *et al.,* 2003, See *et al.,* 2006, Devos, 2010).

Table 1.2: Structural characteristics of four sequenced genomes. Data taken from Devos, 2010.

Species	Haploid genome size (Mb)	Chromosome number	Gene number	Class I transposon content (%)	Class II transposon content (%)
Brachypodium distachyon	320	2n=2x=10	25853	23.3	4.8
Oryza sativa	400	2n=2x=10	28236	25.8	13.7
Sorghum bicolor	800	2n=2x=40	27640	54.5	7.5
Zea mays	2500	2n=2x=20	32540	75.9	8.6

Flowering plants have undergone one or more rounds of polyploidization (also referred to as whole genome duplication or WGD) in their evolutionary history and that has played a significant role in their diversification. Monocots have undergone two WGD events prior to the divergence of cereals and other grasses (Stein, 2007, Jiao *et al.,* 2011). The first evidence of genomic duplication came from the analysis of the rice genome, but was later also identified in the whole-genome sequence of *Brachypodium*, *Sorghum* and maize (Devos, 2010, Luo *et al.,* 2009).The sequenced genomes revealed a considerable amount of gene redundancy and much of this is thought to be due to the result of ancient WGDs (see Soltis *et al.,* 2009). The total number of genes, does not vary greatly among the angiosperms, and typically remains at around 28,000 per haploid genome, but in maize the slightly higher numbers of genes reveal its ancient tetraploid nature.

A significant proportion of the grass genome is repetitive DNA and for example in wheat it accounts for up to 80% of the genome (Flavell *et al.,* 1974b). Thus the variation in genome sizes of grasses can be attributed primarily to the difference in the amount of repetitive DNA (Schmidt and Heslop-Harrison, 1998b, Heslop-Harrison, 2000a, Contento *et al.,* 2005, Bennett and Leitch, 2011). Devos (2010) compared the

four published grass genomes (Table 1.2) and found that the class I or long terminal repeat (LTR) retrotransposons were the most abundant fraction of these genomes. The amount of LTR retrotransposon was related to the size of genome, while the DNA or class II transposons were much more constant across species and did not correlate generally with genome size (Table 1.2 and section 1.11). Possibly this can be explained by DNA transposon being mostly associated with gene-rich regions of the genome and they are cut and pasted rather than replicated (for details see Paterson *et al.,* 2009, Schnable *et al.,* 2009, Vogel *et al.,* 2010).

It is interestingly the chromosomes number that provides structure for genetic linkage groups and allows faithful replication that has fluctuated widely during the evolutionary history of grasses (Heslop-Harrison and Schwarzacher, 2011a). Based upon organizational features and staining properties in cytological preparations, grass chromosomes as chromosomes in general show two distinct regions, the heterochromatin and euchromatin (Flavell, 1986, Schmidt and Heslop-Harrison, 1998b, Schwarzacher, 2008, Endo and Gill, 1996). Euchromatin, stains lightly in cytological preparations and is the gene dense region of the chromosome, with high transcriptional activity and higher levels of recombination at meiosis (Heslop-Harrison and Schwarzacher, 2011a). In contrast heterochromatin has highly condensed chromatin that stains strongly in cytological preparations. In general it is rich in repetitive DNA and transposable elements (TEs). It lacks meiotic recombination and is relatively deficient in genes, and those that are present often have decreased transcriptional activity (Vershinin and Heslop-Harrison, 1998, Heslop-Harrison, 2003, Kubis *et al.,* 2003a). Euchromatin lies at the interstitial and distal regions of the chromosomes while heterochromatic blocks often lie at the telomeric and pericentromeric regions (Figure 1.4).

Physical organization of genes and repeats, and locating them on chromosomal regions is crucial for the understanding of genomic organization and evolution in plants (Heslop-Harrison, 2000b). Availability of full genome sequences from grasses has definitely enhanced our abilities to understand the complexity of the grass genome organization. Nevertheless, reconstruction of the ancestral grass genome is still a major challenge because of the frequent and ubiquitous WGD across angiosperm history. Reconstruction of the ancestral genomes will improve our ability to resolve correlated gene arrangements and shared ancestry of genes among closely and distantly related taxa (Soltis *et al.,* 2009).

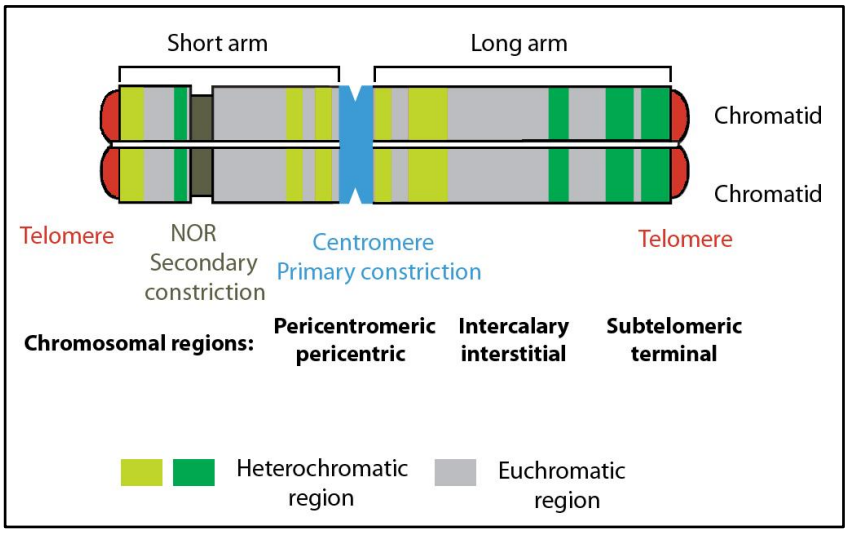


Figure 1.4: The organization and features of plant chromosome, modified from Heslop-Harrison and Schwarzacher, 2011a.

1.10 Cytogenetic structure of wheat

Nutritional significance and the presence of large chromosomes with an average size of ~11.2µ per chromosome always facilitated the cytogenetic investigation of *Triticeae* (Mutti *et al.* 2010). The pioneering cytological work of Sakamura, Sax, Kihara and Sears in the early 20th century, revealed the presence of three different ploidy levels in *Triticeae* species. They also described the main cytogenetic characteristics of these species, such as the basic chromosome number of seven (n=7), large chromosomes, frequent polyploidy and the complicated reticulate pattern of relationships due to repetitive intergeneric hybridizations (see Vershinin *et al.*, 1994, Curtis *et al.*, 2002, Feldman and Levy, 2005, Heslop-Harrison and Schwarzacher, 2011a). However, not all *Triticeae* members have the basic set of n=7 chromosomes. Variation exists from n=2 to n=19 and the ancestral basic chromosome number is still uncertain (Gaut, 2002). Recent phylogenetic reconstruction and comparative studies of grass genome structure suggest that the basic chromosome number could be n=12 in the common ancestor of *Triticeae*, rice, and sorghum. The reduction of chromosome number from n=12, in the common paleo-ancestor was probably driven by non random centric double-strand break repair events (Luo *et al.*, 2009, Murat *et al.*, 2010).

By and large wheats have the basic chromosome number of x=7. Diploid wheat species contain two haploid sets of seven chromosomes, tetraploid contains four and hexaploids contain six and so on. In hexaploid wheat, these chromosomes are assigned to A, B or D-genomes (Figure 1.5). The A and B genomes can be distinguished on the basis of their pairing ability with the diploid A-genome (Sears, 1966). All the A, B and D-genome chromosomes are broadly divided into either homologous (genetically similar) or homoeologous (genetically related) groups (Sears, 1966, Schwarzacher, 1996, Hao *et al.*, 2011). The homoeologous groups are identified on the basis of their ability to compensate for the dose of the lost chromosomal pair from the other genome in nulli-tetrasomic lines (Sears, 1966).

In spite of possessing multiple sets of related chromosomes, hexaploid wheat restricts pairing to true homologues at meiosis and behaves as a diploid. This diploid like behavior is controlled by the *Ph* complex (pairing homologous) comprising of major (*Ph1*) intermediate (*Ph2*) and few minor loci (Okamoto, 1957, Hao *et al.,* 2011, Sutton *et al.,* 2003). Deletion of the single major locus, *Ph1*, from chromosome 5B not only allows pairing of the homoeologous chromosomes from A, B and D genomes but also within the chromosomes of other related species and genera (Griffiths *et al.,* 2006). This property of hexaploid wheat is exploited to make interspecific crosses and manipulating genes from one species to another across the whole group of *Triticeae* (see King *et al.,* 1997a, Lima-Brito *et al.,* 2006).

Bread wheat has a haploid genome size of about 16 billion bp, organized in 21 pairs of the A, B and D genome chromosomes (Heslop-Harrison and Schwarzacher, 2011a). These chromosomes can be identified cytogenetically (Figure 1.5) using different techniques like C-banding, molecular karyotyping, and fluorescent *in situ* hybridization (Mukai *et al.,* 1993, Endo and Gill, 1996, Schwarzacher and Heslop-Harrison, 2000, Schwarzacher, 2003). Genomic *in situ* hybridization (GISH) or fluorescent *in situ* hybridization (FISH) is a powerful technique for chromosomal mapping and genomic analysis. The rapid identification of somatic chromosomes from readily available root meristems has revolutionized cytogenetic research in wheat (Castilho and Heslop-Harrison, 1995, Schwarzacher *et al.,* 1989, Harper *et al.,* 2011, Gill *et al.,* 2011, Schwarzacher *et al.,* 2011). GISH using total genomic DNA as a probe has been able to identify alien fragments or chromosomes in hybrid wheat. It is efficiently used to identify structural rearrangements in chromosomes such as deletions, duplications, translocations, and inversions (Anamthawat-Jonsson *et al.* 1990,

Schwarzacher *et al.* 1992, Heslop-Harrison *et al.* 2003, Schwarzacher 2003a, 2003b). GISH is combined with repetitive DNA sequences, where the unique banding pattern of repetitive DNA along the wheat chromosomes is used to identify genome, chromosome and chromosomal arms (Anamthawat-Jónsson and Heslop-Harrison, 1993, Bodvarsdottir and Anamthawat-Jonsson, 2003, Forsström *et al.,* 2002, Rayburn and Gill, 1986, Contento *et al.,* 2005).

Different cytological markers mapping to specific wheat chromosomes are available (Castilho and Heslop-Harrison, 1995, Vershinin *et al.,* 1994, Gill *et al.*, 1991) along with many other physical maps constructed for all 21 wheat chromosomes (http://wheat.pw.usda.gov). Ideograms for the chromosomes of 'Chinese spring' wheat showing physical mapping and location of different repetitive DNA (Figure 1.5) have already been published, which greatly facilitate the identification of individual wheat chromosomes (see Mukai *et al.,* 1993, Castilho *et al.,* 1996, Taketa *et al.,* 1999, Biagetti *et al.,* 1999).

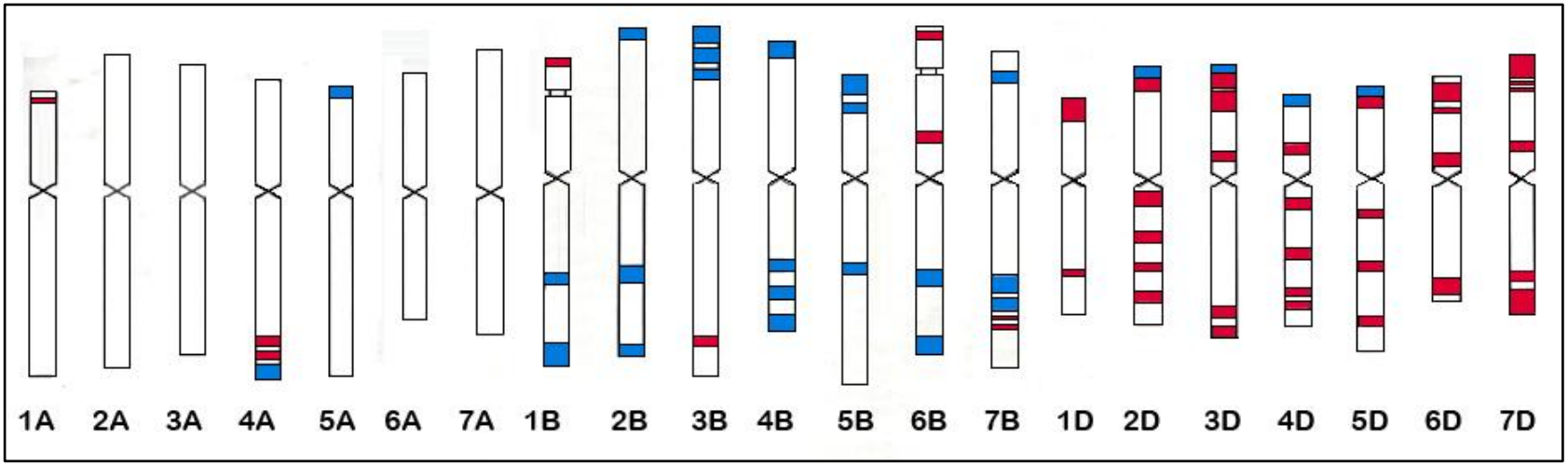


Figure 1.5: Ideogram of‘Chinese spring’ wheat chromosomes, showing the location of pSc119.2 (blue bands) and pAs1 (red bands) sequences. modified from Mukai *et al.,* 1993.

1.11 Repetitive DNA and transposable elements

A major fraction of eukaryotic nuclear DNA is comprised of a variety of repetitive DNA elements that do not encode products used by the cell and sometimes referred to as "selfish" or "junk" DNA (Orgel and Crick, 1980, Salina *et al.,* 2011, Kidwell and Lisch, 2000). In *Triticeae*, these elements account for up to 70–80% of their genome (Flavell *et al.,* 1974a, Heslop-Harrison, 2000a, Charles *et al.,* 2008, Wicker *et al.,* 2003). Recent insights of the plant genome have revealed synteny in gene order and content, but have shown that the repetitive DNA in their genomes varies more widely, and possibly is the main contributing factor of interspecific divergence of genomes (Charlesworth *et al.,* 1994, Bennett and Leitch, 2011, Feuillet and Keller, 2002a, Gaut, 2002, Levy and Feldman, 2002). Therefore, understanding the role and nature of these repeat elements are of great importance for investigating the organizational and phylogenetic relationships as well as their evolutionary dynamics (Vershinin *et al.,* 1996, Vershinin *et al.,* 1995, Luo *et al.,* 2009, Heslop-Harrison, 2000b, Kubis *et al.,* 2003b).

Repeats are classified into two major types according to their genomic organization (Kuhn *et al.,* 2007). The first is composed of sequences of various lengths and composition that occur as tandem repeats, concentrated at one or more distinct positions in the genome and are often referred to as satellite DNAs (Contento *et al.,* 2005, Kubis *et al.,* 1998, Kuhn and Sene, 2005, Murata *et al.,* 1997, Vershinin *et al.,* 1996). The second type is represented by sequences with a dispersed distribution throughout the genome and mainly consists of transposable elements (Salina *et al.,* 2011, Sergeeva *et al.,* 2010, Charles *et al.,* 2008).

Satellite DNAs (satDNAs) consist of highly repeated DNA motifs that are tandemly organised, forming long arrays that may extend from few to tens of thousands of kilobases (Charlesworth *et al.,* 1994, Tsujimoto *et al.,* 1997, Kishii *et al.,* 1999, Kishii and Tsujimoto, 2002, Anamthawat-Jónsson and Heslop-Harrison, 1993, Bodvarsdottir and Anamthawat-Jonsson, 2003). Several unrelated satDNA arrays may be present in the genome, but the main bulk of satDNAs is concentrated in the heterochromatic regions, having no or very few actively transcribing genes (Kuhn and Heslop-Harrison, 2011, Mutti *et al.,* 2010). Many different satDNA families have been described in plant species, showing species or genome specific diversity in their DNA sequence and chromosomal distribution (Bedbrook *et al.,* 1980, Rayburn and Gill, 1986, Vershinin *et al.,* 1994, Vershinin *et al.,* 1995, Nagaki *et al.,* 1995, Bodvarsdottir and Anamthawat-

Jonsson, 2003, Contento *et al.,* 2005). In the current study two highly repetitive DNA families (pSc119.2 and Afa) as examples from the *Triticeae* genomes have been studied and this will be discussed in detail in the results chapter V.

Transposable elements (TEs) on the other hand are dispersed repetitive DNA elements. They are dynamic in nature and are capable of changing their genomic location (Kidwell and Lisch, 2000, Kidwell and Lisch, 2001, Kazazian, 2004). They are divided into two main classes based on their transposition intermediate (Finnegan, 1989, Craig *et al.,* 2002, Wicker *et al.,* 2007). The class I or retrotransposons replicate via reverse transcription of an RNA intermediate before integrating into the genome and follow the "copy & paste" mechanism. The class II or DNA transposons transpose directly from DNA to DNA, these elements are excised from one region and reintegrated elsewhere in the genome by "cut & paste" mechanism (Finnegan, 1989, Wessler, 2006, Charles *et al.,* 2008). TEs may be autonomous or non-autonomous depending upon the presence of sequences that encode for transposase (TPase), the enzyme that catalyses transposition activity. Non-autonomous elements lack functional TPase and their mobility within the genome is limited to the activity of other autonomous elements (Bennetzen, 2000, Bennetzen, 2005, Lander *et al.,* 2001, Feschotte *et al.,* 2002).

Most of the retrotransposons contain either long terminal repeats (LTR) at both ends (LTR retrotransposon) or terminate at a poly-A tail (non-LTR retrotransposon) at their 3´end (Kumar and Bennetzen, 1999, Kazazian, 2004). The LTRs contain regulatory sequences required for the transcription of gag, pol and integrase genes within the LTR retrotransposons. Products of these genes are required for making a cDNA copy and reintegration of the element into a new site within the genome (Kazazian, 2004, Lander *et al.,* 2001). Common non–LTR retrotransposons include SINEs (short interspersed repetitive elements) and LINEs (long interspersed repetitive elements). SINEs are non-autonomous and their transposition is achieved through LINEs machinery (Kumar and Bennetzen, 1999, Kajikawa and Okada, 2002, Dewannieux *et al.,* 2003). Currently LINEs are the only autonomous non-LTR elements within the human genome (Lander *et al.,* 2001).

Retroelements, mainly LTR retrotransposons make up the bulk of plant genomes, and show a direct correlation with the genome sizes in grasses (Table 1.2). LTR retrotransposons show genome-specific amplification, and this is one of the reasons that the genome sizes vary dramatically and that these elements rarely show synteny even between closely related species (Ammiraju *et al.,* 2007). Although other reasons could

be the short life of LTR-retrotransposons as they are removed within a few million years (see Bennetzen, 2005). Furthermore, some chromosomal regions have been reported to be repeat-rich because they accumulate more LTR retrotransposons, or they do not remove them efficiently (Ma *et al.,* 2004, Vogel *et al.,* 2010).

DNA transposons are recognised by their short terminal inverted repeats (TIRs) and a single open reading frame that codes for the TPase enzyme (see De Boer *et al.,* 2007). As the sequence specificity for the integrating DNA elements is limited to a small number of nucleotides, therefore reintegration can occur at many sites. More often, the daughter insertions take place in proximity to the parental copy (Kazazian, 2004). DNA elements are classified into families on the basis of their TPase (Zhang *et al.,* 2004). Some of the important DNA transposons include *CACTA, hAT, Harbinger* and *Mariner* etc. (Kapitonov and Jurka, 2008). Unlike retroelements, DNA transposon content does not vary greatly and with few exceptions are mostly associated with the gene-rich fraction of the genome (Devos, 2010). In humans there are no active DNA transposons and the youngest elements are estimated to have mobilized ~37 MYA (Kazazian, 2004).

Several families of TEs are present at a time in the genomes of eukaryotes, but their relative proportion varies, which may be due to the extent to which genomes have been mined. In rice, *Brachypodium* and *Sorghum*, there is a strong separation between genes and repeats, euchromatic regions consist mostly of genes and the LTR-retrotransposons are located in the heterochromatic regions (Luo *et al.,* 2009, Devos, 2010). Although, TEs may have a much broader distribution along the chromosome compared to satDNAs and may be dispersed widely in the euchromatic regions (Figure 1.4) filling up the intergenic spaces (Feschotte *et al.,* 2002, Devos, 2010, Heslop-Harrison and Schwarzacher, 2011a). Variability of the non-genic sequences that make the bulk of angiosperm nuclear DNA is primarily because of these TEs (Bennetzen, 2005 and Chapter V).

1.12 Epigenetics and chromatin remodelling

1.12.1 Nucleosome as the basic unit of chromatin

The packaging of the double-stranded DNA helix into the nucleosomes is similar in all organisms (Richmond *et al.,* 1984). Packaging prevents DNA from becoming unmanageable, and ensures that it is readily available for processes such as transcription,

replication and repair. DNA of about ~146bp is wrapped in two superhelical turns around the core histone octamer complex, comprising of two copies of each histones H2A, H2B, H3, and H4 (Figure 1.6). Nucleosomes are connected by linker DNA, typically 20 to 35 bp long (Kornberg and Lorch, 1999, Heslop-Harrison, 2000b, Heslop-Harrison and Schwarzacher, 2011a, Luger *et al.,* 1997). Arrays of 10nm chromatin fibres, also known as "beads-on-a-string" are folded into 30nm loops, followed by compaction into a 250nm fibres which undergo helical coiling to form chromatid with a width of approximately 700nm (Horn and Peterson, 2002, Robinson *et al.,* 2006, Maeshima *et al.,* 2010). However, the detailed nature and consequences of packaging of the DNA fibres into the chromosome at higher levels are not clear. Solving the higher levels of chromatin packaging and its genetic control will lead to better understanding of various genetic and epigenetic processes (Heslop-Harrison and Schwarzacher, 2011a). The histone proteins are folded into globular structures, responsible for the interaction with DNA and adjacent histones and form the nucleosome core. Each histone has a long N-terminal tail which extends out the nucleosome core particles (Hacques *et al.,* 1990, Fuchs *et al.,* 2006). The four core histones are subjected to over 100 different post-translational modifications (see Turner 2005, 2009).

1.12.2 Epigenetic phenomena

Hereditary information present in the primary structure of DNA is faithfully transmitted from one generation to the next in the absence of mutation. But some heritable changes having phenotypic and evolutionary consequences do not involve any changes in nucleotide sequence. These are grouped together as epigenetic changes (Liu and Wendel, 2003). For example, covalent modifications of the N-termini of the nucleosome core histones (Figure 1.6) have important roles in gene regulation (Berger, 2002). These long N-terminal tails are subjected to a variety of post-translational modifications such as acetylation, methylation, phosphorylation, ubiquitination, glycosylation, ADP-ribosylation, carbonylation and sumoylation.

These modifications, together with DNA methylation (see below) control the folding of the nucleosomal array into higher order structures. Although histones and their modifications are highly conserved (Turner *et al.,* 1992) recent data show that chromosomal distribution of individual modifications can differ during cell cycle along the chromosomes as well as among and between groups of eukaryotes (Turner *et al.,*

1992, Berger, 2002, Fuchs *et al.*, 2006, Fuchs *et al.*, 2005, Belyaev *et al.*, 1997). During cellular processes the highly condensed chromatin needs to get unpacked to allow access of different molecules to the DNA. This regulated alteration of chromatin structure is termed as chromatin remodelling. It is accomplished by covalent modification of histones or by the action of ATP-dependent remodelling complexes (Aalfs and Kingston, 2000, Lans *et al.*, 2012, Flaus and Owen-Hughes, 2011).

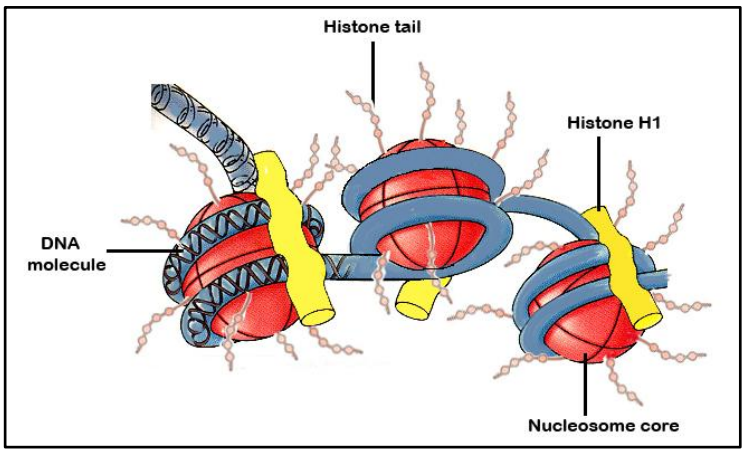


Figure 1.6: Structure of nucleosome showing histone octamer complex wrapped around by ~146bp of DNA (modified from http://youthknowledge.blogspot.com/2011/08/what-are-histones.html).

1.13 Polyploidy a major force shaping the evolution of plants

Whole genome duplication or polyploidy, followed by gene loss and diploidization has long been recognized as a major force in the evolution of eukaryotes, especially plants (Soltis and Soltis, 2000, Soltis *et al.*, 2009, Jiao *et al.*, 2011). Angiosperms represent the largest group of land plants, with more than 300,000 living species. Their widespread occurrence and success has been attributed in part, to the potential innovations associated with gene or whole genome duplications (Soltis *et al.*, 2009, De Bodt *et al.*, 2005, Feldman and Levy, 2009, Heslop-Harrison and Schwarzacher, 2011a). Most flowering plant lineages have experienced one or more episode of ancient polyploidy and the frequency of polyploidy in pteridophytes could be as high as 95% (see Soltis and

Soltis, 1999). Polyploidy, is regarded as a "special class of mutation" that takes place through genomic doubling or through non-reducing gametes (Otto and Whitton, 2000). Doubling of chromosome number which may involve a single genome (autopolyploidy) or a combination of two or more genomes (allopolyploidy) has played a significant role in plant speciation (Soltis and Soltis, 1999, Wendel, 2000).

Allopolyploidy in particular, whereby two or more different genomes are brought together into the same nucleus, results in the variation and ultimately new species are produced by the combination and recombination of two genomes that once were separated by speciation in their evolutionary history (Adams and Wendel, 2005b, Feldman and Levy, 2009, Heslop-Harrison and Schwarzacher, 2011a). Several of our important crop plants, such as bread wheat, oat, cotton, canola, coffee, and tobacco, are allopolyploids. In evolutionary terms, polyploid species have advantages and deliver better than their diploid progenitors. For example polyploids are more vigorous and have a wider ecological dominance than their diploid parental species (Gill *et al.*, 2011, Heslop-Harrison, 2010, Jiao *et al.*, 2011).

Polyploidization was initially thought to be a single event leading to the formation of new species that are genetically uniform but more recent data suggest that new polyploid species originated from multiple events and have much broader genetic base (Soltis *et al.*, 2009, Soltis and Soltis, 2000, Dubcovsky and Dvorak, 2007, van de Wouw *et al.*, 2010). Sequence data also suggest, that the two WGDs that occurred some 319 and 192MYA in the ancestral lineages, before the diversification of extant seed plants and extant angiosperms (Figure 1.2 and section 1.9 above) resulted in the diversification of regulatory genes required for seed and flower development, and these two WGDs events ultimately contributed to the rise and eventual dominance of seed plants and angiosperms (see Jiao *et al.*, 2011).

1.14 Epigenetic phenomena and polyploidy

Genome-wide gene redundancy not only allows the allopolyploids to tolerate more genomic diversity compared to their progenitors, but also generates novel functional variations that are unattainable at diploid level (Adams and Wendel, 2005a, Ma and Gustafson, 2005). But, to ensure increased vigour and fitness in nature, newly formed polyploid species must undergo a series of evolutionary and revolutionary changes in their genomes (Feldman and Kislev, 2007, Feldman and Levy, 2005a, Feldman and

Levy, 2009). They face several important challenges such as chromosomal pairing, the effect of extra gene or genome dosage, regulatory incompatibilities, and reproductive failures, and they need to overcome these to ensure harmonic inter-genomic coexistence (Chen, 2007, Feldman and Levy, 2009). Nascent polyploids accomplish these challenges, through alterations in the DNA or in chromatin structure. Although, the nature and scale of genomic changes required for successful speciation largely remains undetermined (Feldman and Levy, 2005b, Liu *et al.*, 1998b, Ozkan *et al.*, 2001, Heslop-Harrison and Schwarzacher, 2011a).

For understanding, the evolutionary events involved in speciation, synthetic allopolyploids have been used to investigate the early genetic changes contributing to the diploidization process of allopolyploids (Finnegan *et al.*, 1998, Kashkush *et al.*, 2003, Shaked *et al.*, 2001). Analysis of newly synthesized allopolyploids allows identification of genetic and epigenetic changes that are underway soon after the hybridization, because such changes are most evident at this time (Wendel and Wessler, 2000, Ma and Gustafson, 2005). Often the allopolyploid formation is accomplished in one step. The alien and host genome must have a stable relationship in a single nucleus to be adapted successfully in nature (Chen, 2007). To fit better and establish in nature, the polyploid will undergo several genomic changes, some are rapid and non-Mendelian in nature, occurring instantly after the formation of the allopolyploid zygote (Kashkush *et al.*, 2003, Levy and Feldman, 2002, Liu *et al.*, 1998a). Other changes occur sporadically and accumulate over a long period of time (Table 1.3) during the life of the allopolyploid species (Wendel, 2000, Wendel *et al.*, 1995, Chen, 2007).

Allopolyploidization has numerous genetic and epigenetic consequences that vary considerably between different species (Finnegan *et al.*, 1998, Soltis *et al.*, 2009). Some of the well documented genetic changes include sequence elimination, chromosomal rearrangements, transpositions and deletions that can lead to altered gene expression (Gaeta *et al.*, 2007, Pires *et al.*, 2004). On the other hand epigenetic changes including DNA methylation, histone modifications and RNA interference may also alter gene expression levels mainly via DNA methylation or activation of genes that are usually silent at the diploid level (Soltis and Soltis, 2000, Finnegan *et al.*, 1998, Finnegan *et al.*, 1996, Finnegan *et al.*, 2000, Fojtova *et al.*, 2001, Kanno *et al.*, 2010).

Table 1.3: Genomic alterations triggered or facilitated by allopolyploidy (modified from Feldman and Levy, 2009).

Type of modification	Effects
Revolutionary changes (changes that follow immediately after hybridization)	i. Genetic and epigenetic ii. Species specific iii. Cytological diploidization iv. Regulation of gene expression levels v. Heterotic or incompatibility effects in the polyploid iv. Stabilization and establishment of the nascent allopolyploid as a new species in nature
Evolutionary changes	i. Accelerated evolution and increased genetic diversity ii. Sub and neo functionalization iii. Introgressions iv. Biotype specific alterations v. Flexibility and adaptability

1.14.1 DNA methylation

DNA methylation is one of the most extensively studied and well understood epigenetic modification. It is a stable epigenetic mark and is found in the genomes of both prokaryotes and eukaryotes (Bird 2002, Yang *et al.* 2004, Law and Jacobsen 2010). DNA methylation is the conversion of cytosine to 5-methylcytosine (Figure 1.7). It can significantly alter information present in nucleotide sequence without interfering or modifying the pairing properties of both adenine and cytosine (Noyer-Weidner and Trautner 1993, Selker 1999, Jeltsch 2002). Methylation of DNA is brought about by enzymes called DNA methyltransferases (MTase) catalysing the transfer of methyl group from S-adenosyl-methionine to cytosine or adenine residues in newly replicated DNA (Bestor 1994, Finnegan and Kovac 2000, Jones and Baylin 2002, Singal and Ginder, 2010). Most of the naturally occurring methylated bases in DNA are, N6-methyladenine, N4- methylcytosine and 5-methylcytosine (Jeltsch 2002).

In prokaryotes DNA methylation plays an important role in the host restriction modification and occurs on both adenine and cytosine bases (see Wilson and Murray 1991). While in higher eukaryotes, 5-methylcytosine is the most abundant methylated base. In mammals most methylation is found at symmetrical CpG dinucleotides and is estimated to account for ~70–80% of the CpG dinucleotides throughout the genome (Ehrlich *et al.* 1982). Often a small amount of non-CpG methylation is seen in embryonic stem cells (Bird 2002). The remaining unmethylated CpG dinucleotides are mostly found upstream of gene promoters as CpG islands (Cedar and Bergman 2009, Law and Jacobsen 2010). On the other hand, in plants methylation can be seen at

cytosine bases in all sequence contexts as symmetrical CpG and CpNpG (H = A, T or C) as well as non-symmetrical CpHpH sites (Meyer *et al.,* 1994, Grafi *et al.,* 2007, Suzuki and Bird 2008).

In plants four main subfamilies of MTases have been identified. These include domain rearranged methyltransferases (DRM), DNA methyltransferase-1 (MET1), DNA methyltransferase-2 (Dnmt2) and chromomethyltransferase (CMT). DRMs are similar to human Dnmt3, which is required for establishing methylation patterns during development (Finnegan *et al.,* 1998, Finnegan and Kovac 2000, Sharma *et al.,* 2009). In eukaryotes the different MTases known are involved in either maintenance methylation or de novo methylation (Law and Jacobsen 2010). Maintenance methylation is the addition of methyl group to cytosines in the hemimethylated DNA after replication. However, methylation of cytosines in the non-methylated DNA is referred to as de novo methylation. This process is responsible for establishing new methylation patterns that are then maintained by maintenance MTases (Bird, 2002, Suzuki and Bird 2008, Sharma *et al.,* 2009).

1.14.2 DNA methylation and transcriptional repression

Despite the long held view that DNA methylation might act as a negative regulator of transcription, the precise mechanism involved in the inhibition of transcription still remains obscure (Finnegan and Kovac 2000, Fuchs *et al.,* 2006, Law and Jacobsen 2010). To date three possible mechanisms have been proposed by which DNA methylation may inhibit gene expression (Singal and Ginder, 1999). The first mechanism explains the direct interference of methylated bases with the binding sites of transcription factors in their respective promoter regions (Razin and Cedar 1991, Weiss and Cedar 1997, Bird 2002). Many transcription factors recognize sequences that contain CpG residues and methylation of these bases inhibits their active binding (Meehan *et al.,* 1989). But in contrast, some transcription factors are not sensitive to methylation of their binding sites, and some do not have CpG dinucleotide in their binding sites (Tate and Bird 1993, McGough *et al.,* 2008). The second possible mechanism could be the proteins that recognize methylated sites may add to the repressive potential of methylated DNA (Boys, 1993, McGough *et al.,* 2008, Slotkin *et al.,* 2009). Two methyl cytosine binding proteins 1 and 2 (MeCP-1 and MeCP-2) have been identified and shown to bind to methylated CpG residues in all kinds of sequences

(see Singal and Ginder, 1999). Although in vertebrates DNA methylation has been argued to inhibit transcription initiation, methylation has also been shown to block transcription elongation in Neurospora through a mechanism that may be mediated through MeCP-1 or MeCP-2 (Rountree and Selker 1997). The third mechanism by which methylation may mediate or inhibit transcription is by altering chromatin structure (Onodera *et al.,* 2005, Suzuki and Bird 2008, Turner 2009, Matzke *et al.,* 2009, Law and Jacobsen 2010). Experiments using microinjection of certain methylated and non-methylated gene templates into nuclei have shown that methylation inhibits transcription only after chromatin is assembled. Therefore, in addition to stabilizing the inactive state, methylation also prevents activation by blocking the access of transcription factors (Singal and Ginder, 1999).

Previously, small non-coding RNAs (ncRNAs) were considered insignificant and as transcriptional noise. However, recently these ncRNAs have also been reported in relation to establishing and maintaining the transcriptional state of chromatin (Matzke *et al.,* 2009, Slotkin *et al.,* 2009, Serra and Esteller 2011). These reports provide strong evidence that small interfering RNAs (siRNAs) and microRNAs (miRNAs) have an active role in the suppression of many aspects of genes and mutagenic activities of TEs (Matzke *et al.,* 2009, Slotkin *et al.,* 2009, Naumann *et al.,* 2011). In such cases, these siRNAs (24 nucleotides long) target the *de novo* methyltransferases DRM to complementary DNA sequences to establish DNA methylation leading to silencing. The mechanism is also referred to as RNA-directed DNA methylation (Law and Jacobsen 2010, Naumann *et al.,* 2011).

Flowering plants have highly developed and elaborate transcriptional machinery assigned for this sequence-specific gene silencing (Kanno *et al.,* 2010). The process require two plant-specific RNA polymerases (Pol IV and Pol V, both themselves are related to Pol II) that interact with proteins of the RNA interference machinery and generates long and short ncRNAs required for epigenetic modification (Matzke *et al.,* 2009, Verdel *et al.,* 2009, Naumann *et al.,* 2011).

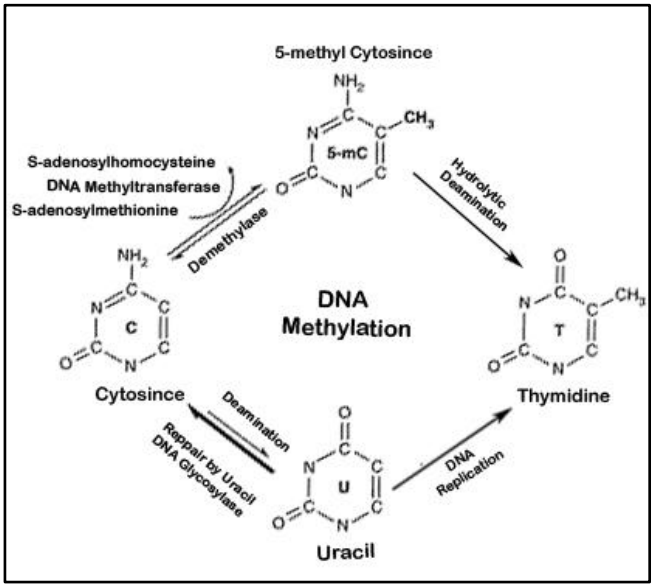


Figure 1.7: Schematic representation of the biochemical pathways for cytosine methylation, demethylation, and mutagenesis of cytosine and 5-mC, modified from Singal and Ginder, 2010.

1.15 *Wheat streak mosaic virus* (WSMV)

WSMV is the type specimen of genus *Tritimovirus* within the family *Potyviridae.* Under electron microscope, the WSMV appears as a flexuous rod shape particle of 700nm long and 15nm wide (see Sivamani *et al.*, 2000). The virus has a single stranded 9,384bp positive sense RNA genome, with a 3′-polyadenylated tail. The RNA genome is translated as a polyprotein, which is subsequently processed into at least ten mature proteins (Figure 1.8) by the viral proteinases (Stenger *et al.*, 1998, Fahim *et al.*, 2010b, Tatineni *et al.*, 2011).

P1 is the first mature protein that enables the virus to establish symptoms, infection and has a role in gene silencing (Choi *et al.*, 2000, Stenger *et al.*, 2007a). The second protein, Helper component-Protease (HC-Pro) is required for transmission of WSMV by the WCM and plays an essential role in viral amplification and systemic movement (see Fahim *et al.*, 2010b, Tatineni *et al.*, 2011). P3 is the third mature protein and is involved in cell-to-cell movement of the virus (Choi *et al.*, 2005). Both 6K1 and

6K2 are the small and least characterized proteins. However, 6K1 is believed to be involved in host-range definition and pathogenicity, while the 6K2 appears to have a role in RNA replication (Sáenz *et al.,* 2000, Spetz and Valkonen, 2004). The Cylindrical Inclusion (CI) is the largest among the processed WSMV proteins, and it has been reported in cell to cell movement of the virus. The viral genome-linked protein, Nuclear inclusion "a" (NIa-VPg) has been identified with a role in host-specific infection (see Tatineni *et al.,* 2011). While the Nuclear inclusion "b" (NIb) protein, which is larger than NIa is mostly found in association with the replication complexes of viral genome in the cytoplasm of host cells, and acts as an RNA dependent RNA polymerase (Fellers *et al.,* 1998, Sivamani *et al.,* 2000). The Capsid Protein (CP) protein, along with encapsidation, plays a role in systemic movement, infection and transmission of the virus (López-Moya *et al.,* 1999).

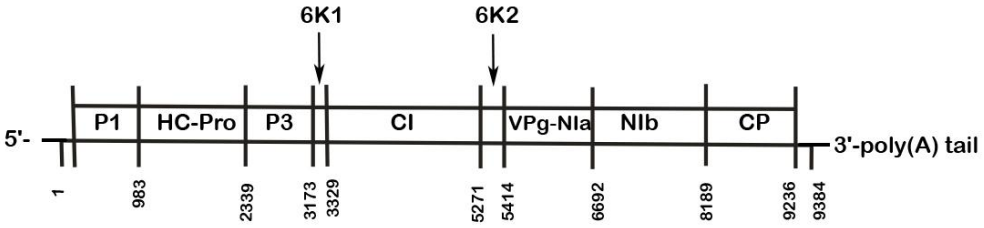


Figure 1.8: Genomic map showing the organization of WSMV modified from Stenger *et al.,* (1998) and Fahim *et al.,* (2010b). The RNA genome is represented by bar with nucleotide sequence positions below. The translated polyprotein is processed by viral proteinases into mature proteins. The name of each protein is given inside or above the boxes, and include P1, HC-Pro, P3, 6K1, CI, 6K2, NIa, NIb and CP.

1.16 Aims of the study

Wheat streak mosaic virus (WSMV) is one of the potentially devastating diseases of wheat and often results in 100% losses of both forage and grain wheat. Lack of effective resistance in most wheat cultivars and rapid spread of WSMV in the entire wheat growing world made me to design a project to confront the potential new challenges of future wheat production. I was highly interested to understand the possible genomic and evolutionary implications of the transferred natural resistance *i.e.* the introgressed alien chromatin of *Th. intermedium*, frequently used as a source of WSMV-resistance.

Chief aims of the project included:

- Explore potential natural sources of WSMV-resistance in wheat-*Th. intermedium* hybrid lines
- Characterize the nature and size of introgressed *Th. intermedium* chromatin and identify novel sources of WSMV-resistance
- Test the efficacy of known PCR-markers linked to WSMV-resistance and identify new potential markers
- Apply PCR-based markers to assess the molecular breakpoints and loss of any important wheat gene(s) in lines carrying wheat-*Th. intermedium* recombinant chromosomes. Based on the results, the aim was to suggest potential line(s) for future breeding and isolating the resistant gene(s)
- To understand the genomic organization, diversity, amplification and chromosomal localization of tandemly repeated DNA sequences (Afa and pSc119.2) from wheat and *Th. intermedium* and compare them with other members of the grass family
- To use repetitive DNA as chromosomal markers and find their role (if any) in alien introgression and chromosomal re-arrangements.
- To assess genome-wide, and alien chromosome specific epigenetic modifications in the DNA methylation patterns that may be accompanied with alien transfers

CHAPTER II: MATERIALS AND METHODS

2.1 Materials

2.1.1 *Triticeae* species and hybrids

Thirty-five diploid and polyploid *Triticeae* species were used in the current study. They are listed in Table 2.1 and Table 2.2 along with their genomic constitution, ploidy level and source they were obtained from. Pedigree analysis of the wheat-*Th. intermedium* hybrid lines showing the original crosses made by Robert (Bob) Graybosch and his co-workers at University of Nebraska-Lincoln Agriculture & Horticulture, USA is shown in Table 2.3.

2.1.2 Germination of seed and multiplication of seed stock

Seeds were germinated in darkness at 22°C for 48-72 hrs in sterile Petri dishes containing water-soaked filter papers. Newly emerged root tips of about 1-2cm long were collected and processed for *in situ* hybridization or immunostaining experiments (section 2.2.13). After collecting the root tips, the seedlings were transferred into new Petri dishes and kept under the same conditions for another day or two to recover, before they were grown in soil under greenhouse conditions of 25°C temperature and 16 hrs of day light, in the Department of Biology, University of Leicester. Three seedlings were grown in 3 litre pots containing well-watered compost (Scotts Professional, UK), the same plants were used for both DNA extractions and seed multiplication. During the flowering season, initial ears were collected to study meiosis and then 5-6 ears per plant were bagged to prevent cross-pollination and mixing. Seeds were harvested on maturity, labelled and then stored with desiccant at 4°C. Ears that emerged later or were not properly bagged were discarded and not used in the study.

Table 2.1: List of *Triticeae* species used in the study.

Species	**Line/ variety/landrace**	**Genome**	**Chromosome No. (2n)**	**Source**	**Remarks**
Triticum aestivum	CS N1B T1A	AABBDD	42	1	Substitution line
Triticum aestivum	CS N4D T4B	AABBDD	42	1	Substitution line
Triticum aestivum	CS N4B T4D	AABBDD	42	1	Substitution line
Triticum aestivum	Beaver	AABBDD	42	2	Wheat-rye translocation line
Triticum aestivum	Chinese Spring	AABBDD	42	3	Wheat cultivar
Triticum durum		AABB	28	3	Durum wheat or macaroni wheat
Aegilops tauschii		DD	14	3	Jointed goatgrass

Source 1: Steve Reader (JIC, Norwich, UK), *Source* 2: Plant Breeding Institute (Cambridge, UK), *Source* 3: Molecular Cytogenetics Laboratory 201, Seed Stock (University of Leicester, UK).

*a Lines used in final WSMV-resistance screen with molecular markers (chapter IV)

*b Lines used in 1BS mapping study (chapter IV)

Table 2.2: List of wheat and *Thinopyrum* lines from Robert (Bob) Graybosch (University of Nebraska-Lincoln Agriculture & Horticulture, USA).

Population	Species	Line/ variety/landrace	Genome	Chromosome No. (2n)	Remarks
KS102	*Triticum aestivum*	KS95H102	AABBDD	42	Wheat-*Th.intermedium* hybrid wheat
KS10-1	*Triticum aestivum*	KS96HW10-1	AABBDD	42	Wheat-*Th.intermedium* hybrid wheat
MILL	*Triticum aestivum*	Millennium	AABBDD	42	Wheat cultivar
I	*Triticum aestivum*	N02Y5018	AABBDD	42	Wheat-*Th.intermedium* hybrid wheat
	Triticum aestivum	N02Y5019	AABBDD	42	Wheat-*Th.intermedium* hybrid wheat
	Triticum aestivum	N02Y5021	AABBDD	42	Wheat breeding line
	Triticum aestivum	N02Y5025	AABBDD	42	Wheat-*Th. intermedium* hybrid wheat
	Triticum aestivum	N02Y5003	AABBDD	42	Wheat-*Th. intermedium* hybrid wheat
II	*Triticum aestivum*	N02Y5057	AABBDD	42	Wheat-*Th. intermedium* hybrid wheat
	Triticum aestivum	N02Y5075	AABBDD	42	Wheat-*Th. intermedium* hybrid wheat
	Triticum aestivum	N02Y5078	AABBDD	42	Wheat-*Th. intermedium* hybrid wheat
	Triticum aestivum	N02Y5082	AABBDD	42	Wheat breeding line
	Triticum aestivum	N02Y5096	AABBDD	42	Wheat breeding line
III	*Triticum aestivum*	N02Y5105	AABBDD	42	Wheat breeding line
	Triticum aestivum	N02Y5106	AABBDD	42	Wheat-*Th. intermedium* hybrid wheat
	Triticum aestivum	N02Y5109	AABBDD	42	Wheat-*Th. intermedium* hybrid wheat
	Triticum aestivum	N02Y5117 or MACE	AABBDD	42	Wheat-*Th. intermedium* hybrid wheat

Table 2.2: continued

	Triticum aestivum	N02Y5121	AABBDD	42	Wheat cultivar
IV	*Triticum aestivum*	N02Y5154	AABBDD	42	Wheat-*Th. intermedium* hybrid wheat
	Triticum aestivum	N02Y5149	AABBDD	42	Wheat-*Th. intermedium* hybrid wheat
	Triticum aestivum	N02Y5156	AABBDD	42	Wheat-*Th. intermedium* hybrid wheat
	Triticum aestivum	N02Y5163	AABBDD	42	Wheat-*Th. intermedium* hybrid wheat
	Triticum aestivum	N02Y2016	AABBDD	42	Wheat-*Th. intermedium* hybrid wheat
	Thinopryum intermedium	Beef maker	JJJ^SJ^SSS	42	Intermediate wheat-grass
	Thinopryum intermedium	Hay maker	JJJ^SJ^SSS	42	Intermediate wheat-grass
	Thinopryum intermedium	Rostov 31	JJJ^SJ^SSS	42	Intermediate wheat-grass
	Thinopryum intermedium	Reliant	JJJ^SJ^SSS	42	Intermediate wheat-grass
	Thinopryum intermedium	Manaska	JJJ^SJ^SSS	42	Intermediate wheat-grass

Table 2.3: Pedigree analysis of wheat-*Th. intermedium* hybrid lines segregating for *Wsm1*.

Population	Line/variety/land race	Pedigree
KS102	KS95H102	KS91H184/KS89H20//TAM 107
KS10-1	KS96HW10-1	KS91HW29//Rio Blanco/KS91H184
MILL	Millennium	Arapahoe/Abilene//NE86488
Pop-I	N02Y5018	CO850034//T-57/5*TAM107/3/(KS91H174/Rio Blanco/KS91HW29//VISTA)
	N02Y5019	CO850034//T-57/5*TAM107/3/(KS91H174/Rio Blanco/KS91HW29//VISTA)
	N02Y5021	CO850034//T-57/5*TAM107/3/(KS91H174/Rio Blanco/KS91HW29//VISTA)
	N02Y5025	CO850034//T-57/5*TAM107/3/(KS91H174/Rio Blanco/KS91HW29//VISTA)
	N02Y5003	CO850034//T-57/5*TAM107/3/(KS91H174/Rio Blanco/KS91HW29//VISTA)
Pop-II	N02Y5057	YUMA//T-57/3/LAMAR/4/4*YUMA/5/(KS91H184/ARLIN S/KS91HW29//NE89526)
	N02Y5075	YUMA//T-57/3/LAMAR/4/4*YUMA/5/(KS91H184/ARLIN S/KS91HW29//NE89526)
	N02Y5078	YUMA//T-57/3/LAMAR/4/4*YUMA/5/(KS91H184/ARLIN S/KS91HW29//NE89526)
	N02Y5082	YUMA//T-57/3/LAMAR/4/4*YUMA/5/(KS91H184/ARLIN S/KS91HW29//NE89526)
	N02Y5096	YUMA//T-57/3/LAMAR/4/4*YUMA/5/(KS91H184/ARLIN S/KS91HW29//NE89526)
Pop-III	N02Y5105	Yuma // T-57 /3/ CO850034 /4/4* Yuma /5/ (KS91H184 / Arlin S / KS91HW29)// NE89526)
	N02Y5106	Yuma // T-57 /3/ CO850034 /4/4* Yuma /5/ (KS91H184 / Arlin S / KS91HW29)// NE89526)
	N02Y5109	Yuma // T-57 /3/ CO850034 /4/4* Yuma /5/ (KS91H184 / Arlin S / KS91HW29)// NE89526)
	N02Y5117 (MACE)	Yuma // T-57 /3/ CO850034 /4/4* Yuma /5/ (KS91H184 / Arlin S / KS91HW29)// NE89526)
	N02Y5121	Yuma // T-57 /3/ CO850034 /4/4* Yuma /5/ (KS91H184 / Arlin S / KS91HW29)// NE89526)
Pop-IV	N02Y5149	MO8/REDLAND//KS91H184/3*RIO BLANCO
	N02Y5154	MO8/REDLAND//KS91H184/3*RIO BLANCO
	N02Y5156	MO8/REDLAND//KS91H184/3*RIO BLANCO
	N02Y5163	MO8/REDLAND//KS91H184/3*RIO BLANCO
	N02Y2016	MO8/REDLAND//KS91H184/3*RIO BLANCO

/ represents cross, * represent generation number (e.g. 3* represents F3, 4* represents F4 and so on)
Final crosses for lines in population I-IV were made by C. James Peterson, USDA-ARS.
Sources: Wells *et al.,* 1982, Seifers *et al.,* 1995, Divis *et al.,* 2006, Graybosch *et al.,* 2009 (personal communication).

2.1.3 Standard solutions and media

Table 2.4: Solutions and media. Unless indicated the solutions were autoclaved and stored at room temperature (RT).

Solution	Preparation/final concentration
CTAB buffer (pH 7.5 - 8.0)	2% (w/v) cetyltrimethylammonium bromide, 100mM Tris-HCl, 1.4M NaCl, 20mM EDTA.
DNA Wash buffer	76 % ethanol, 10mM ammonium acetate. No autoclaving.
10x TE buffer [*1] (pH 8.0)	100mM Tris (tris-hydroxymethylamino-methane)-HCl, 10mM EDTA (ethylene-diamine-tetra-acetic acid).
6x Gel loading buffer	0.25% Bromophenol blue, 0.25% Xylene cyanol FF, 60% Glycerol. No autoclaving and stored at 4°C.
50x TAE [*1] (pH 8.0)	242g of Tris-base, 57.1ml of glacial acetic acid, 100ml of 0.5M EDTA. Final volume 1000ml with sterile distilled water.
Ethidium Bromide (10 mg/ml)	1g Ethidium bromide, 100ml of sterile distilled water. No autoclaving and stored at 4°C.
Ampicillin	10mg/ml (dissolved in distilled water). No autoclaving and stored at -20°C.
20x SSC (saline sodium citrate, pH 7.0) [*1]	0.3M NaCl, 0.03M sodium citrate.
10x PBS (phosphate buffered saline, pH 7.4) [*1]	1.3M NaCl, 70mM Na2HPO4, 30mM NaH2PO4.
Detection buffer (FISH)	4x SSC, 0.2% (v/v) Tween 20.
10x KPBS (potassium phosphate buffered saline, pH 7.4) [*1]	1.28M NaCl, 20mM KCl, 80mM Na2HPO4, 20mM NaH2PO4.
10x Enzyme buffer (pH 4.6) [*1]	40mM citric acid, 60mM tri-sodium citrate. No autoclaving and stored at 4°C.
1x Enzyme solution	3% (w/v) pectinase (Sigma), 1.8% (w/v) cellulase (Calbiochem), 0.2% (w/v) cellulase (Onozuka RS) in 1x enzyme buffer. No autoclaving and stored at -20°C.
4% Paraformaldehyde (pH 7.0)	4g paraformaldehyde (Agar Scientific) dissolved in distilled water. Final volume 100ml, no autoclaving and used fresh.
McIlvaine's buffer (pH 7.0)	0.1M citric acid, 0.2M di-sodium hydrogen phosphate.
Blocking DNA [*2]	Autoclaved at 114°C for 5

Table 2.4: continued

100µg/ml DAPI [*3]	5g of DAPI (4′,6-diamidino-2-phenylindole) dissolved in Sigma water. Final volume 50ml. No autoclaving and stored at -20°C.
50x Denhardts solution	1% Ficoll type 400 (Sigma), 1% polyvinylpyrrolidone (Sigma) and 1% bovine serum albumin (Amersham Biosciences). Filter sterilized and stored at -20°C.
Southern denaturing solution	0.25M NaOH, 1M HCl.
Southern depurinating solution	0.25M HCl.
Southern neutralizing solution (pH 7.5)	0.5M Tris-HCl, 3M NaCl.
Southern Transfer buffer	0.4M NaOH.
Buffer 1 (probe detection, pH7.5 0)	100mM Tris-HCl, 15mM NaCl
Buffer 2 (probe detection)	0.5% (w/v) Blocking Reagent (Roche Diagnostics) in buffer 1
Buffer 3 (probe detection, pH9.5)	100mM Tris-HCl, 100mM NaCl, 50 mM MgCl2
Salmon sperm DNA[*4]	1mg/ml of sheared salmon sperm DNA.
Wash buffer 1 (Southern hybridization, pH 7.5)	0.1M Maleic acid, 0.15 M NaCl, 0.3% (v/v) Tween 20
Buffer 1 (Southern hybridization, pH 7.5)	0.1M Maleic acid, 0.15 M NaCl
Buffer 2 (Southern hybridization)	1% (w/v) Blocking Reagent (Roche Diagnostics) in buffer 1
Buffer 3 (Southern hybridization, pH 9.5)	0.1M Tris-HCl, 0.1 M NaCl
SOB medium (super optimal broth, pH 7.0)	20g of Tryptone, 5g Yeast extract, 0.5g NaCl, 10ml 250mM KCl. Final volume 1000ml with sterile distilled water.
LB medium (Luria-Bertani, pH 7.0)	10g Tryptone, 5g Yeast extract, 10g NaCl. Final volume 1000ml with sterile distilled water.

*1 Diluted with distilled water to appropriate concentration

*2 Genomic DNA from 'Chinese Spring' was sheared into pieces and applied 4-20x of the probe concentration to block the repetitive DNA sequences.

*3 DAPI was diluted in water for stock of 100µg/ml and then diluted with McIlvaine's buffer to final concentration of 4µg/ml.

*4 Salmon sperm DNA was denatured in boiling water for 10 mins and placed on ice for 10 mins before adding it to the hybridization mixture.

2.2 Methods

2.2.1 Isolation of genomic DNA

Total genomic DNA was isolated from young leaves using CTAB method (Doyle and Doyle, 1990) with minor modifications. One gram of fresh and healthy leaves were collected from a single individual, washed with distilled water, wrapped in aluminium foil, frozen in liquid nitrogen and quickly grounded to fine powdered with the help of pestle and mortar while kept cold to prevent enzymatic degradation. A half spatula of PVP (Polyvinylpyrrolidone, Sigma) was added before the powdered leaf was taken into a 50ml Falcon tube with 10ml of pre-heated CTAB buffer (Table 2.4) containing 50µl of β-mercaptoethanol. Tubes were incubated at 60°C for 30 mins in a shaking water bath. An equal volume of absolute chloroform : isoamyl alcohol (24:1) was added to each tube and mixed by repeated inverting for 3 mins, followed by centrifugation at 5000 rpm at RT for 10 mins. The top aqueous supernatant was carefully transferred to a new Falcon tube using 1ml blue tip cut at the end. The chloroform : isoamyl alcohol washing and centrifugation steps were repeated once more, and then the DNA was precipitated with 0.6 volume of pre-chilled isopropanol added to the supernatant, mixed gently by inverting and then kept on ice for 10 mins. Precipitated DNA was spooled out with a sterile glass rod or spun down at 3000 rpm for 3 mins, dried and washed with 5ml of wash buffer (Table 2.4) for 20 mins, and then air dried before resuspending DNA in 1ml of 1x TE buffer (Table 2.4) at RT overnight. DNA was incubated at 37°C for 1 hr with 2µl of 10mg/ml RNase A (Bioline) to get rid of RNA and then re-precipitated with 1x volume of sodium acetate 3M (pH 6.8) and 2x volume pre-chilled absolute ethanol. DNA was spun down as before and resuspended in 500µl of 1x TE buffer at RT overnight. Adequate measures were taken at all the times to avoid contamination of the genomic DNA samples from any DNA or dust present in the surrounding. The DNA samples were stored in a -20°C freezer.

2.2.2 Agarose gel electrophoresis

Both genomic and PCR amplified DNA fragments (section 2.2.5) were separated by agarose gel electrophoresis. Agarose gels [0.8 - 3% (w/v)] were prepared by boiling agarose (Molecular Grade, Bioline) in 1x TAE (Table 2.4) and poured into sealed gel

trays after adding ethidium bromide (final concentration of 0.5µg/ml) inside a fume hood. Gel combs were placed to make wells and then left at RT to solidify. DNA samples were mixed with appropriate amount of 6x gel loading buffer (Table 2.4) and loaded along with DNA ladder, Hyperladder I (Bioline) or Q-Step 2 (YorkBio) with known band concentrations (ladder pattern in appendix) on 7V/cm for 45-60 mins, and visualized with GeneFlash (Syngene) gel documentation system.

2.2.3 Concentration and quality of DNA

The concentration and quality of genomic DNA were assessed through gel electrophoresis and spectrophotometer (Helyos) at a wavelength of 260nm (Sambrook and Russell 2001). For electrophoresis 1µl DNA was loaded on 0.8% (w/v) agarose gel (section 2.2.2), while for spectrophotometer, 1:50 sample dilutions (total volume 200µl) of genomic DNA were used. High molecular weight DNA samples with no visible shearing on gels and spectrophotometer O.D260/O.D.280 ratio of 1.8 or above was used for subsequent PCR amplifications and southern hybridization experiments.

2.2.4 PCR markers and primer design

A variety of PCR markers including, repetitive DNA (transposable elements and tandem repeats), IRAP (inter retroelements amplified polymorphism), SSRs (simple sequence repeats), ESTs (expressed sequence tags), EST-SSRs and one RFLP (restriction fragment length polymorphism) were used (for details see result chapters). Unless obtained from published or unpublished studies, primer pairs were designed using Primer 3 program (http://www.frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) with the annealing temperature set from 50-60°C, and optimal length of 20 bases preferably with 50% GC content for the amplification of products between 120bp to 442bp in size, and were ordered from Sigma.

2.2.5 Polymerase Chain Reaction (PCR)

DNA was amplified by PCR using a *T*professional Gradient Thermocycler (Biometra) in a 15µl reaction mixture containing 100ng of template DNA, 1x Kapa Biosystems buffer A [750mM Tris-HCl pH 8.8, 200mM (NH4)2SO4, 15mM MgCl2, 0.1% Tween 20], 1.5mM MgCl, 200µM of dNTPs (Bioline), 0.6µM of each primer and 0.5 U of Kapa Taq DNA polymerase. Unless mentioned PCR conditions were: 94°C for 4 mins, followed by 30 cycles at 94°C for 1 min, 45 - 62°C (depending upon the annealing temperature of different primer sets) for 45 secs, 72°C for 2 mins, and final extension of 72°C for 7 mins was followed by holding the block at 16°C. Amplification and polymorphism of the PCR products were analysed by 1.5-3% agarose gels (section 2.2.2). Final assessment and labelling etc. of the gels was carried out in Adobe® Creative Suite® 3 Photoshop®.

Plasmid DNA was amplified in a final volume of 50µl containing 1× PCR buffer [16mM (NH4)2SO4, 67mM Tris-HCl, 0.1% Tween 20 (Bioline)], 1.5mM MgCl2, 200µM of dNTPs (Bioline), 0.4µM of each M13 primer, 0.5U of Taq DNA Polymerase (Bioline) and 0.5µl of recombinant plasmid DNA. PCR cycling conditions were: 94°C for 5 mins, 35 cycles of 94°C 30 secs, 50°C for 30 secs and 72°C for 45 secs, followed by 72°C for 5 mins and holding at 16°C.

2.2.6 Cleaning and purification of PCR products

After analyzing agarose gels, selected PCR bands were excised, washed and purified with the QIAGEN Minielute Gel Extraction Kit according to manufacturer's instructions. Following the removal of residual contaminants, 1µl of the recovered DNA was reloaded on 0.8% (w/v) agarose gel (section 2.2.2) to confirm the size and concentration of eluted DNA, before using it in probe labelling, cloning or sequencing.

2.2.7 Cloning of PCR products

Purified PCR fragments were cloned in pGEM®-T Easy vectors, using pGEM®-T Easy Vector System I kit (Promega) following the manufacturer's protocol with little modification. The cloning site of in pGEM®-T Easy vector has a single overhanging 3′

deoxythymidine (T) nucleotide (Figure 2.1) that can be ligated to a single base deoxyadenosine (A) to the 3′ end of the PCR products generated by Taq polymerase.

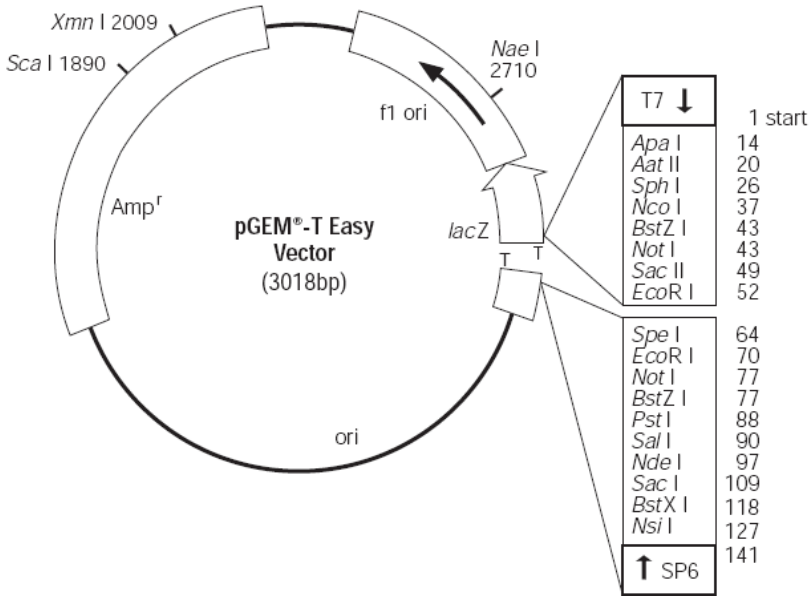


Figure 2.1: pGEM®-T Easy Vector circular map (http://www.promega.com/).

2.2.7.1 Ligation reaction and transformation of competent *E. coli* cell

The ligation reactions were set up in a small 300µl tube. For a final volume of 10µl, 5µl of 2x Rapid Ligation Buffer (60mM Tris-HCL pH 7.8, 20mM MgCl2, 20mM DTT, 2mM ATP, 10% PEG from Promega), 0.5µl of the pGEM-Teasy vector, 1µl of T4 DNA Ligase and 3.5µl of purified PCR product were mixed and incubated at RT for 1 hr, or at 4ºC overnight.

For transformation, 5µl of the ligation reaction was added to 50µl of the competent *E. coli* (α -Select Bronze Efficiency, Bioline) cells and was kept on ice for 20 mins before a heat shock of 42ºC for 45 secs, which was again followed by 2 mins on ice. Pre warmed 900µl of SOB media (Super Optimal Broth) was added to each reaction tube on ice and then incubated at 37ºC for 1.5 hr in an orbital shaker at 230 rpm to allow the growth of transformed competent cells. After the incubation, 50µl, 100µl & 200µl

of culture was plated on three LB agar plates, containing 100µg/ml ampicillin, 40µg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactosidase (X-gal) and 500µM isopropyl-β-Δ-thiogalactopyranoside (IPTG). Plates were incubated at 37ºC for 14-16hrs.

2.2.7.2 Screening of recombinant clones and isolation of transformed *E. coli* cells

Recombinant clone selection was based on screening for white colonies that were indicative of pGEM®-T Easy vector with an insert. The pGEM®-T Easy vector contains lacZ gene (Figure 2.1) encoding for β-galactosidase, that breaks down the chromogenic X-gal substrate and results in blue colonies. Successful transformation results in the disruption of the plasmid β-galactosidase gene (lacZ) and colonies appear white due to their inability to metabolize X-gal. Single white colonies were picked with a sterile toothpick and inoculated in 10ml LB medium with 40µg/ml of ampicillin and incubated overnight at 230 rpm in an orbital shaker at 37ºC. To recover transformed *E. coli* cells, 750µl of medium were spin down in a 1.5ml eppendorf tube at 13000 rpm for 1 min, the supernatant was carefully decanted and this process was repeated 3-4 times until a pellet of appreciable size was obtained.

2.2.7.3 Purification of plasmid DNA, verification of insert size and storage of *E. coli* cells

Recombinant plasmid DNA was recovered from the pellet of *E. coli* cells with QIAGEN Minprep Kit following manufacturer's instructions. The size of insert was confirmed either with PCR (section 2.2.5) using universal M13 primers (forward: 5′-GTA AAA CGA CGG CCA GT-3′, reverse: 5′-GGA AAC AGC TAT GAC CAT-3′) or by digesting the plasmid DNA with *EcoR*I, to release the cloned fragment. Both M13 and *EcoR*I sites are located near the multiple cloning site in pGEM®-T Easy vector (Figure 2.1).

For restriction ~300ng of plasmid DNA (pUC19 or pGEM®-T Easy vector) was digested with *EcoR*I (New England BioLabs) in a final volume of 20µl, according to manufacturer guidelines in the presence of appropriate NEB buffer in a 37°C water bath for at least 2 hrs. Once the clone size was confirmed, 500µl of the overnight culture was mixed with 500µl of sterilized 50% glycerol in a 1.5ml eppendorf tube, frozen quickly by dipping in liquid Nitrogen and kept in -80°C freezer.

2.2.8 Sequencing of PCR amplicons and sequence analysis

Selected cleaned PCR products were commercially sequenced at Genome Enterprise Limited (Norwich Research Park, UK), either by sending the PCR products directly along with custom primers, or after cloning in pGEM®-T Easy vector and using recombinant plasmid DNA (200-400ng) with universal M13 forward and or reverse primers. All Afa-family clones were sequenced in both directions. While the dimers of pSc119.2 sequences were sequenced with M13F or M13R and trimers, tetramers were sequenced in both directions.

DNA sequences in the form of chromatograms were downloaded from Genome Enterprise Limited website server, and opened using bioinformatics software Chromas version 1.45 (Conor McCarthy, Griffith University, Australia). The DNA sequences were copied and saved in FASTA format, the pGEM®-T Easy vector sequences flanking the inserts were identified and deleted from the FASTA file. Multiple sequence alignment was performed using default settings of the Jalview Multiple Alignment Editor V 1.3 (EMBL-EBI Version) and improved by eye when necessary in BioEdit (Hall, 1999). BLASTN search was used to screen GenBank for homologous DNA sequences. Insertions-deletions (Indels) were excluded from the estimates as per Tang *et al.,* (2011). Sequences clustered in clades were compared with and without indels and virtually no difference was observed. Phylogenetic reconstruction and estimation of nucleotide variability (p-distance) were conducted in MEGA5 program (Tamura *et al.,* 2011). The evolutionary history was inferred by using Maximum Likelihood (ML) method based on the Tamura 3-parameter model (Tamura, 1992). Nodal support was assessed via bootstrapping, and the bootstrap consensus tree inferred from 1000 replicates (Felsenstein, 1985). All positions containing gaps or missing data was eliminated from the dataset by selecting the "complete deletion option" in MEGA5 (Tamura *et al.,* 2011).

2.2.9 Probe used

pTa71 contains a 9kb *Eco*RI fragment of the repeat unit of 25S-5.8S-18S rDNA isolated from *T. aestivum* (Gerlach and Bedbrook, 1979)and was linearised with *EcoRI* before labelling (section 2.2.7.3).

pTa794 contains 410bp fragment of 5S rDNA of *T. aestivum* (Gerlach and Dyer, 1980).

pSc119.2, CS13 or PET5 contain a 120bp tandemly repeated DNA sequence isolated from *Secale cereale* (McIntyre *et al.,* 1990), *T. aestivum* (Contento *et al.,* 2005) and *Th. intermedium* (see Chapter V). .

dpTa1 containing a tandem repeat with a monomeric length of 340bp isolated from *T. aestivum* was subcloned by Vershinin *et al.,* (1994) and is homologous to pAs1 (Rayburn and Gill, 1986)and the 340bp Afa-repeat sequences (Nagaki *et al.,* 1998a) and isolated here from *T. aestivum* cv. 'Chinese spring' and *Th. intermedium* (see also Chapter V).

LTR-probe is a 500bp sequence amplified with LTR6150 and Afa1-F primers. BLAST search showed 85-95% coverage of this sequence with TEs of grasses (see below).

Cas2-probe is a 1311bp sequence of CACTA element *(Caspar)* from wheat related species and present at the junction between terminal repeats and the sequence encoding transposase (Sergeeva *et al.,* 2010).

Total genomic DNA from *Th. intermedium* and *Ae. tauschii* was sheared to 3-5kb pieces by autoclaving before labelling.

2.2.10 DNA labelling

DNA *in situ* hybridization followed the method described by Schwarzacher & Heslop-Harrison (2000) with minor modification. Both cloned and genomic DNA was labelled with biotin-16-dUTP and digoxigenin-11-dUTP (Roche Diagnostics) in separate reactions, for non-radioactive detection after *in situ* hybridization and Southern hybridization. Description of probes is given in the result chapters.

2.2.10.1 PCR labelling of probe

Clones of less than 500bp size were labelled through PCR amplification, using universal M13 primers, by adding 1µl of biotin-16-dUTP or digoxigenin-11-dUTP (1mM, Roche Diagnostics) or 1µl of water as control to the standard PCR mixture (section 2.2.5) and amplified as described.

2.2.10.2 Random primers labelling of probe

Single stranded DNA is amplified using a random mixture of all oligonucleotides with the Klenow fragment of *E. coli* DNA polymerase I (Schwarzacher and Heslop-Harrison., 2000). Total genomic DNA and clones larger than 500bp in size were labelled with random primer labelling kits, ordered from Invitrogen (www.invitrogen.com).

Genomic DNA was sheared to 3-5kb pieces by autoclaving at 110°C for 4 mins before labelling. The fragment sizes were estimated by running the autoclaved DNA on 1% agarose gel (section 2.2.2).

Probes between 500bp-2kb were labelled with BioPrime® DNA Labelling System (Cat. No. 18094-011) for biotin and Random Primer DNA Labelling System (Cat. No. 18187-013) for digoxigenin incorporation. Genomic DNA and large clones of several kb were labelled with BioPrime® Array CGH Labelling System (Cat. No. 18095-011). Labelling reactions were performed in a final volume of 50 µl, following manufacturer's instruction with little modifications.

Labelling was achieved with 200ng of the purified clone DNA (section 2.2.7.3) or 1µg of sheared genomic DNA mixed with 20µl of 2.5x respective Random Primer Solution, denatured in boiling water for 5 mins and then chilled on ice for 5 mins. To this mixture, 5µl of 10x dNTP Mix and 1µl of 40U Klenow Fragment was added and incubated at 37ºC for 2 hrs, for biotin labelling with BioPrime® DNA Labelling System. For digoxigenin labelling with Random Primer DNA Labelling System, 2µl of dATP, dCTP, dGTP and 1µl of dTTP together with 1µl digoxigenin-11-dUTP (1mM) and 2µl of Klenow Fragment (3U) were mixed with the denatured DNA mixture and incubated at RT overnight. Labelling reactions with BioPrime® Array CGH Genomic Labelling System involved the addition of 3µl of biotin-16-dUTP or digoxigenin-11-dUTP (1mM), 3µl of 10x dUTP Nucleotide Mix and 1µl of Exo- Klenow Fragment (40U) to the denatured DNA mixture. The reaction was incubated at 37ºC for 2 hrs.

All polymerization reactions were stopped at the end of incubation by adding 5µl of Stop Buffer (0.5M EDTA pH 8.0). Labelled probes were purified to remove any unincorporated nucleotides, enzyme and salts using NucleoSpin® Extract II Kit (MACHERY-NAGEL), following manufacturer's instructions (http://www.mn-net.com/tabid/1452/default.aspx) and stored at -20ºC freezer.

2.2.10.3 Testing of labelled nucleotides in probes

The efficiency of labelled nucleotide incorporation was estimated by a colorimetric dot blot test. A positively charged nylon membrane (Hybond-N^+, Amersham Biosciences) of appropriate size (depending on the number of probes) was marked at the edge with pencil and soaked in buffer 1 (Table 2.4) at RT for 5 mins, and then dried between filter papers. Labelled probes (0.8-1µl) along with a positive control were micro-pipetted on the membrane, air-dried for 5 mins and then re-soaked in buffer 1, for 2 mins. The membrane was incubated at RT for 30 mins in buffer 2 (Table 2.4). Excess of buffer 2, was drained and the membrane was then incubated under a plastic cover slip at 37ºC for 30 mins, with 0.75U/ml of conjugated antibody solution (anti-biotin-alkaline phosphatase and anti-digoxigenin-alkaline phosphatase, Roche Diagnostics) diluted 1:500 in buffer 1. The membrane during incubation was slowly agitated from time to time and then washed with buffer 1. The membrane was equilibrated in buffer 3 (Table 2.4) for 3 mins and then detected with INT/BCIP (Roche Diagnostics). The stock solution of INT/BCIP [33mg/ml 2-(4-iodophenyl)-5-(4-nitrophenyl)-3-phenyltetrazolium chloride and 33mg/ml 5-bromo-4-chloro-3-indolyl-phosphate, toluidine-salt in DMSO] was diluted 1:500 in buffer 3 and the membrane detected at RT for 10-15 mins in the dark. Labelled probe(s) appeared as a dark brown dot on the membrane due to the colorimetric reaction of the detection reagents. Labelling efficiency was judged by colour density in comparison to the control.

The efficiency and concentration of PCR labelled probes was checked with agarose gel, 1µl of PCR product was loaded on a 1% agarose gel (section 2.2.2). Successful incorporation of label nucleotide was indicated by retardation of the same size band in biotin and digoxigenin incorporated probes compared with the unlabelled control reaction. These probes were used in both fluorescent *in situ* hybridization (section 2.2.12) and non-radioactive Southern hybridization (section 2.2.16.2.1).

2.2.11 Chromosome preparations

2.2.11.1 Collection and fixation of root tips

Newly emerging root tips of around 1-2cm length were collected from germinated seeds (section 2.1.2) and synchronized with 20-24 hrs ice water treatment. Root tips were then fixed in absolute ethanol: glacial acetic acid (3:1) at RT for 16 hrs. For long term fixation, root tips were left at RT for 2 hrs in the fixative and then transferred into new fixative, 70% or absolute ethanol and stored at -20ºC for several months.

2.2.11.2 Chromosomes preparation

Chromosomal preparations were carried out as described by Schwarzacher and Heslop-Harrison (2000). Fixed root tips were washed twice for 10 mins in 1x enzyme buffer (Table 2.4) to get rid of the fixative and then digested at 37°C for 1 hr, with 3% (w/v) pectinase (Sigma, 450U/ml), 1.8 % (w/v) cellulase (Calbiochem, 4000U/g) and 0.2 % (w/v) cellulase (Onozuka RS, 5000U/g). After digestion, root tips were washed in 1x enzyme buffer for 15 mins. Chromosomal preparations were made on clean glass slides (SuperFrost®, Menzel-Glaser, Thermo Scientific) under a stereo microscope. A single root tip was put in a drop of 45% or 60% glacial acetic acid, the root cap and other permanent tissues were removed by using fine needles and forceps. The meristematic tissue was dissected, separated and then squashed under a No. 1, 18mm x18mm cover slip by applying thumb pressure.

For meiotic chromosomes, anthers were checked with 45% acetic acid for appropriate stages and then fixed directly in absolute ethanol : glacial acetic acid (3:1) at RT for at least 4hrs. For digestion, anthers were washed twice for 10 min in 1x enzyme buffer (Table 2.4) to get rid of the fixative and then digested at 37°C for 90 mins (or until the material become soft) with the same enzyme as used for root tip chromosomal preparation but for digesting the thick callose wall 0.4-2% (w/v) Cytohelicase (Sigma, 3000-4000U/g, final concentration of 20-40U/ml) was added. After digestion, anthers were washed in 1x enzyme buffer for 15 mins and chromosomal preparations were made from single anther as above.

The cover slips (from both mitotic and meiotic slides) were removed with a razor blade after freezing the slides on dry ice for 5-10 mins. Slides were air dried at

RT, scanned, labelled and then used or stored at -20ºC in slide boxes together with silica gel. Slides stored at -20ºC were raised to RT before pre hybridization. This was achieved by keeping the slide boxs at 37ºC for 20 min and then at RT for 10 mins.

2.2.12 Fluorescent *in situ* hybridization

Fluorescent *in situ* hybridization (FISH) was carried out according to Schwarzacher and Heslop-Harrison (2000).

2.2.12.1 Pre hybridization

Slides were re-fixed in fresh absolute ethanol : glacial acetic acid (3:1) for 15 mins and dehydrated with absolute ethanol twice for 10 mins. Slides were allowed to air-dry and then incubated under a plastic cover slip with 200µl of RNase A (100µg/ml, Bioline) diluted in 2x SSC (Table 2.4) at 37°C for 1 hr. The slides were washed in 2x SSC at RT for 5 mins and re-fixed in freshly prepared 4% (w/v) paraformaldehyde (Table 2.4) at RT for 10 mins and then washed twice in 2x SSC for 5 mins. Slides were then dehydrated in a series of 70%, 85% and absolute ethanol for 2 mins and then air dried. Before probing, slides were re-scanned for the possible loss of cells that may occur during storage or pre hybridization steps

2.2.12.2 Hybridization

A total of 40µl probe mixture was applied per slide, containing 50% (v/v) formamide, 20% (w/v) dextran sulphate, 2x SSC, 25-100ng probe, 0.025µg of salmon sperm DNA and 0.125%SDS (sodium dodecyl sulphate) as well as 0.125mM EDTA (ethylene-diamine-tetraacetic acid). For genomic *in situ* hybridization (GISH) autoclaved genomic DNA from ‘Chinese spring’ was added to the mixture as blocking DNA (Table 2.4). The hybridization mixture was denatured at 80ºC for 10 mins followed by immediate cooling on ice for 10 mins. Probe and chromosomal DNA was denatured together on a Hybaid Omniblock at 75ºC for 7 mins under a plastic cover slip and slowly cooled to the hybridization temperature of 37ºC for 16-20 hrs with vibration set up to 3.

The formamide concentration, Na^+ ion amount in SSC and temperature of the probe mixture determine stringency of hybridization. Unlabelled blocking DNA and

salmon sperm DNA out-compete nonspecific hybridization, while dextran sulphate increases the volume without diluting the probe. SDS helps the penetration of probe and EDTA stops nucleases (Schwarzacher and Heslop-Harrison 2000). The above concentrations of formamide and salt at 37°C allowed sequences of 75-80% homology to form duplexes.

2.2.12.3 Post hybridization washes

Hybridization was followed by post-hybridization washes to remove the hybridization mixture and any unbound probe. A slightly higher stringency than the hybridization stringency was used to remove non-specific or weakly bound probes and to minimize background signal. Cover slips were floated off by incubating the slides in 2x SSC at 35-40ºC. Two stringent washes were carried out with 20% (v/v) formamide and 0.1x SSC at 42ºC of 5 mins each, an equivalent to 85% stringency. Slides were then washed twice in 2x SSC at 42°C for 5 min, followed by cooling down to RT.

2.2.12.4 Slides detection

Slides were incubated in detection buffer (Table 2.4) for 5 mins and then blocked at 37°C for 30 mins with 200µl of 5% (w/v) BSA (bovine serum albumin, Sigma) made in detection buffer. Hybridization sites were detected with 40-50µl of 2µg/ml streptavidin conjugated to Alexa594 (Molecular Probes) and 4µg/ml antidigoxigenin conjugated to FITC (flourescein isothiocyanate, Roche Diagnostics) made up in 5% BSA solution. Slides were incubated at 37ºC for 1hr in humid chamber, followed by two washes in detection buffer at 42ºC for 10 mins each.

2.2.12.5 Mounting of slides

Chromosomes were counterstained with 100µl of 4µg/ml DAPI (Table 2.4) diluted in McIlvaine's buffer (Table 2.4) for 30 mins in dark. The slides were then rinsed in detection buffer, before final mounting in 80µl of antifade solution (Citiflour, Agar Scientific) under a No. 0, 24mm x 40mm coverslip. The slides were stored at 4ºC overnight, to allow binding of the antifade solution to the fluorophores that stabilizes the fluorescence when viewed under the microscope.

2.2.12.6 Photography and image processing

The *in situ* hybridization slides were analyzed on a Zeiss epifluorescence microscope with single band pass filters equipped with a CCD camera (ProgRes™ C12, Optronics, model S97790). The *in situ* hybridization signals were analyzed using Filter Set 10 (excitation = BP450-490, bean splitter = FT510 and emission = BP515-565) for digoxigenin-labelled probe and Filter Set 15 (excitation = BP546/12, bean splitter = FT580 and emission = LP590) for biotin-labelled probe whereas the DAPI-stained chromosomes were analyzed with UV band pass filter (Filter Set 01, excitation = BP365/12, bean splitter = FT395 and emission = LP397). Each metaphase was captured in three different filter sets and then overlayed and analyzed using Adobe Photoshop CS3. Only those functions that treat all pixels of the image equally were used for colour balance and processing.

2.2.13 Immunostaining with anti-5-methylcytosine antibody combined with FISH

The immunostaining procedure was modified from Houben *et al.,* (2003). Pre hybridization and hybridization of the slides was carried out as described above (section 2.2.12.1 and section 2.2.12.2). After post-hybridization washes (section 2.2.12.3), slides were equilibrated in 1x PBS 0.5% (v/v) Tween 20 at RT for 5 mins before blocking with 5% (w/v) BSA (Sigma) prepared in 1x PBS 0.5% (v/v) Tween 20 at RT for 30 mins. Slides were then incubated under a plastic cover slip with 70 µl of monoclonal anti-methylcytosine antibody (Calbiochem®, Cat. No. NA81) diluted 1:200 in 1xPBS buffer at 37ºC for 1.5 hrs or at 4°C overnight. Slides were then washed twice in 1x PBS and once in 1x PBS 0.5% (v/v) Tween 20 at RT for 5 mins.

FISH hybridization and anti-5-meC sites were detected together in a humid chamber under a plastic cover slip, using 70µl of antibodies mixture diluted in 1x PBS at 37°C for 1 hr. For methylated sites, Alexa Fluor® 594 or Alexa Fluor® 488 conjugated goat anti-mouse secondary antibody (Molecular Probes) was diluted 1:100, while for hybridization sites 2µg/ml streptavidin conjugated to Alexa594 (Molecular Probes) or 4µg/ml antidigoxigenin conjugated to FITC (flourescein isothiocyanate, Roche Diagnostics) were applied.

Finally, slides were washed twice in 1x PBS and once in 1x PBS 0.5% (v/v) Tween 20 at RT for 5 mins and then counterstained with 4µg/ml DAPI and mounted as described before (section 2.2.12.5 and section 2.2.12.6).

2.2.14 Re-probing of slides

FISH/GISH and immunostained slides were re-probed to see probes with different labels and label combinations simultaneously on the same cell following Schwarzacher and Heslop-Harrison (2000) with little modification. Traces of immersion oil were carefully wiped from coverslips of selected slides. Slides were kept at 37°C for 10 mins to reduce the viscosity of the antifade mount and were removed by lifting them with a razor blade. Slides were washed in 4x SSC containing 0.2% (v/v) Tween 20 at RT once for 5 mins and then twice for 30-60 mins, followed by two washes in 2x SSC at RT for 5 mins. Preparations were denatured with 70% formamide 2x SSC at 70°C for 2 mins and then dehydrated in an ice-cooled ethanol series of 70%, 85% and absolute for 2 mins and air dried. Hybridization, washes and detection then followed the standard protocol from section 2.2.12 onward.

2.2.15 Restriction enzyme digestion

Genomic DNA from selected lines was digested with restriction endonucleases, including isoschizomers *Msp*I-*Hpa*II and *Bst*NI-*Scr*FI as well as *Mcr*BC (New England BioLabs). Several concentrations of restriction endonucleases and genomic DNA were tested to achieve optimal digestion and matching concentrations of DNA. Approximately 4µg of genomic DNA was digested with 5U/µg of restriction enzyme in the presence of appropriate NEB buffer according to manufacturer's instructions in a final volume of 20µl. Digested DNA was loaded on 1-2% agarose gels along 2µg of uncut genomic DNA as control. Gel electrophoresis was carried out at a slow speed of 30V in 1x TAE buffer for 15hrs, and then visualized by staining with 0.5µg/ml of ethidium bromide (section 2.2.2).

2.2.16 Southern hybridization

2.2.16.1 Transfer of DNA onto charged nylon membrane

After gel electrophoresis, the DNA fragments were transferred onto a positively charged nylon membrane (Hybond N^+, Amersham Biosciences). Agarose gel was depurinated for 10 mins with 5x gel volume of 0.25N HCl and denatured for 30 mins with 5x gel volume of Southern denaturing solution (Table 2.4). Gel was then neutralized with 5x gel volume of Southern neutralizing solution (Table 2.4) for 30 mins at RT slowly shaking and then washed with sterile distilled water before setting up the gel for alkaline transfer. The size of gel was reduced by cutting its edges with a scalpel and then placed upside down in a set up for upward capillary action in a tray containing 10-15x gel volume of 0.4N NaOH. A support was placed in the tray with a bridge on it made of a single piece of 3MM Whatman filter paper in direct contact with 0.4N NaOH on either side of the support for continuous capillary action. The positively charged nylon membrane of appropriate size was marked at the edge with a pencil and soaked with 0.4N NaOH before placing on the top of the gel. Cling film was placed around the edge of the membrane to stop the movement of 0.4N NaOH except through the gel, followed by 2 sheets of 3MM Whatman filter papers a bit larger in size than the membrane and a stack of 8-10cm paper towels. On the top of paper towels about 0.5kg weight was kept to create a constant pressure and allow the flow of liquid and transfer of DNA fragments from gel on to the membrane. For complete transfer the gel was kept in this set up for 16 hrs and then the efficiency of transfer was checked by re-staining the agarose gel in 0.5µg/ml of ethidium bromide solution for 10-15 mins, washed in distilled water for 5 mins and observed under UV light. After transfer the nylon membrane was soaked in 6x SSC for 2 mins to remove any gel pieces, and dried between filter papers before baking at 80°C oven for 10 mins, and then the DNA was covalently linked to the membrane by exposure to $7x10^4$ J/cm^2 of UV light (DNA side facing up) in a UVP CL-1000 Ultraviolet Crosslinker (McKinley Scientific, UK) and stored at 4°C before hybridization.

2.2.16.2 Membrane hybridization

Both radioactive and non-radioactively labelled probes were used for southern hybridization purposes.

2.2.16.2.1 Non-radioactive hybridization

For non-radioactive Southern, digoxigenin labelled probes (section 2.2.10) were used for membrane hybridization. The membrane was re-hydrated with 4x SSC 0.5% (w/v) SDS and pre-hybridized at 55°C for 4hrs in a Thermohybaid Hybridization oven (Ashford, UK) using 5ml of pre-hybridization solution per 100cm^2 of nylon membrane containing 5x Denhardts solution (Table 2.4), 4x SSC, 0.5% (w/v) SDS and 100µg/ml of sheared Salmon sperm DNA (Table 2.4). The pre-hybridization solution was discarded and replaced with an identical hybridization solution having 3-4µl (corresponding to ~150ng) of digoxigenin labelled probe and hybridized at 55°C for 16 to 18 hrs with constant rotation.

2.2.16.2.2 Post-hybridization washes and detection

High stringency washes were carried out by washing the membrane twice in 2x SSC 0.1% (w/v) SDS at 56°C for 5 mins (64% stringency) and then twice in 0.2x SSC 0.1% (w/v) SDS for 15 mins each at 56°C (equivalent to 82% stringency). Detection was carried out at RT. Membranes were rinsed for 5 mins in 100 ml of washing buffer 1 (Table 2.4), followed by 100 ml of buffer 2 (Table 2.4) for 30 mins. They were then incubated for 30 mins with 20 ml of antibody conjugate solution [anti-digoxygenin alkaline phosphatase (Roche Diagnostics) with final dilution of 0.1U/ml in buffer 2]. After the antibody incubation, the membrane was washed twice for 15 mins with buffer 1 (Table 2.4), and then equilibrated for 5 mins with buffer 3 (Table 2.4).

The membranes were finally incubated in dark for 5 mins in with 500µl of CDP-star solution (Roche Diagnostics) diluted 1:100 in buffer 3. The excess of CDP-Star solution was drained and then the membrane was wrapped in a cling film and transferred to auto radiographic cassette in complete darkness. The chemiluminescence was recorded by keeping X-ray film (FUJI Medical X-Ray film) of appropriate size

below the membrane. Different exposure times from 1-15 mins were given to detect all possible signals. X-ray films were developed using automatic photographic developing machine and scanned with EPSON Expression Pro 1600, and images were processed with Adobe Photoshop CS3.

2.2.16.2.3 Radioactive hybridization

For radioactive hybridization the membrane was soaked in 4x SSC 0.5% (w/v) SDS and then transferred into a hybridization bottle with 25 ml of pre-hybridization buffer containing 5x Denhardts solution (Table 2.4), 4x SSC 0.5% (w/v) SDS and 100µg/ml of sheared Salmon sperm DNA (Table 2.4). The membrane was pre-hybridized in a Thermohybaid Hybridization oven (Ashford, UK) for 4 hrs at 65°C with constant rotation.

While the membrane was in the pre-hybridization step, the radioactively labelled probe was freshly prepared, using RadPrime DNA Labelling System (Cat. No. 18428-011, Invitrogen) following manufacturer's instructions with little modifications. 100-150 ng of DNA was diluted in 22.5µl of water (Sigma) and denatured in boiling water for 5 mins followed by 5 mins on ice. The labelling reaction contained: 20µl of Random primer buffer, 4µl of dNTPs (excluding dCTP), 2.5µl of α-^{32}P-dCTP (3000Ci/mmol) supplied by Amersham Biosciences, UK and 1µl of 40U Klenow Fragment. The reaction was left at 37°C for 45 mins, after which the reaction was stopped with 5µl of Stop Buffer (0.5 M EDTA, pH 8.0). To avoid radioactive contamination probes were not cleaned for hybridization.

The pre-hybridization solution was replaced by 25ml of identical hybridization solution and 50µl of freshly made probe. The membrane was hybridized at 65°C for 16 hrs. The hybridization solution was collected and stored at -20°C for possible re use. The membrane was washed twice for 5 mins at RT with 50ml of 2x SSC 0.5% (w/v) SDS, then twice with 50ml of 2x SSC 0.1% (w/v) SDS at 65°C for 30 mins, and twice with 0.1X SSC 0.1% (w/v) SDS for 30 mins at 65°C. After the final wash, the membranes were dried between tissue paper, wrapped in cling film and the sites of hybridisation were visualised by exposing the membrane against X-ray film (FUJI Medical X-Ray film) in an autoradiographic cassette with intensifying sheet (Fisher) above the X-ray film and kept at -20°C from 20 hrs (> 250 counts/secs) to 15 days (< 30

counts/secs). These X-ray films were then processed as described above (section 2.2.16.2.2).

For re-probing, the radioactively labelled probes were stripped by immersing the membrane in 200 ml of boiling 0.1xSSC 0.1% (w/v) SDS for 5 mins, in a flat tray shaking gently. The same process was repeated 3-4 times or until the radioactivity detected with a Geiger counter was less than 5 counts/secs. These stripped membranes were placed against X-ray film for at least 24 hrs at RT to check any radioactivity left. In case no background signals were produced, membranes were re-probed for the second time.

CHAPTER III: CHARACTERIZATION OF ALIEN CHROMATIN AND ITS ORGANIZATION IN WHEAT BREEDING LINES CONFERRING RESISTANCE TO *WHEAT STREAK MOSAIC VIRUS*

3.1 Introduction

Every year, about 10–16% of the global harvest an equivalent of US$220 billion is lost to different plant pests and diseases (section 1.5). *Wheat streak mosaic virus* (WSMV), is among one of the most important yield-limiting diseases, which can cause significant loss to both forage and grain production in wheat (Fahim *et al.,* 2010a, Liu *et al.,* 2011, Schwarzacher *et al.*, 2011) The first report about WSMV came from United States (Mckinney, 1937), but in a short period of time, the disease has spread widely and distributed throughout the wheat growing world (Lanoiselet *et al.,* 2008, Oldfield, 1970, Sanchez-Sanchez *et al.,* 2001, Navia *et al.,* 2006, Coutts *et al.,* 2008, Kudela *et al.,* 2008). The disease is transmitted to healthy plants via an insect vector, the wheat curl mite (WCM) *Aceria tosichella* Kiefer (Coutts *et al.,* 2008). Normally yield losses vary from 2.5-5% but in some localised epidemics can cause 100% crop failure (Christian and Willis, 1993, McNeil *et al.,* 1996, Stenger *et al.,* 2002).Volunteer wheat plants, time of infection and temperature are some important factors contributing to the severity of the disease (Hunger *et al.,* 1992, Bockus *et al.,* 2001, Thomas and Hein, 2003, Thomas *et al.,* 2004).

3.1.1 Symptoms of WSMV

Infected plants exhibit a variety of symptoms, but the most common symptoms associated with WSMV include, the appearance of greenish yellow streaks, rolled up leaf margins and mosaic to severely chlorotic leafy tissue (Atkinson and Grant, 1967, Hunger *et al.,* 1992, Baley *et al.,* 2001, Fahim *et al.,* 2010a). The streaks usually run parallel along the leaf axis and the infected plants exhibit stunted growth. The yield of infected crop is reduced significantly, as the infected plants produce fewer tillers with low quality grain or the plants may even remain vegetative. In more extreme cases the

disease ends up with death of the plant and elimination of the crop (Hunger *et al.,* 1992, Sharp *et al.,* 2002, Lanoiselet *et al.,* 2008, Velandia *et al.,* 2010).

WSMV can infect a broad range of hosts, including many cultivated and wild species of the family Poaceae (Christian and Willis, 1993, Coutts *et al.,* 2008). Both the virus and WCM survive on the 'green bridges' provided by volunteer wheat and other grasses (Coutts *et al.,* 2008). However, environmental factors that influence plant growth and multiplication of the WCM plays a major role in the development of symptom and epidemics. These factors include temperature, light, soil fertility, growth stage of the plant, time and season of infection and the genotype or cultivars used (Hunger *et al.,* 1992, Martin *et al.,* 1984, Seifers *et al.,* 1995, Baley *et al.,* 2001, Atkinson and Grant, 1967). Winter infections are more devastating than the spring infection (Hunger 2004) as the mites get more time to multiply and establish its population within the crop and to spread the disease into the neighbouring fields (Hunger *et al.,* 1992, Thomas and Hein, 2003).

In certain epidemics of *Wheat streak mosaic*, more than one type of infectious particle is isolated from the same extract which is due to the fact, that WSMV can interact with related viruses such as, *High plains virus* (HPV) and *Triticum mosaic virus* (TriMV) to co-infect a single host (Seifers *et al.,* 2009a, Stenger *et al.,* 2007a). Both these viruses are also transmitted by the WCM and results in more severe infection due to disease synergism (Tatineni *et al.,* 2010).

3.2 Transmission of WSMV

Persistent epidemics and initiation of diseases into new areas depend upon the prevalence of infectious inocula. For WSMV, the effective transmission of the viral propagules is achieved via two known sources, the WCM and infectious seeds (Jones *et al.,* 2005, Dwyer *et al.,* 2007, Lanoiselet *et al.,* 2008). In the absence of primary host, both WCM and its vector over-summer on cereals and other volunteer grasses, until wheat is available for infection (Harvey *et al.,* 2001, Thomas *et al.,* 2004, Thomas and Hein, 2003).

3.2.1 Management and control of WSMV

Lack of remedial control options (Slykhuis, 1955) and identification of only one gene of WSMV-resistance within the primary and secondary genetic pool of wheat, makes WSMV one of the major threat to global wheat production (Seifers *et al.,* 2007, Divis *et al.,* 2006, Graybosch *et al.,* 2009, Liu *et al.,* 2011, Mutti *et al.,* 2011, Schwarzacher *et al.,* 2011). WSMV can infect both winter and spring wheat cultivars (Langham *et al.,* 2001) and among cereal infecting viruses, ranks second after Barley *yellow dwarf virus* (BYDV) in severity (Ellis *et al.,* 2003). Recent studies have revealed that WSMV reduces the root biomass and water use efficiency, making it a serious concern for regions with limited water resources (Price *et al.,* 2010). Of the few available options to control WSMV, some important include:

3.2.2 Cultural practices

Outbreaks of WSMV are mainly associated with the presence of volunteer wheat and other cereals that serve as reservoirs for both the virus and mites (Thomas *et al.*, 2004). Eliminating these potential sources of spread before planting can prevent or reduce the risks of WSMV infection (Slykhuis, 1955, Christian and Willis, 1993). Late plantation is an effective cultural practice that helps in reducing the losses to WSMV infestation (Hunger *et al.*, 1992). But in regions, where wheat is grown for dual purposes (grazing followed by recovery for grain production) such wheat is grown early and are at a higher risk of WSMV infection and its subsequent spread in the region (Velandia *et al.,* 2010, Fahim *et al.,* 2010a). In such cases, removal of volunteer grasses alone is helpful in minimizing the sources of primary infection, but may not reduce the chances of WSMV epidemics (Thomas and Hein, 2003).

WSMV causes chlorosis, streaking, necrosis and stunting (Graybosch *et al.,* 2009) that ultimately results in modification of the cell make-up, pigment concentrations, water and nutrient uptake and gaseous exchange. These changes altogether modify the reflection properties of wheat canopy area (West *et al.,* 2003). Satellite and remote sensing, that monitor the change in the reflected light from the canopy has been used with success to monitor WSMV epidemics (Riedell and Blackmer, 1999, Mirik *et al.,* 2006). It provides the quickest, efficient and inexpensive means for identifying the WSMV infection over a large area (Mirik *et al.,* 2006).

However, remote sensing is essentially a monitoring tool for crop losses (Richardson *et al.,* 2004) and is not a remedial approach for the control of WSMV.

3.2.2.1 Chemical control measures for WSMV

Utilization of chemicals is one of the most effective strategies for controlling losses to plant diseases (De Waard *et al.,* 1993). However, there is no chemical treatment available for WSMV (Hull, 2004, Chen *et al.,* 1999a, Qi *et al.,* 2007). Often, the use of herbicides a few weeks before the growing season is highly recommended, as it eliminates the alternative hosts that act as green bridges (Jiang and Gill, 1994, Jiang *et al.,* 2005, Wegulo *et al.,* 2008). Few miticides are effective in controlling mite populations, but their efficiency is reduced by the habitat of mites, along with the lack of information concerning optimum timing and conditions for chemical application (Hein and 2010, Velandia *et al.,* 2010). Furthermore, the persistence and mobility of pesticides in the environment is hazardous and its cost lowers the net profits and revenues of wheat production (De Waard *et al.,* 1993, Chen *et al.,* 1999a, Lu *et al.,* 2011).

3.2.2.2 Engineered resistance to WSMV

Recently pathogen-derived resistance or transgenic virus-resistant plant strategies have been employed for the control of WSMV. In transgenic wheat, resistance to a virus is derived from the expression of viral genes (Barton and Brill, 1983, Sanford and Johnston, 1985, Sivamani *et al.,* 2000, Fahim *et al.,* 2012).Transgenic wheat varieties resistant to WSMV have been developed successfully, either by disrupting the life cycle of the virus through expression of viral coat protein (Sivamani *et al.,* 2002) and replicase gene (Sivamani *et al.,* 2000) or by developing transgenic resistance based on RNA interference (RNAi) designed to target the nuclear inclusion protein 'a' (NIa) gene of WSMV (Fahim *et al.,* 2010b). Although, transgenic wheat display considerable to complete WSMV-resistance in controlled environments, but field trials have revealed yield penalties and failure of some transgenic wheat to resist WSMV infestation (Sharp *et al.,* 2002). Nevertheless, emerging research data about the critical environmental impacts of transgenic crops, cultural sensitivity and undetermined future do not put transgenic crops at the forefront for practical utilization (Altieri, 2000).

3.2.2.3 Natural resistance to WSMV

Most wheat cultivars (*T. aestivum* L., 2n=6x=42, ABD) lack effective WSMV-resistance and in cases where resistance is present, it is ineffective at high temperatures (Seifers *et al.,* 1998, Seifers *et al.,* 2007, Liu *et al.,* 2011). Therefore, the exploration of WSMV-resistance in both cultivated and wild *Triticeae* and its subsequent incorporation in wheat backgrounds is of utmost importance to ensure the successful production of wheat over the coming years (Cox *et al.,* 2002).

Some perennial wheat grasses from the tertiary gene pool of wheat, like *Th. intermedium* syn. *Agropyron intermedium* (Host) Barkworth and Dewey (2n=6x=42, $JJJ^{s}J^{s}SS$) and *Th. ponticum* (Podp.) Barkworth and Dewey (2n=10x=70, $JJJJJJ^{s}J^{s}J^{s}J^{s}$) provide large reservoirs of useful agronomic traits (Mujeeb-Kazi and Hettel, 1995, Li and Wang, 2009, Chen *et al.,* 1999a, Chen *et al.,* 2003b). Both show high levels of resistance to WSMV and its vector, and can be readily crossed with wheat (Li *et al.,* 2005a, Wells *et al.,* 1982, Harvey *et al.,* 2003, Schwarzacher *et al.,* 2011). These perennial wheat grasses have been used for the enrichment and genetic diversity of wheat (Tsitsin, 1965, Fedak *et al.,* 2001, Qi *et al.,* 2007, Divis *et al.,* 2006, Mutti *et al.,* 2011).

Many genes of disease resistance including those for WCM and WSMV-resistance have been successfully transferred into the wheat backgrounds as chromosomal segments (Graybosch *et al.,* 2009, Li and Wang, 2009, Fahim *et al.,* 2011, Sears, 1966, Feldman and Sears, 1981). The amount of alien chromatin involved in these transfers varies from a single gene to chromosomal arms or entire chromosomes (Wells *et al.,* 1973, Friebe *et al.,* 1996b, Friebe *et al.,* 1991, Friebe *et al.,* 2009, Qi *et al.,* 2007, King *et al.,* 1997a, Singh *et al.,* 2008b, Singh *et al.,* 1998, Bockus *et al.,* 2001). Translocations involving small alien fragments, have less likelihood of linkage drag, but in cases may be further minimised with chromosomal engineering (Friebe *et al.,* 2009, King *et al.,* 1992, Harper *et al.,* 2011, Gill *et al.,* 2011, Heslop-Harrison and Schwarzacher, 2011a). This alien derived, WSMV-resistance in wheat is durable and provides benefits in the presence of virus and has limited detrimental effects on the essential agronomic and end-use quality traits of wheat (Baley *et al.,* 2001, Divis *et al.,* 2006, Graybosch *et al.,* 2009). In fact, the most promising sources of WCM and WSMV-resistance have been obtained from the intergeneric crosses of wheat with *Th. intermedium* (Wells *et al.,* 1973, Mutti *et al.,* 2011, Chen *et al.,* 1999a, Schwarzacher *et*

al., 2011, Cox *et al.,* 2002, Qi *et al.,* 2009). Several *Th. intermedium* derived lines, with WSMV-resistance have been released as commercial wheat cultivars (Martin *et al.,* 2007, Graybosch *et al.,* 2009, Mutti *et al.,* 2011).These lines have incorporated *Th. intermedium* chromatin in the form of 4DS (Wells *et al.,* 1982, Wells *et al.,* 1973, Friebe *et al.,* 1996a) or 4AS (Haber *et al.,* 2007) chromosomal translocations. Though the size of known *Th. intermedium* fragments vary considerably among different wheat germplasm (see Friebe *et al.,* 2009, Fahim *et al.,* 2011 and below).

In all known sources of WSMV-resistance, the *Th. intermedium* chromatin has essentially three different chromosomal origins. One source contains *Th. intermedium* telosome that was initially believed to be a group-4 long arm, but later on confirmed to be homoeologous to group-7 long arm (Friebe *et al.,* 2009, Liu *et al.,* 2011). The second source is a J^s-genome chromosome present in the Zhong series of wheat-*Th. intermedium* amphiploids and designated as J^s2 (Chen *et al.,* 1999a, Chen *et al.,* 2003a). But neither of the two sources has been exploited. Previously both sources lack compensating Robertsonian translocations (Friebe *et al.,* 2009). However, very recently group-7 Robertsonian translocation lines have been reported (Liu *et al.,* 2011). The most extensively studied source carries the short arm of the group-4 or J^S genome chromosome of *Th. intermedium* and is designated as 4Ai#2S (Chen *et al.,* 1998a, Friebe *et al.,* 2009). This source carries a compensating Robertsonian translocation, and the chromosomal arm of *Th. intermedium* replaces the short arm of wheat chromosome 4D in the form of T4DL·4Ai#2S chromosomal translocation (Divis *et al,* 2006, Friebe *et al.,* 2009).

3.2.3 Deployed natural resources of WSMV-resistance

To date, two genes of WSMV-resistance, named as *Wsm1* (Friebe *et al.,* 1991) and *Wsm2* (Lu *et al.,* 2011) have been used in wheat cultivars improvement. The *Wsm1* is a *Th. intermedium* origin gene (Chen *et al.,* 1998a). It was transferred into wheat germplasm CI 17884 by Wells and his co-workers (Wells *et al.,* 1973, Wells *et al.,* 1982, also see Figure 1.3). This resistance is present on the short arm of *Th. intermedium* chromosome 4Ai#2 (Chen *et al.,* 1998a) and provides effective resistance against WSMV infection and WCM colonization (Chen *et al.,* 2003a, Chen, 2005, Li *et al.,* 2005, Friebe *et al.,* 2009). Commercial winter wheat cultivars 'Mace' contain the *Wsm1* gene (Graybosch *et al.,* 2009). The *Wsm2* gene was recently mapped to wheat

chromosome 3BS in CO960293-2 germplasm (Lu *et al.,* 2011). Its origin is not clear, as none of its parents were found resistant to WSMV. Perhaps, it is the first substantial resistance to WSMV originating in bread wheat itself (Haley *et al.,* 2002). The *Wsm2* has been incorporated in the released winter wheat cultivars 'RonL' (Seifers *et al.,* 2007) and 'Snowmass' (Haley *et al.,* 2011). Another alien derived gene of *Th. intermedium* origin, the *Wsm3* was mapped to the T7BS·7S#3L recombinant chromosome (Liu *et al.,* 2011). Homozygous lines with this recombinant chromosome have been reported effective against WSMV but this resistance is yet to be exploited in wheat germplasm improvement.

Although *Wsm1* confers resistance and advantages in the presence of the virus but the original lines carrying *Wsm1,* were frequently associated with undesirable traits of yield and bread-making qualities (Seifers *et al.,* 1995). Some wheat lines incorporating this alien chromatin were reported to have poor performance and significantly lower yield of 11 to 28% in the absence of virus (Baley *et al.,* 2001, Sharp *et al.,* 2002). Lines involved in this study were obtained by backcrossing and hybridization of the Nebraska-adapted winter wheat lines with Kansas developed materials carrying *Wsm1* (Divis *et al.,* 2006, Graybosch personal communication). These lines were then evaluated for potential linkage drag associated with this alien chromatin (Divis *et al., 2006* and next line). Sister-lines from six breeding populations were assessed for agronomic and quality traits under virus-free, and under a naturally occurring WSMV-infection. In the absence of virus, no significant difference for grain yield was observed between resistant (R) and susceptible (S) lines. However, under infection R-lines had a significantly higher yield compared to S-lines (see Figure 3.1A&B). In brief, no negative effects of grain yield or bread making quality were linked to *Wsm1* (Divis *et al.,* 2006, Graybosch personal communication).

The resistance offered by *Wsm1, Wsm2* and *Wsm3* genes is temperature-dependent. Strains of WSMV fail to infect wheat lines incorporating either of the genes up to 18°C (Liu *et al.,* 2011). However, resistance offered by *Wsm2* is ineffective above 18°C (Seifers *et al.,* 2007). The *Th. intermedium* origin *Wsm-1* gene provides resistance to both WSMV and TriMV (Tatineni *et al.,* 2010). The *Wsm1* resistance is effective at higher temperatures and is stable enough to give complete protection from under field conditions up to 20°C (Seifers *et al.,* 1995, Seifers *et al.,* 2006, Graybosch *et al.,* 2009). Recently in green house experiments lines carrying the *Wsm3* gene were reported stable at a temperature of 24°C (Liu *et al.,* 2011).

In this part of the study, FISH/GISH was applied, using total genomic DNA probes from *Th. intermedium* and *Ae. tauschii* to wheat-*Th. intermedium* hybrid lines, to determine the size of alien chromatin, chromosomal break point (BP) and the wheat genome involved in recombination. Similarly, multi-target *in situ* hybridization was carried out to physically map highly repetitive DNA sequences on wheat chromosomes to characterize the specific the chromosomes and chromosomal arms involved in translocation.

3.3 Materials and methods

3.3.1 Plant material

Wheat genotypes comprising of reference and experimental lines used in this study is given along with their pedigree analysis in Materials and Methods Chapter II. Experimental lines are derived from four breeding populations of Divis *et al.,* (2006) and designated as I, II, III and IV. Three reference lines, two resistant (R) KS95H102, KS96HW10-1 from populations KS102, KS10-1and one susceptible (S) line Millennium from population MILL were also used as control.

Experimental lines included in this study were rated consistently as R or S to WSMV in both green house and under field experiments (Figure 3.1A&B, also see Divis *et al.,* 2006). Before this study, no information was available about the cytogenetic structure of the experimental lines (Table 3.1). However, the ultimate source resistance in all R-lines was either KS91H184 or KS91H174, both being selected from populations that had been randomly mated for several generations with CI 17884 and then screened for WSMV resistance (Figure 3.2, Divis *et al.,* 2006, Graybosch personal communication). The CI 17884 is a WSMV-resistance line carrying the *Wsm1* gene on a chromosomal arm translocated from *Th. intermedium* (Wells *et al.,* 1982 and Figure 1.3).

All analysis of the experimental lines including the nature and size of alien chromatin as well the identification of recombinant chromosomes was carried out in this study (see results and discussion below). These lines (Table 3.1) as a whole are often referred to as wheat-*Th. intermedium* hybrid lines in this report. For the cytogenetic and genomic analysis, seeds of these lines were kindly provided by R. A. Graybosch University of Nebraska-Lincoln Agriculture & Horticulture, USA.

A B C D

Figure 3.1A: Field resistance of the winter wheat lines (A) ‘Tomahawk’ (B) N02Y5117 or ‘Mace’ (C) N02Y5003 and (D) N02Y5149 to *Wheat streak mosaic virus*. ‘Tomahawk’ is a WSMV-susceptible line, while N02Y5117 or ‘Mace’, N02Y5003 and are N02Y5149 WSMV-resistant lines. *Source of photograph:* Bob Graybosch.

A B C

Figure 3.1B: Field resistance of the winter wheat lines (A) N02Y5149 (B) N02Y5117 or ‘Mace’ and (C) ‘Tomahawk’ to *Wheat streak mosaic virus*. Line N02Y5149 and N02Y5117 or ‘Mace’ and are WSMV-resistant lines, while ‘Tomahawk’ is a WSMV-susceptible line. *Source of photograph:* Bob Graybosch.

3.3.2 Fluorescent *in situ* hybridization

Information about probes used, labelling procedures and *in situ* hybridization are given in M&M chapter II (section 2.2.9-2.2.12).

3.4 Results

3.4.1 Characterization of recombinant wheat lines

Recombinant wheat chromosomes were identified with multi target *in situ* hybridization using genomic and repetitive DNA probes simultaneously to the spread chromosomes. Genomic probe revealed the presence of alien fragments, while the unique banding patterns of repetitive DNA were helpful in identifying, and designating the recombinant chromosomes. The same strategy was applied to identify or rule out the possibility of any reciprocal translocation and targeting the chromosomal arm harbouring the WSMV-resistance gene within the wild *Th. intermedium* genome. For each line 10-15 cells were analysed. Length of the alien fragment was determined as the percent distance from the centromere to the discriminating hybridization sites over the total arm length and was calculated as, mean % arm length with standard deviation (mean ± standard deviation) from 10 recombinant chromosomes of each line.

Among the randomly selected experimental R and S-lines from the four breeding populations (Table 3.1), 75% individuals incorporated alien chromatin of variable sizes (Table 3.2, Figures 3.5-3.24). Genome identification of the recombinant wheat chromosomes was facilitated by the use of *Ae. tauschii* genomic DNA probe, which labelled the entire D-complement of the wheat genome (Figure 3.17B). Additional repetitive DNA sequences were then applied to target the chromosomal arms involved in the translocations. Successful mapping of the Afa/dpTa1 family (D-genome), pSc119.2 (abundant in the B-genome with some sites on A and D-genome chromosomes), pTa794 and pTa71 probes revealed characteristic banding patterns (see Figure 1.5 and Mukai *et al.,* 1993, Castilho *et al.,* 1996, Pedersen and Langridge 1997, Biagetti *et al.,* 1999) that enabled recombinant chromosomes to be identified as 1B, 3D and 4D respectively. The results (summarized in Table 3.1) are consistent with previous findings (Friebe *et al.,* 1991, 2009, Divis *et al.,* 2006, Graybosch *et al.,* 2009) and

confirm WSMV-resistance is strongly correlated with the presence of *Th. intermedium* chromatin (Table 3.1 and below).

Table 3.1: FISH screening results of the wheat-*Th. intermedium* hybrid lines, 75% of the experimental lines show alien chromatin.

Sr. No	Population	Line	Final rating	Recombinant chromosomes			Figure list
				4D	1B	3D	
Reference wheat-*Th. intermedium* lines							
1	KS102	KS95H102	R	+/+	-/-		3.2
2	KS10-1	KS96HW10-1	R	+/+	-/-		3.3
3	MILL	Millennium	S	-/-	-/-		3.4
Experimental wheat-*Th. intermedium* lines							
4	Pop-I	N02Y5018	R	+/+	+/+		3.5
5		N02Y5019	S	-/-	+/+		3.6
6		N02Y5021	S	-/-	-/-		3.7
7		N02Y5025	R	+/+	-/-		3.8
8		N02Y5003	R	-/-	+/+		3.9
9	Pop-II	N02Y5057	R	+/+	-/-		3.10
10		N02Y5075	R	+/+	-/-		3.11
11		N02Y5078	R	+/+	-/-		3.12
12		N02Y5082	S	-/-	-/-		3.13
13		N02Y5096	S	-/-	-/-		3.14
14	Pop-III	N02Y5105	S	-/-	-/-		3.15
15		N02Y5106	R	+/+	-/-		3.16
16		N02Y5109	R	-/-	-/-	+/+	3.17
17		N02Y5117 (Mace)	R	+/+	-/-		3.18
18		N02Y5121	S	-/-	-/-		3.19
19	Pop-IV	N02Y5149	R	+/+	+/+		3.20
20		N02Y5154	R	+/+	-/-		3.21
21		N02Y5156	S	-/-	+/+		3.22
22		N02Y5163	S	-/-	+/+		3.23
23		N02Y2016	R	+/+	+/+		3.24

Final rating = field evaluation response of a line to WSMV in trails (Divis *et al.*, 2006)
+/+ = when two similar fragments are seen homozygous condition
-/- = when no fragment is detected
No heterozygous condition was found
For characterization of chromosomes see Figures 3.25 and 3.26

3.4.2 Detailed description of lines

Reference Lines

In situ hybridization (ISH) of the reference populations, revealed the presence of a whole *Th. intermedium* chromosomal arm translocated on the wheat in both resistant line KS95H102 (Figure 3.2) and KS96HW10-1 (Figure 3.3). This alien arm can be recognized by its terminal pSc119.2 and a strong centromeric Afa or dpTa1 sites (Figure 3. 26). Several other dpTa1 sites were consistently found on the other half of this recombinant chromosome, of wheat origin. This recombinant wheat chromosome

was identified as 4D and the translocation has been described previously as 4Ai#2S.4DL (Friebe *et al.*, 2009). 'Millennium' the reference susceptible line did not show any detectable alien chromatin of *Th. intermedium* (Figure 3.4).

Population-I

FISH showed heterogeneity among the lines of population-I, in terms of both the presence and size of the alien fragments (Table 3.1 and Table 3.2). Like the reference resistant lines, two R-lines, N02Y5018 (Figure 3.5) and N02Y5025 (Figure 3.8) show alien chromatin in the form of 4Ai#2S.4DL translocation. However in contrast to reference lines, an additional small fragment of *Th. intermedium* is seen on another large chromosome of N02Y5018 (Figure 3.5). This recombinant chromosome was identified as 1B by the specific 5S and 45S rDNA sites (Figure 3.25). This fragment is most probably originating from the small arm of the homoeologous group-1 of *Th. intermedium* and the translocation was speculated as 1Ai#1-1BS.1BL. FISH was also able to map the small alien fragments at the distal end of 1BS chromosome in both N0Y5019 (Figure 3.6) and N02Y5003 (Figure 3.9) lines. However, these two lines vary in their WSMV response. Line N0Y5019, cannot resist WSMV infection while N02Y5003 was consistently rated as resistant or moderately resistant in field trials (see Figure 3.1A&B). N02Y5021, the only WSMV-susceptible line in population-I, that did not show any *Th. intermedium* chromatin (Figure 3.7).

Population-II

Individuals of population II were genetically homozygous in the sense, that all the three resistant lines from this population including N02Y5057 (Figure 3.10), N02Y5075 (Figure 3.11) and N02Y5078 (Figure 3.12) show *Th. intermedium* chromatin identical to the reference resistant lines, in the form of the 4Ai#2S.4DL chromosomal translocation (Table 3.1). Furthermore, no alien chromatin of *Th. intermedium* origin was detected in both of the tested susceptible lines N02Y5082 (Figure 3.13) and N02Y5096 (Figure 3.14) of this population.

Population-III

Two resistant lines N02Y5106 (Figure 3.16) and N02Y5117 or 'Mace' (Figure 3.18) have been tested and have *Th. intermedium* chromatin similar to the reference resistant lines. However, no large *Th. intermedium* fragments resembling 4Ai#2S.4DL was

detected in WSMV-resistant line N02Y5109 (Figure 3.17A&B). The size of the observed alien fragment was considerably larger than the recombinant 1B chromosome. GISH with *Ae. tauschii* DNA indicated that a D-genome of wheat is recipient of this alien fragment (Figure 3.17B). Multi target FISH with pSc119.2 or dpTa1 combined with *Th. intermedium* genomic DNA recognized the recombinant chromosome as 3D, and the translocation was speculated as 3Ai#1-3DL.3DS. FISH analysis of line N02Y5105 (Figure 3.15) and N02Y5121 (Figure 3.19) revealed that *Th. intermedium* fragments are missing in these lines. Furthermore, both these lines do not confer resistance to WSMV in the field and were consistently rated as S-lines.

Population-IV

Similar to the reference resistant lines, the R-lines from population-IV, N02Y5149 (Figure 3.20), N02Y5154 (Figure 3.21) and N02Y2016 (Figure 3.24) have the 4Ai#2S.4DL chromosomal translocation. Line N02Y5149 also has incorporated another small fragment at the distal end of 1BS arm (Figure 3.20 and Table 3.1). The 1BS recombinant chromosome was also detected in the WSMV-susceptible lines N02Y5156 (Figure 3.22) and N02Y5163 (Figure 3.23) of population-IV.

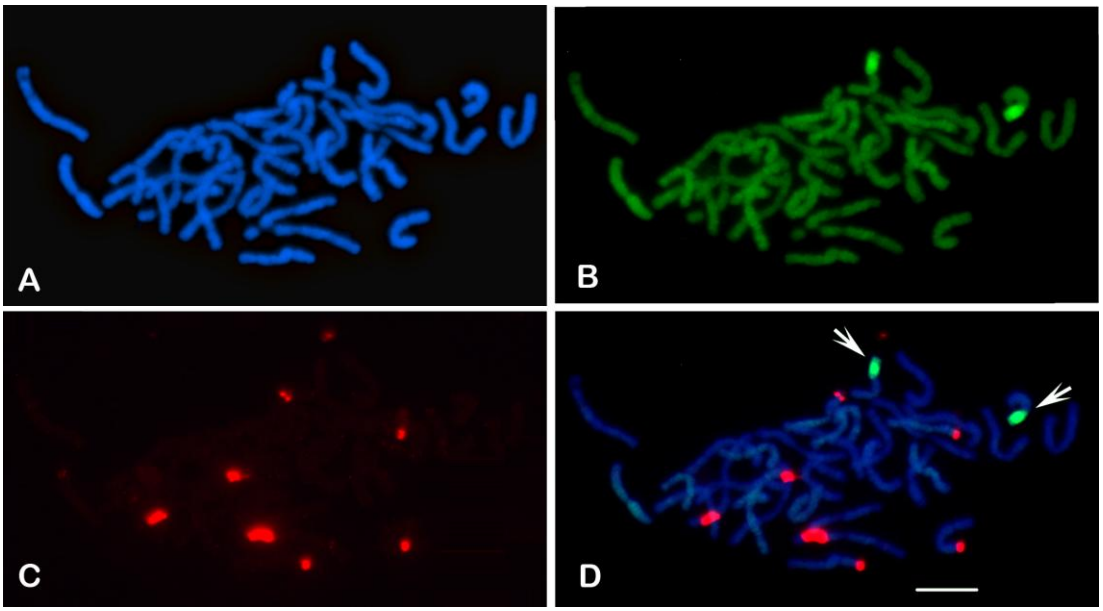


Figure 3.2: Root-tip metaphase chromosomes of the reference resistant line KS95H102 (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with digoxygenin 11-dUTP (detected in green). The genomic *in situ* hybridization allows the detection of *Th. intermedium*-origin chromosome segments. (C) Hybridization pattern of the pTa71 clone labelled with biotin 16-dUTP (detected in red) showing the physical location of major 45S rDNA sites on 1A, 1B, 6B, 5D and 7D of wheat. (D) Overlay of A, B and C images, alien chromosomal arms are indicated by arrow. Bar represents 10µm.

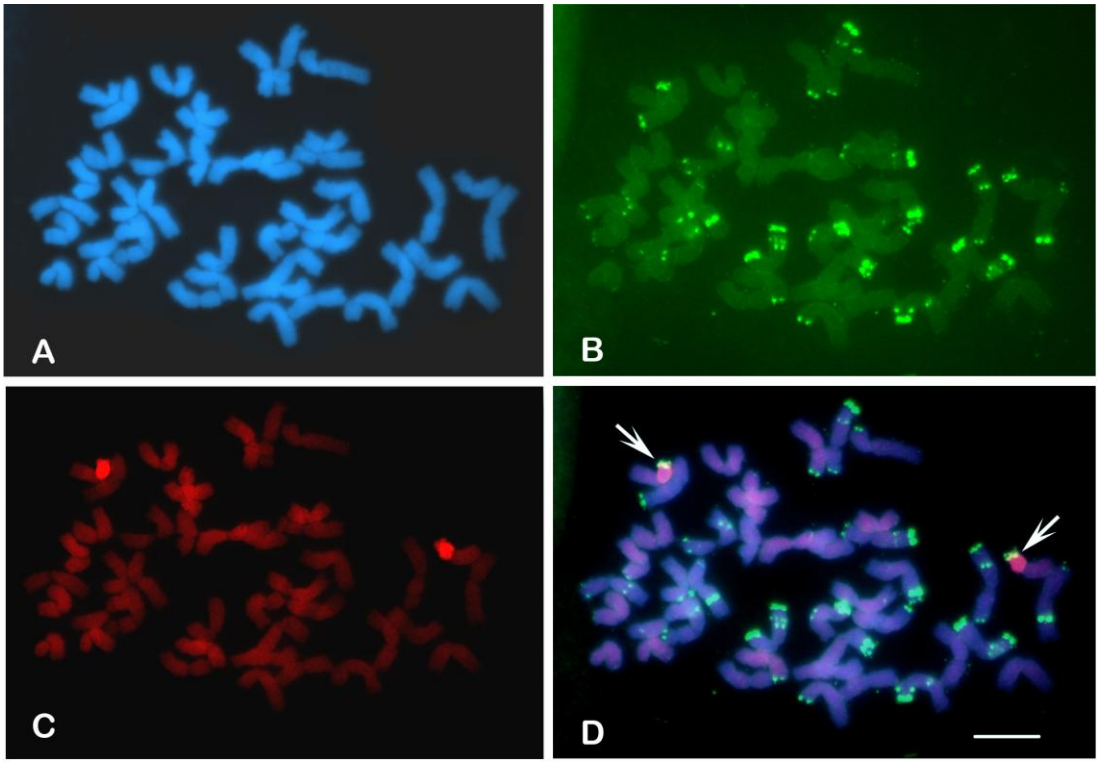


Figure 3.3: Root-tip metaphase chromosomes of the reference resistant line KS96HW10-1 (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) Hybridization pattern of the pSc119.2 DNA sequence labelled with digoxygenin 11-dUTP (detected in green) hybridizing to B and some A-genome chromosomes. (C) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with biotin 16-dUTP (detected in red). The genomic *in situ* hybridization allows the detection of *Th. intermedium*-origin chromosome segments. (D) Overlay of A, B and C images, alien chromosomal arms with the telomeric pSc119.2 sites are indicated by arrow. Bar represents 10μm.

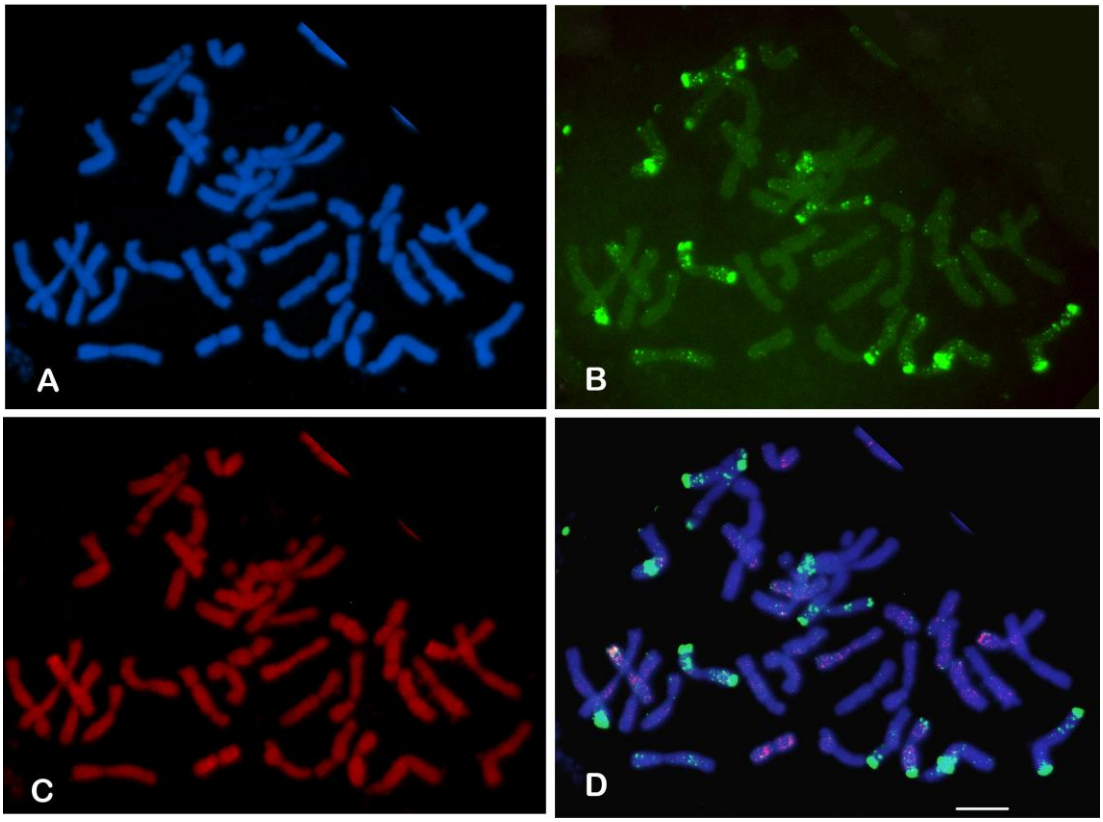


Figure 3.4: Root-tip metaphase chromosomes of the reference susceptible line Millennium (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) Hybridization pattern of the dpTa1 DNA sequence labelled with digoxygenin 11-dUTP (detected in green) that hybridize preferentially to the D-genome chromosomes. (C) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with biotin 16-dUTP (detected in red). The genomic *in situ* hybridization could not detect any *Th. intermedium*-origin chromosome segments. (D) Overlay of A, B and C images. Bar represents 10µm.

Population-I

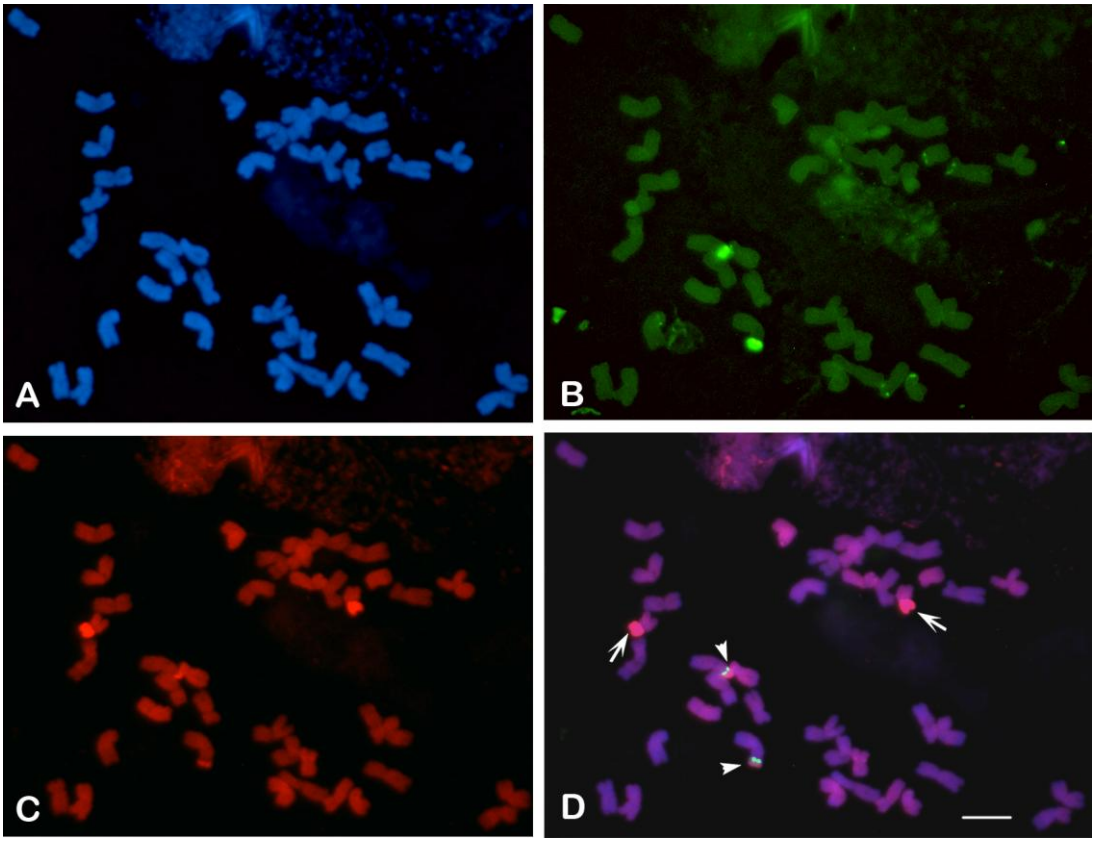


Figure 3.5: Root-tip metaphase chromosomes of the WSMV resistant-line N02Y5018 (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) Hybridization pattern of the pTa794 clone labelled with digoxygenin 11-dUTP (detected in green) showing the physical location of 5S rDNA sites in wheat. (C) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with biotin 16-dUTP (detected in red). The genomic *in situ* hybridization allows the detection of *Th. intermedium*-origin chromosome segments. (D) Overlay of A, B and C images, indicating alien chromosomal arm (arrows) and small secondary segments (arrows head) present above the 5S rDNA sites. Bar represents 10µm.

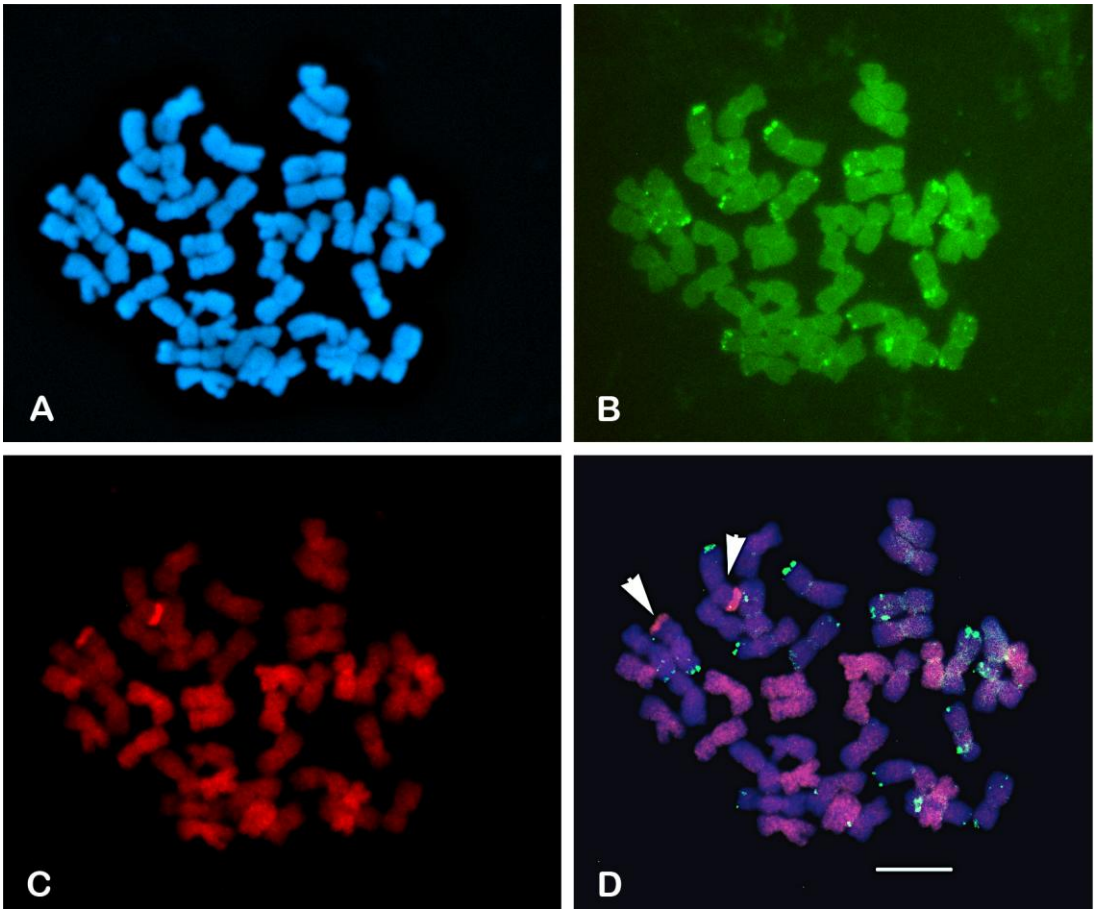


Figure 3.6: Root-tip metaphase chromosomes of the WSMV susceptible-line N02Y5019 (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) Hybridization pattern of the pSc119.2 DNA sequence labelled with digoxygenin 11-dUTP (detected in green) hybridizing to B and some A-genome chromosomes. (C) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with biotin 16-dUTP (detected in red). The genomic *in situ* hybridization allows the detection of *Th. intermedium*-origin chromosome segments. Weak hybridization signals on all D-genome chromosomes show the affinity of *Th. intermedium* to D-genome of wheat. (D) Overlay of A, B and C images, alien chromosomal segments are indicated by arrows head. Bar represents 10µm.

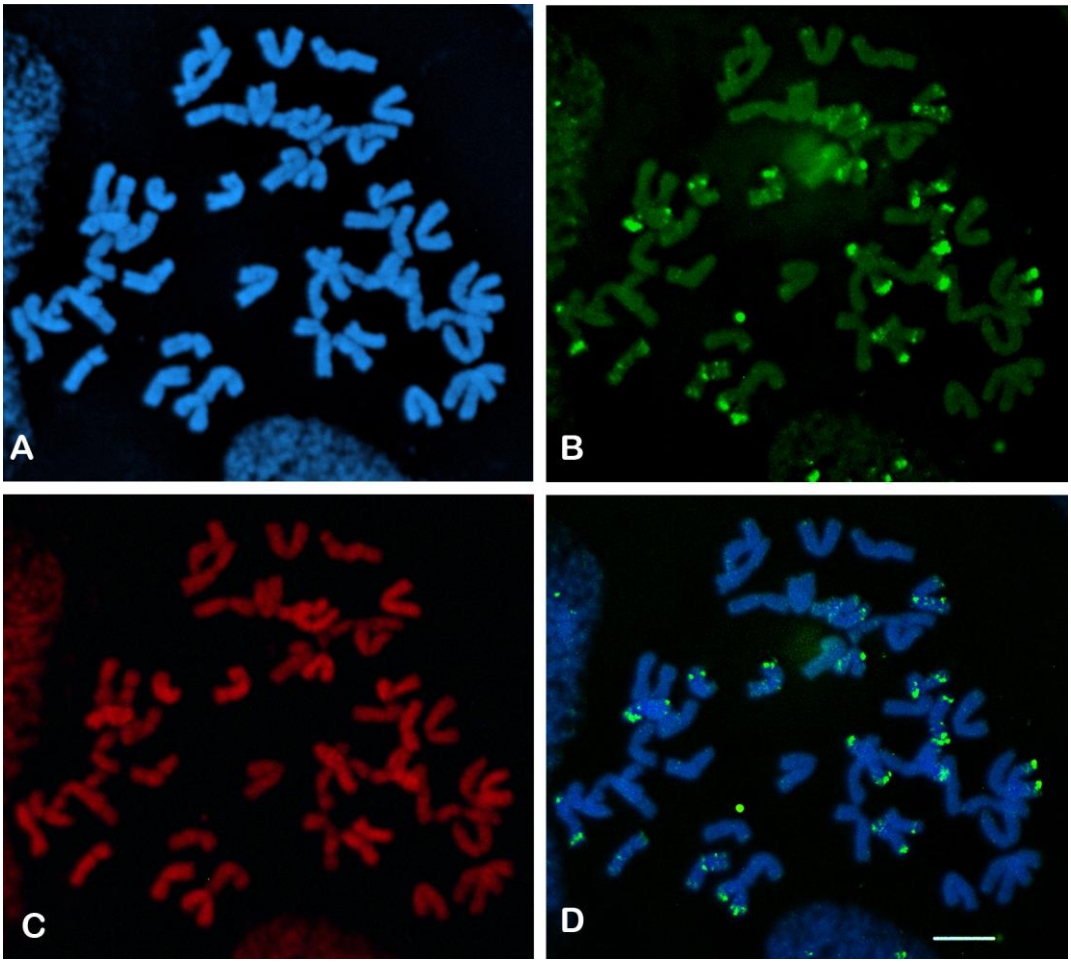


Figure 3.7: Root-tip metaphase chromosomes of the WSMV susceptible-line N02Y5021 (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) Hybridization pattern of the dpTa1 DNA sequence labelled with digoxygenin 11-dUTP (detected in green) that hybridize preferentially to the D-genome chromosomes. (C) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with biotin 16-dUTP (detected in red). The genomic *in situ* hybridization could not detect any *Th. intermedium*-origin chromosome segments. (D) Overlay of A, B and C images. Bar represents 10µm.

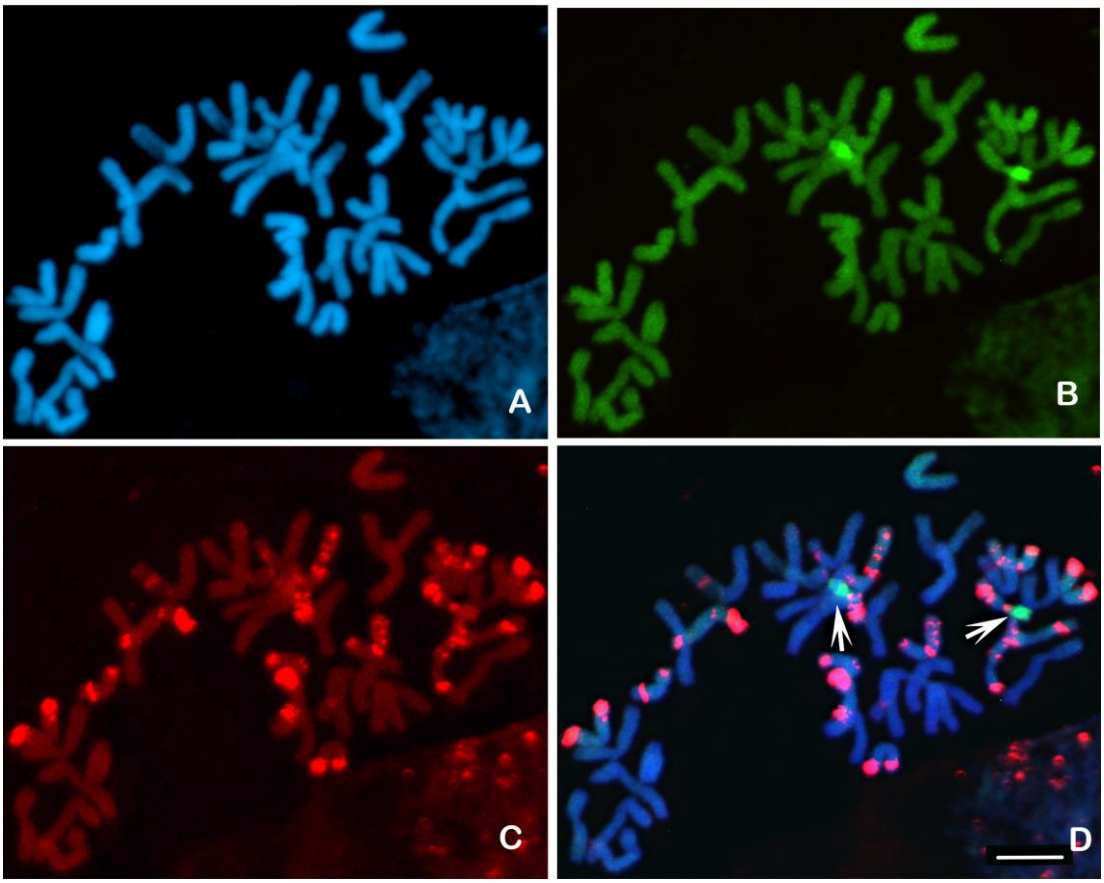


Figure 3.8: Root-tip metaphase chromosomes of the WSMV resistant-line N02Y5025 (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with digoxygenin 11-dUTP (detected in green). The genomic *in situ* hybridization allows the detection of *Th. intermedium*-origin chromosome segments. (C) Hybridization pattern of the dpTa1 DNA sequence labelled with biotin 16-dUTP (detected in red) that hybridize preferentially to the D-genome chromosomes. (D) Overlay of A, B and C images, alien chromosomal arm are indicated by arrows. Bar represents 10µm.

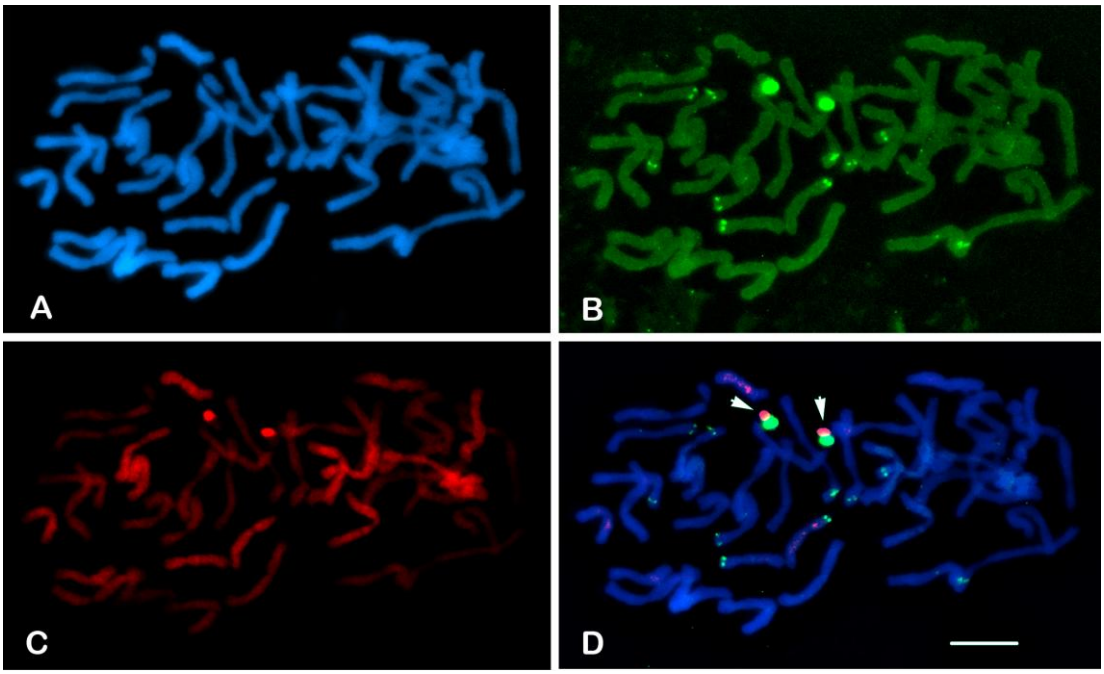


Figure 3.9: Root-tip metaphase chromosomes of the WSMV resistant-line N02Y5003 (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) Hybridization pattern of the pTa794 clone labelled with digoxygenin 11-dUTP (detected in green) showing the physical location of 5S rDNA sites in wheat. (C) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with biotin 16-dUTP (detected in red). The genomic *in situ* hybridization allows the detection of *Th. intermedium*-origin chromosome segments. (D) Overlay of A, B and C images, indicating alien chromosomal segments (arrows head) present above the 5S rDNA sites. Bar represents 10μm.

Population-II

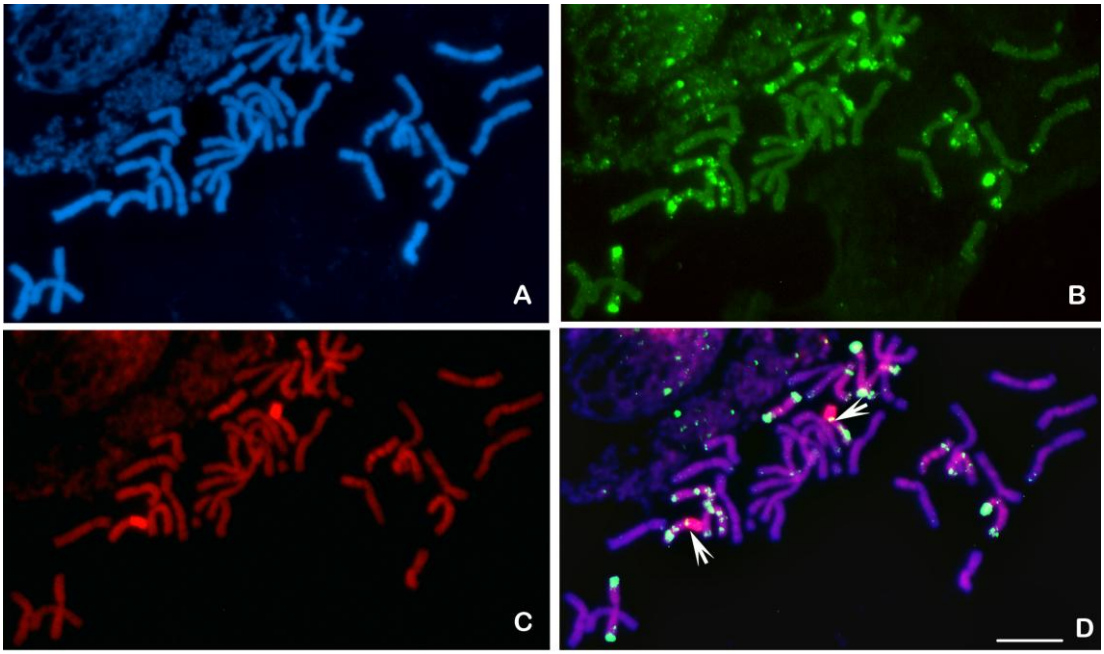


Figure 3.10: Root-tip metaphase chromosomes of the WSMV resistant-line N02Y5057 (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) Hybridization pattern of the dpTa1 DNA sequence labelled with digoxygenin 11-dUTP (detected in green) that hybridize preferentially to the D-genome chromosomes. (C) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with biotin 16-dUTP (detected in red). The genomic *in situ* hybridization allows the detection of *Th. intermedium*-origin chromosome segments. (D) Overlay of A, B and C images, alien chromosomal arms with centromeric dpTa1 sites are indicated by arrows. Bar represents 10µm.

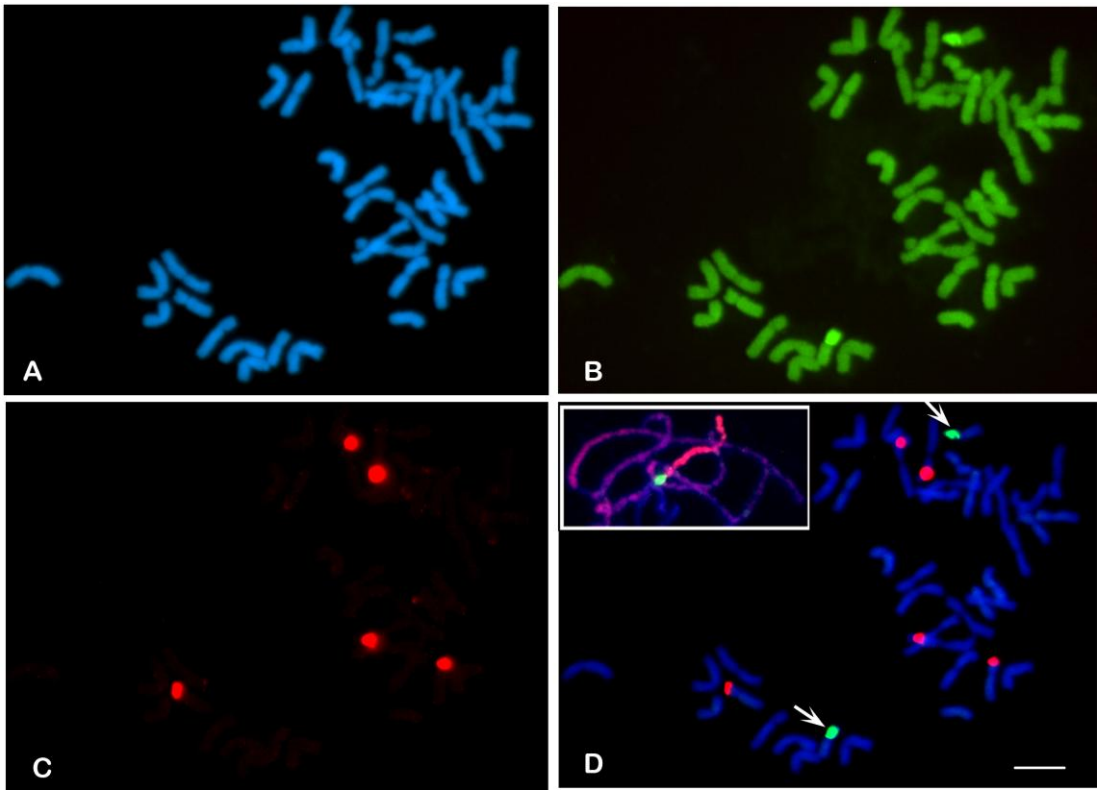


Figure 3.11: Root-tip metaphase chromosomes of the WSMV resistant-line N02Y5075 (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with digoxygenin 11-dUTP (detected in green). The genomic *in situ* hybridization allows the detection of *Th. intermedium*-origin chromosome segments. (C) Hybridization pattern of the pTa71 clone labelled with biotin 16-dUTP (detected in red) showing the physical location of 45S rDNA sites in wheat. (D) Overlay of A, B and C images, alien chromosomal arm are indicated by arrows. The insert is a part of meiotic pachytene of the same line showing a fully paired *Th. intermedium* arm (red) with a large Afa site near the centromere (green). Bar represents 10µm.

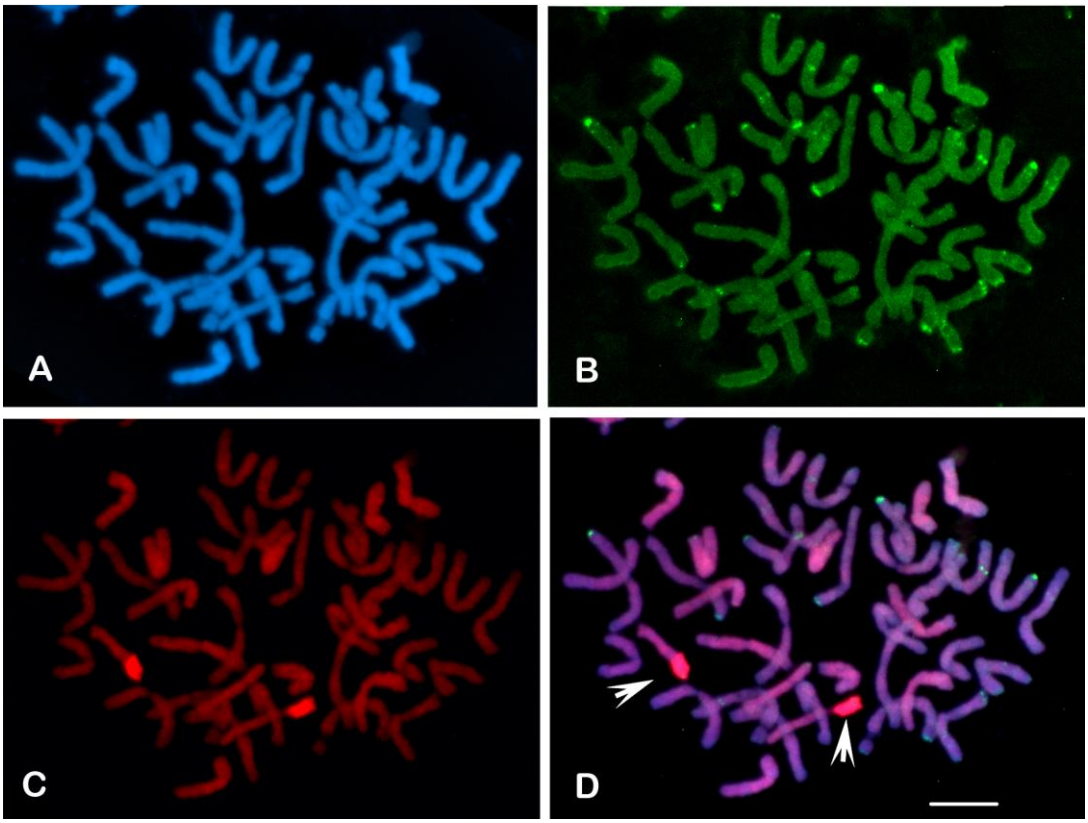


Figure 3.12: Root-tip metaphase chromosomes of the WSMV resistant-line N02Y5078 (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) Hybridization pattern of the pSc119.2 DNA sequence labelled with digoxygenin 11-dUTP (detected in green) hybridizing to B and some A-genome chromosomes. (C) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with biotin 16-dUTP (detected in red). The genomic *in situ* hybridization allows the detection of *Th. intermedium*-origin chromosome segments. (D) Overlay of A, B and C images, alien chromosomal arm are indicated by arrows. Bar represents 10μm.

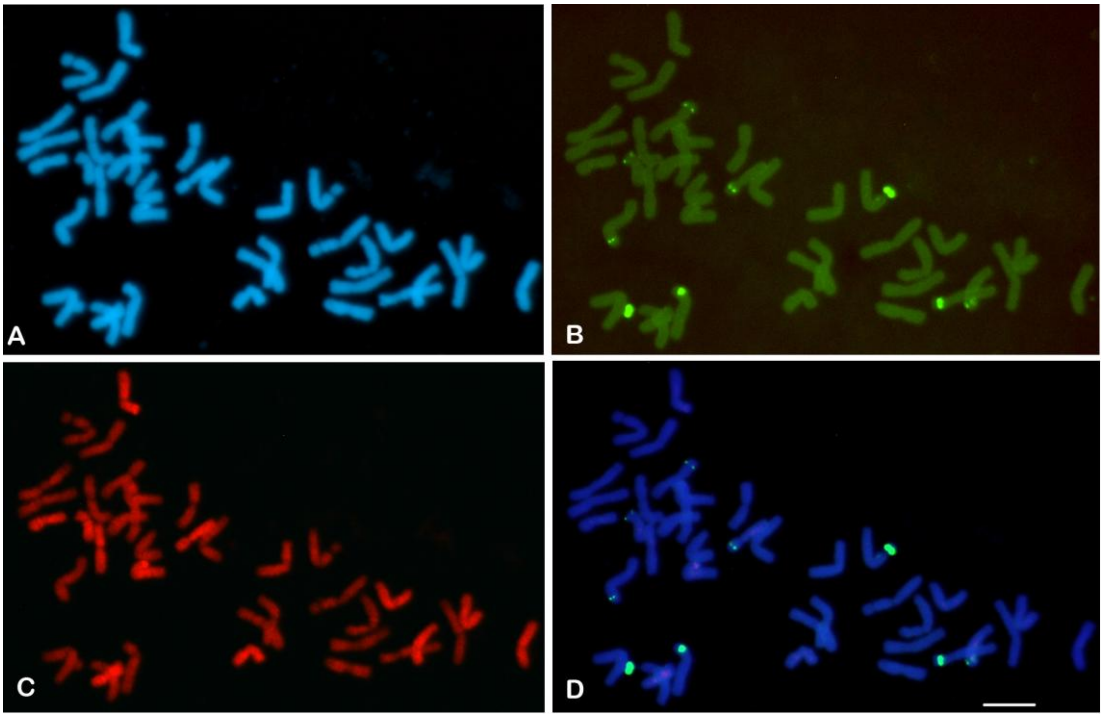


Figure 3.13: Root-tip metaphase chromosomes of the WSMV susceptible-line N02Y5082 (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) Hybridization pattern of the pTa794 clone labelled with digoxygenin 11-dUTP (detected in green) showing the physical location of 5S rDNA sites in wheat. (C) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with biotin 16-dUTP (detected in red). The genomic *in situ* hybridization could not detect any *Th. intermedium*-origin chromosome segments. (D) Overlay of A, B and C images. Bar represents 10μm.

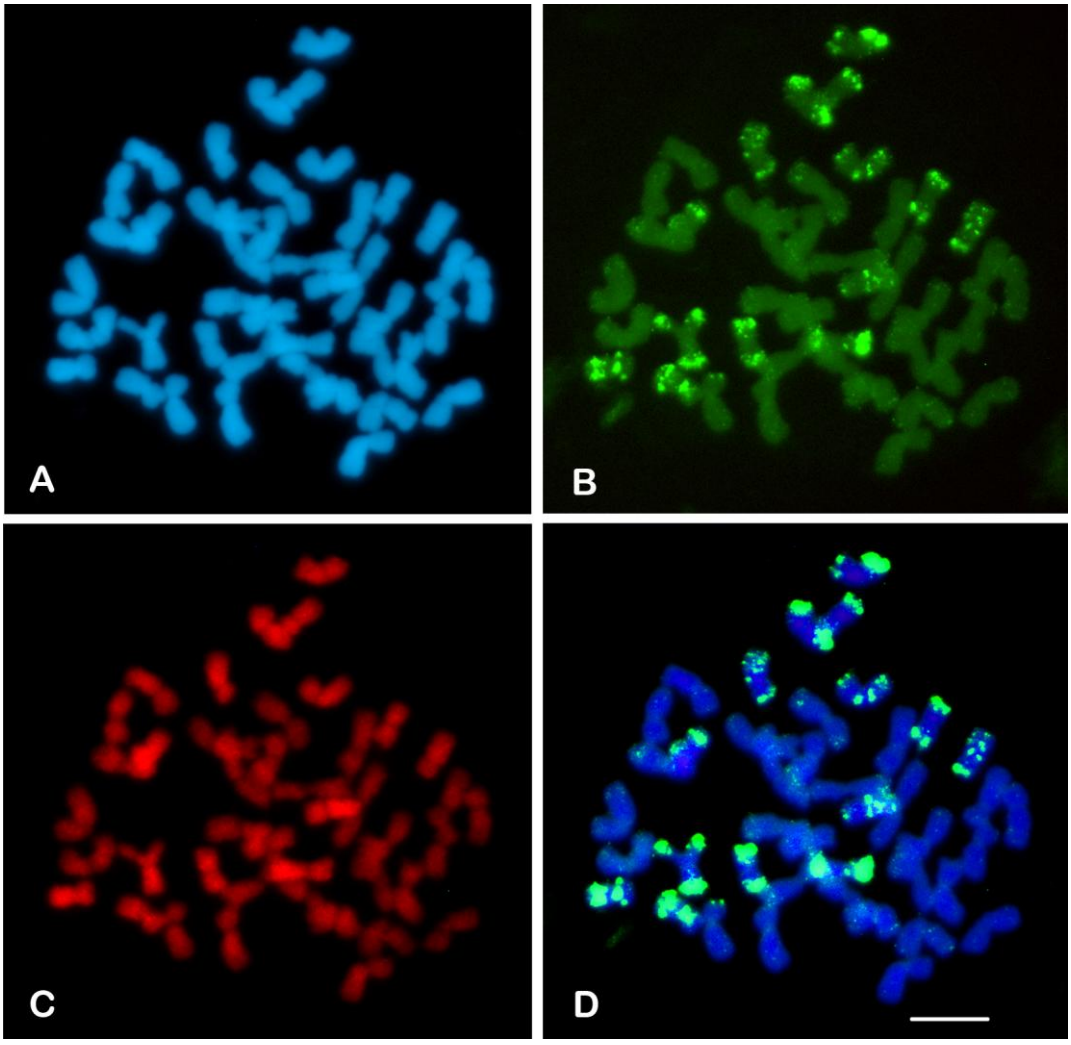


Figure 3.14: Root-tip metaphase chromosomes of the WSMV susceptible-line N02Y5096 (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) Hybridization pattern of the dpTa1 DNA sequence labelled with digoxygenin 11-dUTP (detected in green) that hybridize preferentially to the D-genome chromosomes. (C) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with biotin 16-dUTP (detected in red). The genomic *in situ* hybridization could not detect any *Th. intermedium*-origin chromosome segments. (D) Overlay of A, B and C images. Bar represents 10µm.

Population-III

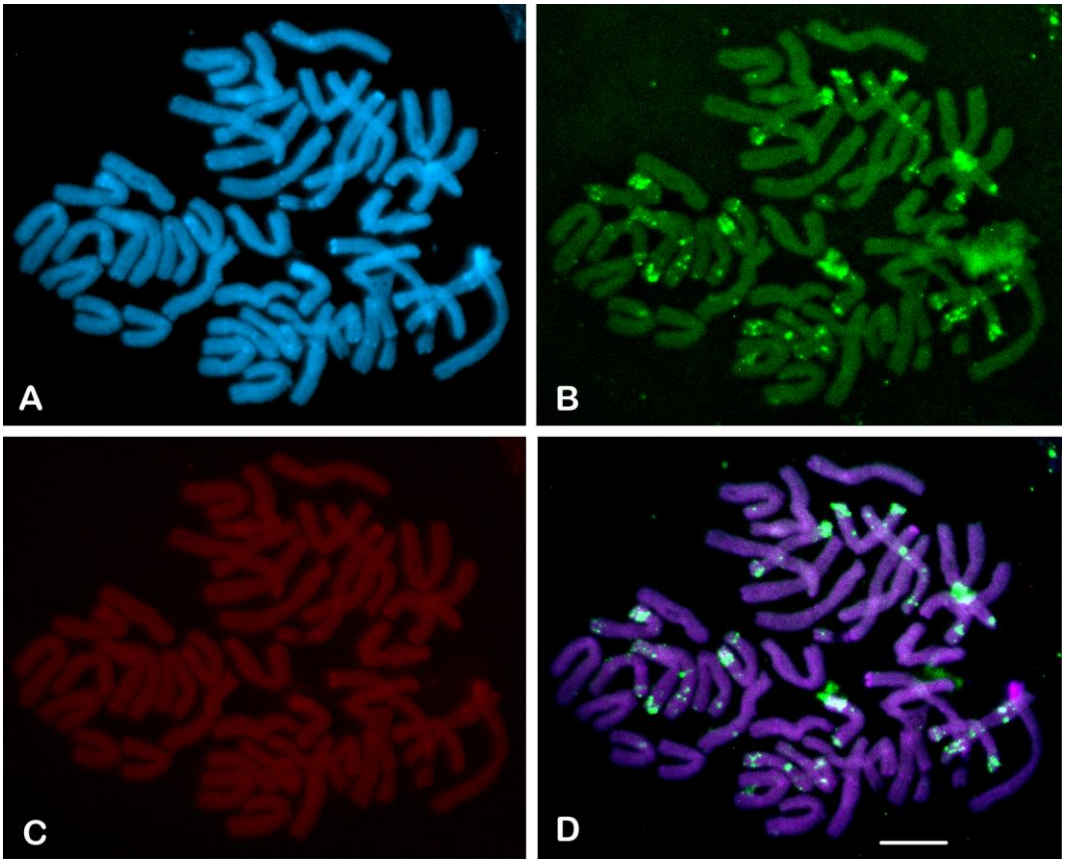


Figure 3.15: Root-tip metaphase chromosomes of the WSMV susceptible-lineN02Y5105 (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) Hybridization pattern of the dpTa1 DNA sequence labelled with digoxygenin 11-dUTP (detected in green) that hybridize preferentially to the D-genome chromosomes. (C) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with biotin 16-dUTP (detected in red). The genomic *in situ* hybridization could not detect any *Th. intermedium*-origin chromosome segments. (D) Overlay of A, B and C images. Bar represents 10µm.

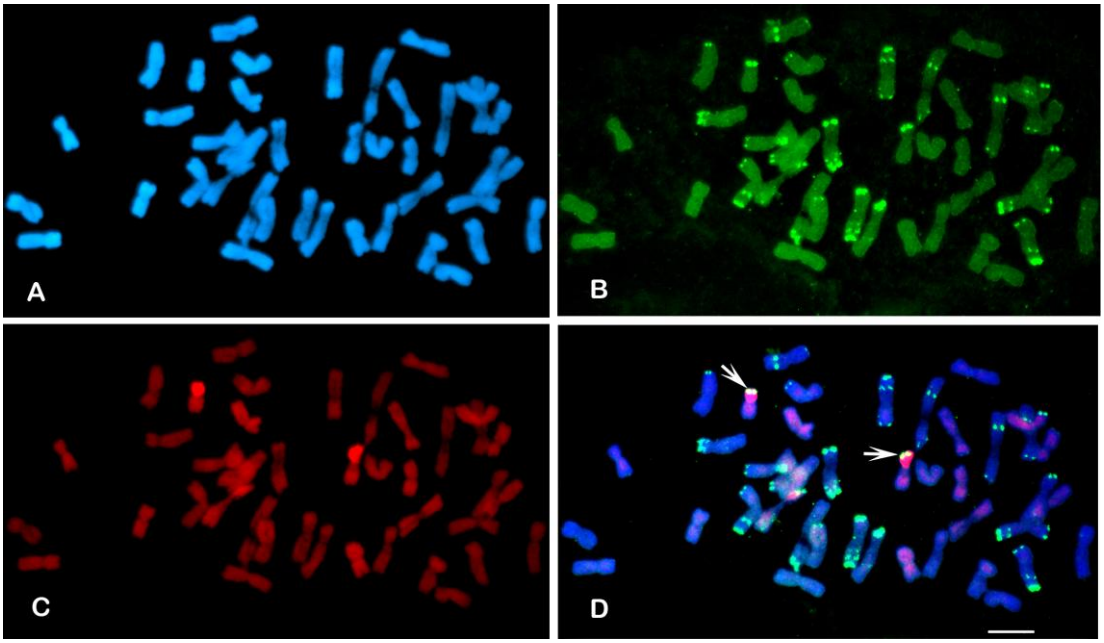


Figure 3.16: Root-tip metaphase chromosomes of the WSMV resistant-line N02Y5106 (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) Hybridization pattern of the pSc119.2 DNA sequence labelled with digoxygenin 11-dUTP (detected in green) hybridizing to B and some A-genome chromosomes. (C) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with biotin 16-dUTP (detected in red). The genomic *in situ* hybridization allows the detection of *Th. intermedium*-origin chromosome segments. (D) Overlay of A, B and C images, alien chromosomal arms with the telomeric pSc119.2 sites are indicated by arrow. Bar represents 10µm.

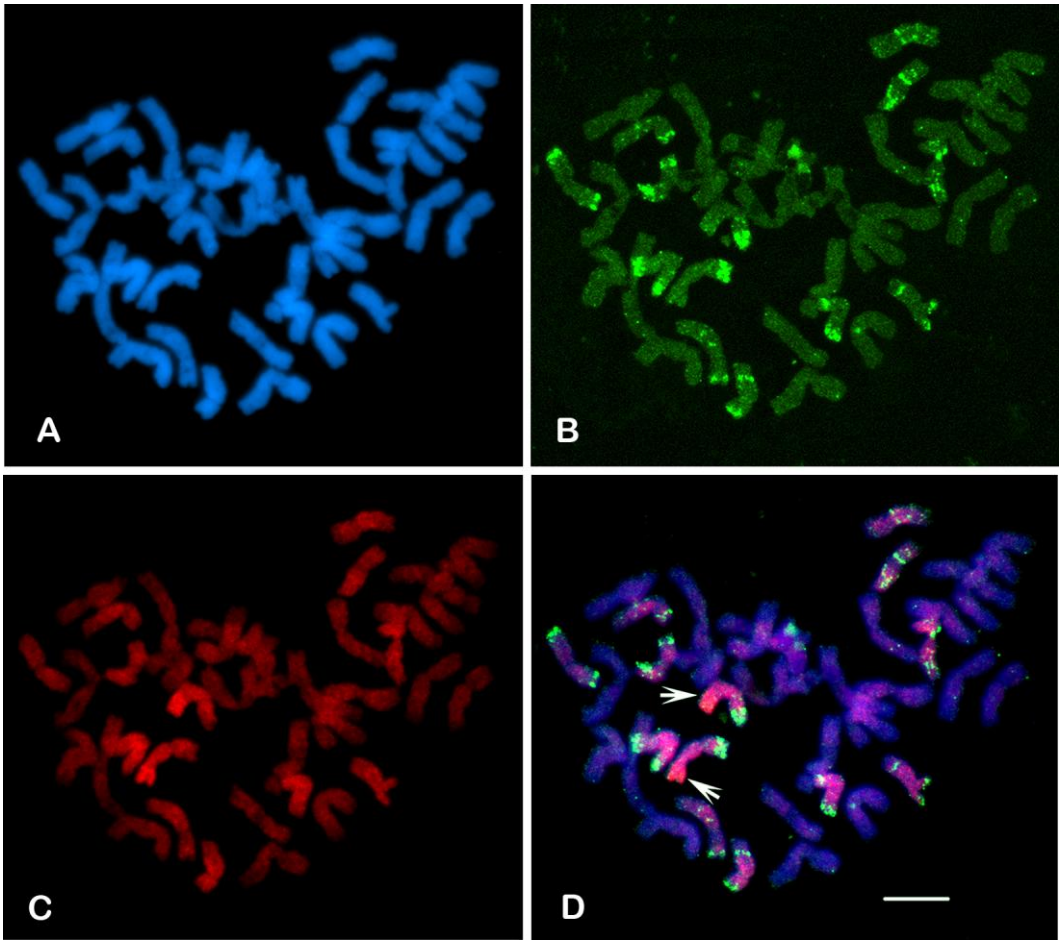


Figure 3.17A: Root-tip metaphase chromosomes of the WSMV resistant-line N02Y5109 (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) Hybridization pattern of the dpTa1 DNA sequence labelled with digoxygenin 11-dUTP (detected in green) that hybridize preferentially to the D-genome chromosomes. (C) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with biotin 16-dUTP (detected in red). The genomic *in situ* hybridization allows the detection of *Th. intermedium*-origin chromosome segments. (D) Overlay of A, B and C images, alien chromosomal segments are indicated by arrows. Bar represents 10μm.

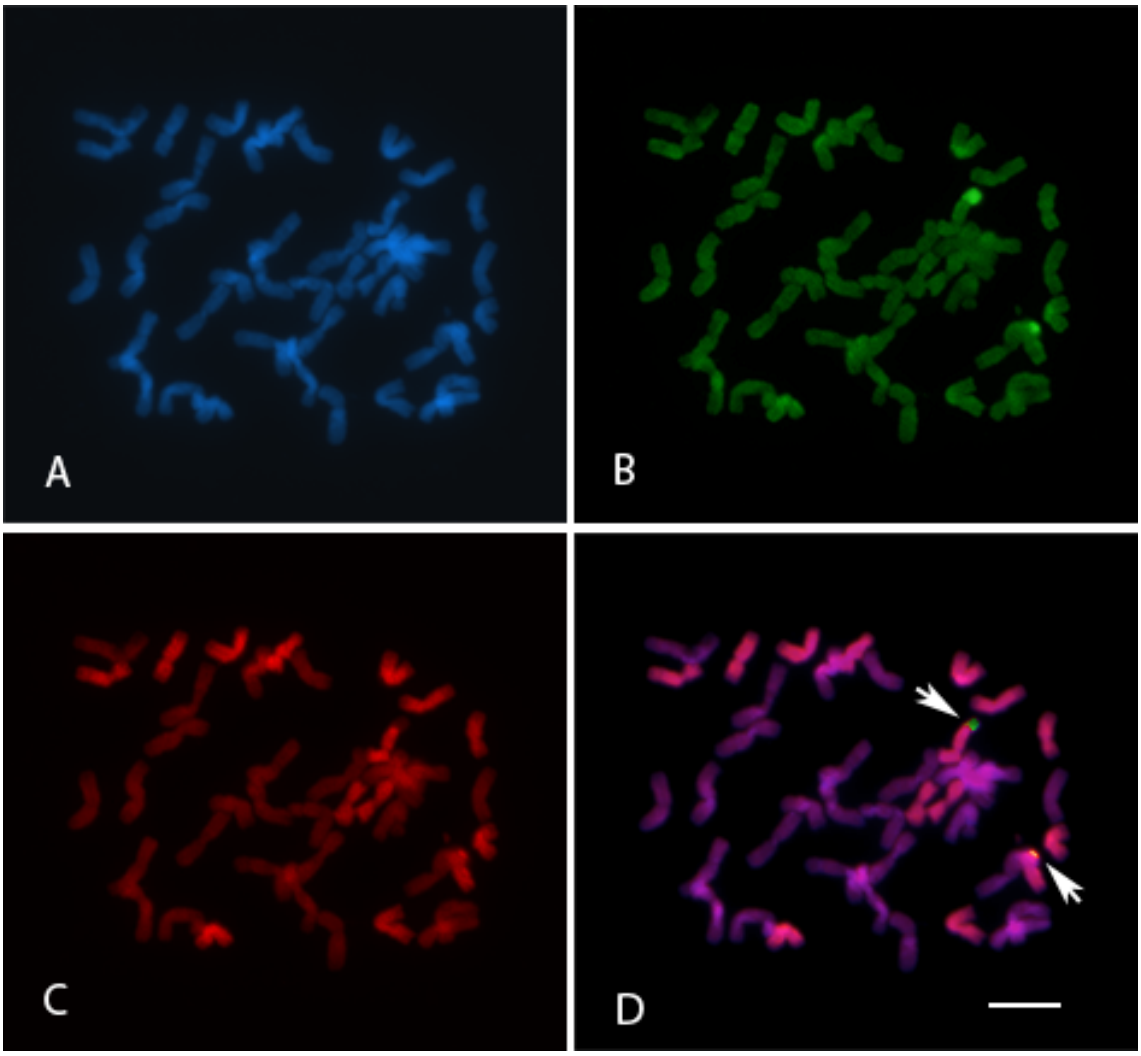


Figure 3.17B: Root-tip metaphase chromosomes of the WSMV resistant-line N02Y5109 (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with digoxygenin 11-dUTP (detected in green) that allows the detection of *Th. intermedium*-origin chromosome segments (C) *In situ* hybridization of the total genomic DNA from *Ae. tauschii* labelled with biotin 16-dUTP (detected in red) allows the detection of D-genome chromosome of wheat. (D) Overlay of A, B and C images, alien chromosomal segments are indicated by arrows. Bar represents 10μm.

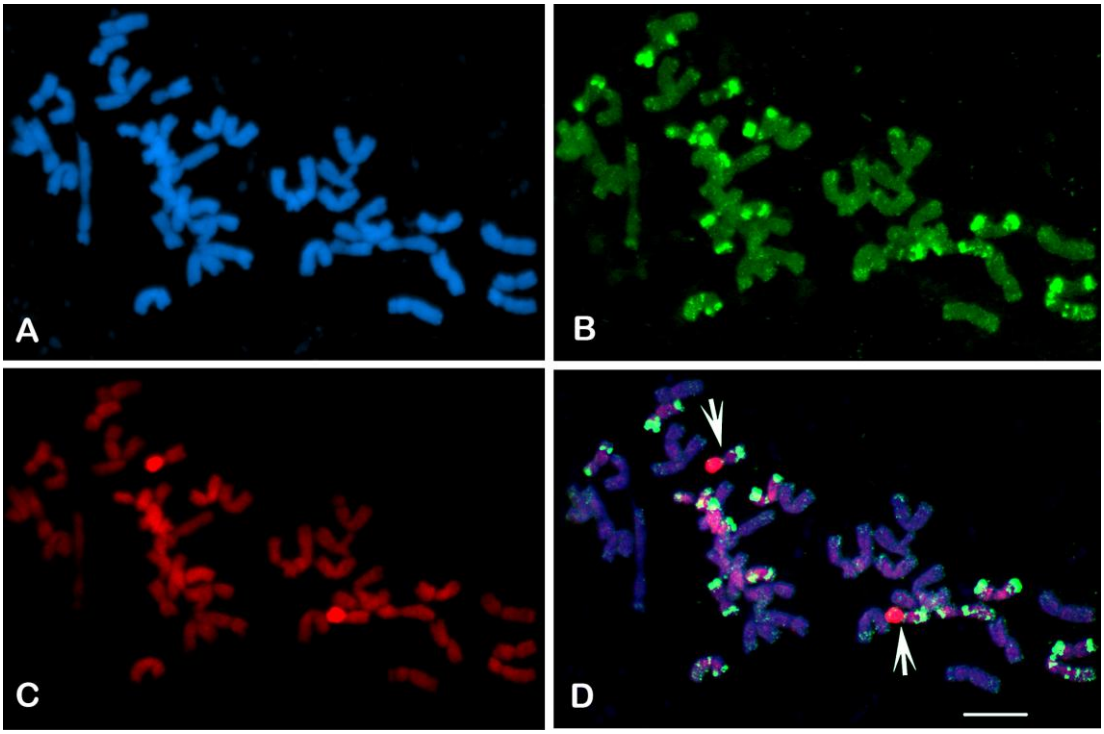


Figure 3.18: Root-tip metaphase chromosomes of the WSMV resistant-line N02Y5117 or Mace (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) Hybridization pattern of the dpTa1 DNA sequence labelled with digoxygenin 11-dUTP (detected in green) that hybridize preferentially to the D-genome chromosomes. (C) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with biotin 16-dUTP (detected in red). The genomic *in situ* hybridization allows the detection of *Th. intermedium*-origin chromosome segments. (D) Overlay of A, B and C images, alien chromosomal arm are indicated by arrows. Bar represents 10µm.

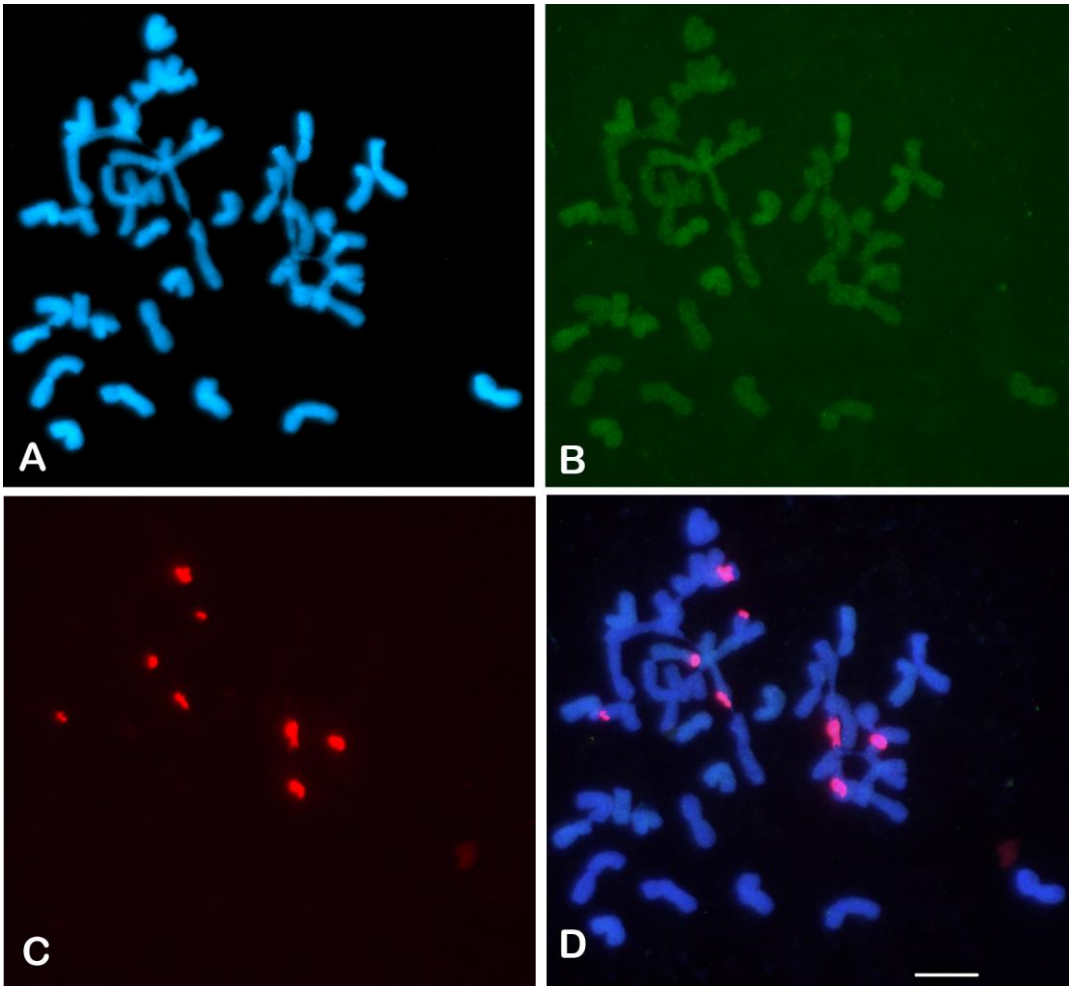


Figure 3.19: Root-tip metaphase chromosomes of the WSMV susceptible-line N02Y5121 (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) Hybridization pattern of the pT71 clone labelled with digoxygenin 11-dUTP (detected in green) showing the physical location of major 45S rDNA sites in wheat. (C) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with biotin 16-dUTP (detected in red). The genomic *in situ* hybridization could not detect any *Th. intermedium*-origin chromosome segments. (D) Overlay of A, B and C images. Bar represents 10μm.

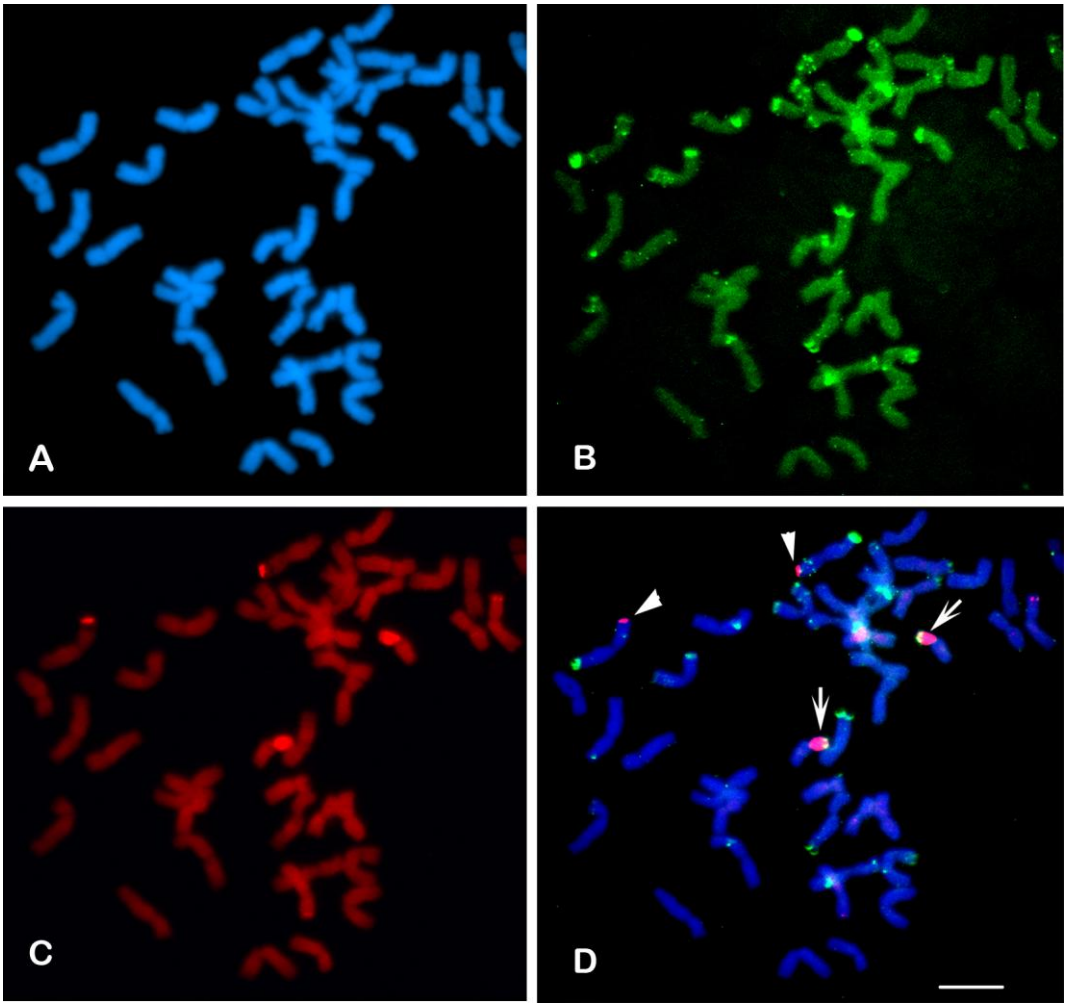


Figure 3.20: Root-tip metaphase chromosomes of the WSMV resistant-line N02Y5149 (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) Hybridization pattern of the pSc119.2 DNA sequence labelled with digoxygenin 11-dUTP (detected in green) hybridizing to B and some A-genome chromosomes. (C) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with biotin 16-dUTP (detected in red). The genomic *in situ* hybridization allows the detection of *Th. intermedium*-origin chromosome segments. (D) Overlay of A, B and C images, indicating to alien chromosomal arm with telomeric pSc119.2 sites (arrows) and small secondary segments (arrows head). Bar represents 10µm.

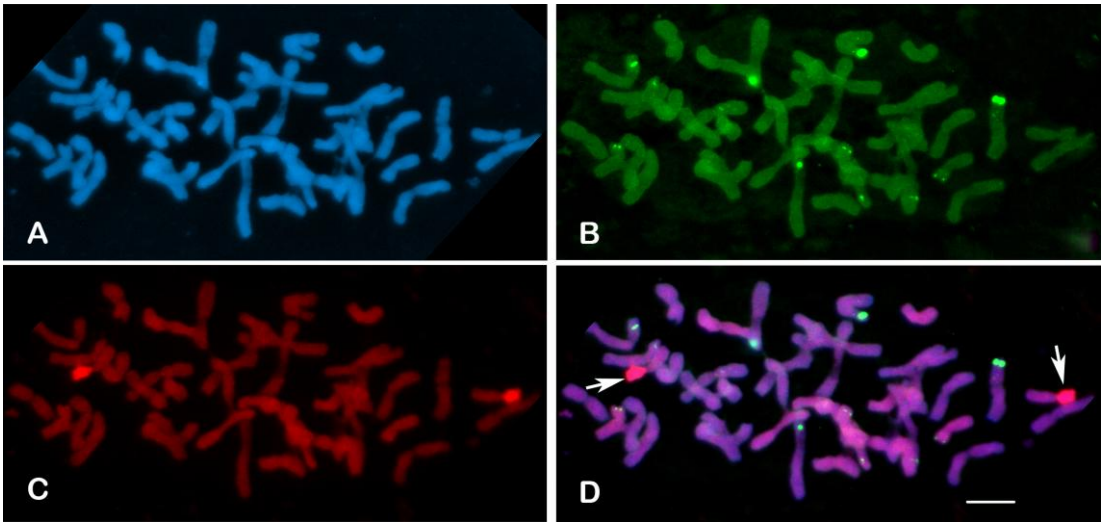


Figure 3.21: Root-tip metaphase chromosomes of the WSMV resistant-line N02Y5154 (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) Hybridization pattern of the pTa794 clone labelled with digoxygenin 11-dUTP (detected in green) showing the physical location of 5S rDNA sites in wheat. (C) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with biotin 16-dUTP (detected in red). The genomic *in situ* hybridization allows the detection of *Th. intermedium*-origin chromosome segments. (D) Overlay of A, B and C images, alien chromosomal arms are indicated by arrows. Bar represents 10µm.

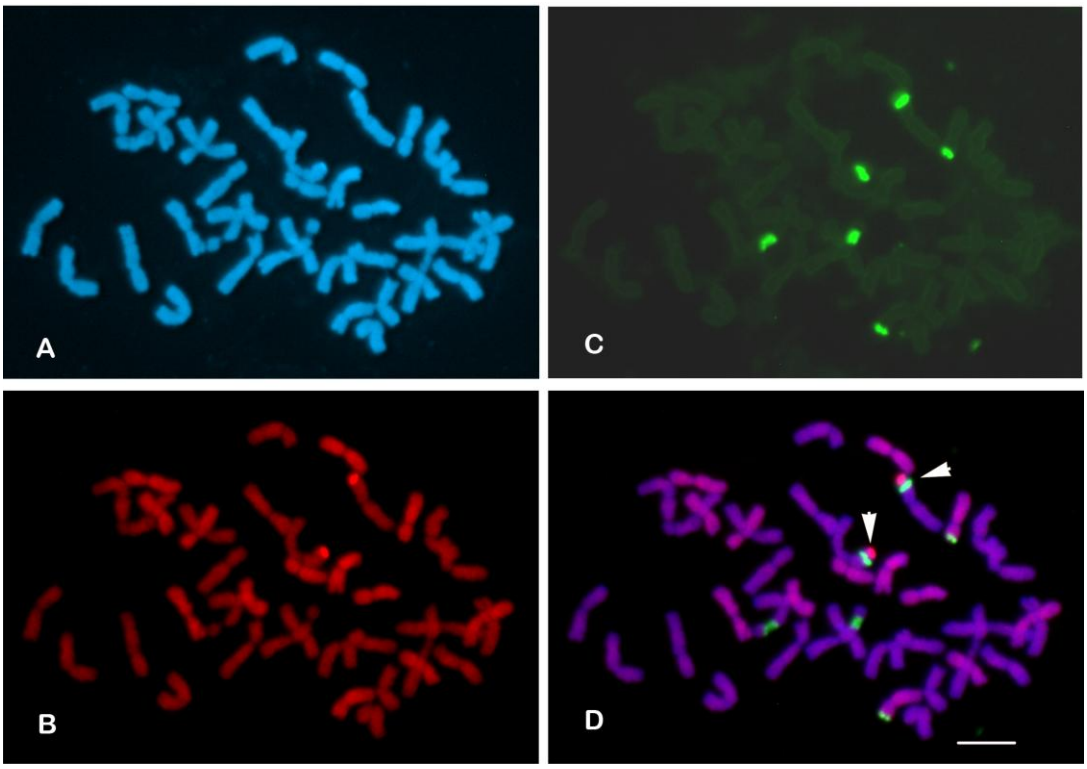


Figure 3.22: Root-tip metaphase chromosomes of the WSMV susceptible-line N02Y5156 (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) Hybridization pattern of the pTa71 clone labelled with digoxygenin 11-dUTP (detected in green) showing the physical location of 45S rDNA sites in wheat. (C) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with biotin 16-dUTP (detected in red). The genomic *in situ* hybridization allows the detection of *Th. intermedium*-origin chromosome segments. (D) Overlay of A, B and C images, alien chromosomal segments are indicated by arrows head. Bar represents 10µm.

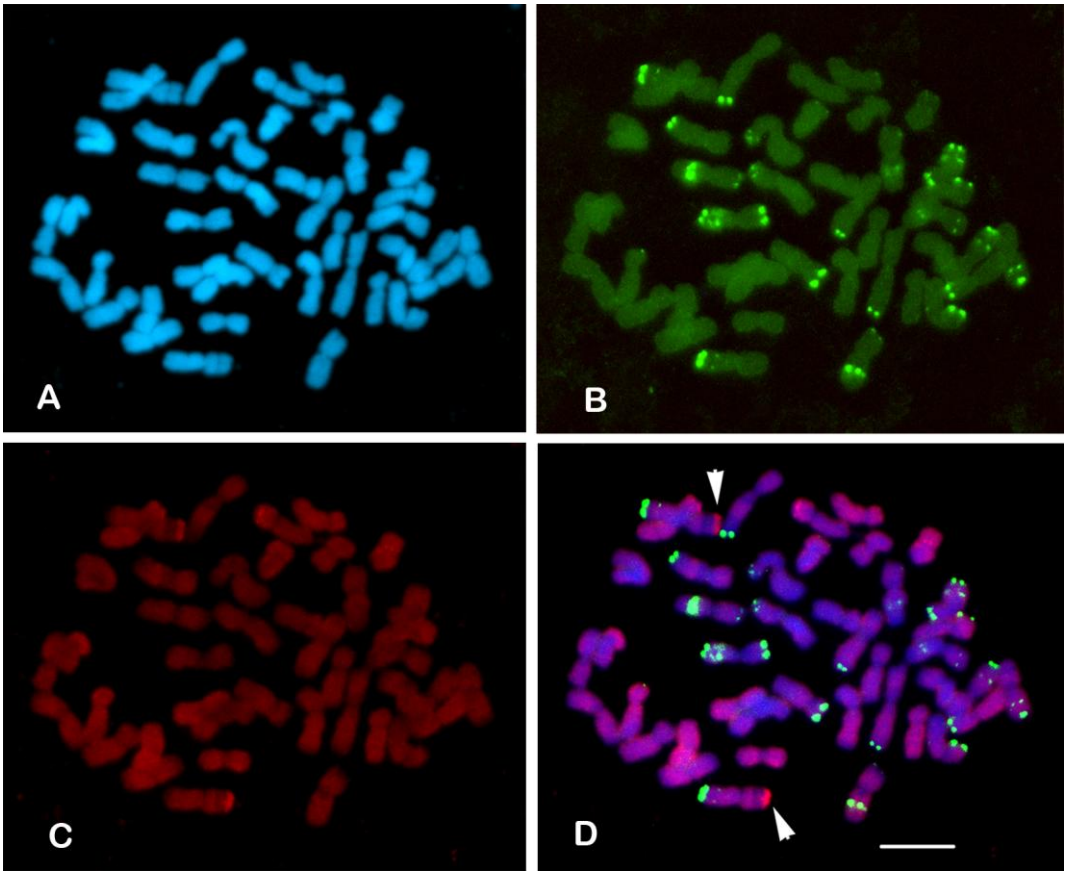


Figure 3.23: Root-tip metaphase chromosomes of the WSMV susceptible-line N02Y5163 (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) Hybridization pattern of the pSc119.2 DNA sequence labelled with digoxygenin 11-dUTP (detected in green) hybridizing to B and some A-genome chromosomes. (C) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with biotin 16-dUTP (detected in red). The genomic *in situ* hybridization allows the detection of *Th. intermedium*-origin chromosome segments. (D) Overlay of A, B and C images, alien chromosomal segments are indicated by arrows head. Bar represents 10µm.

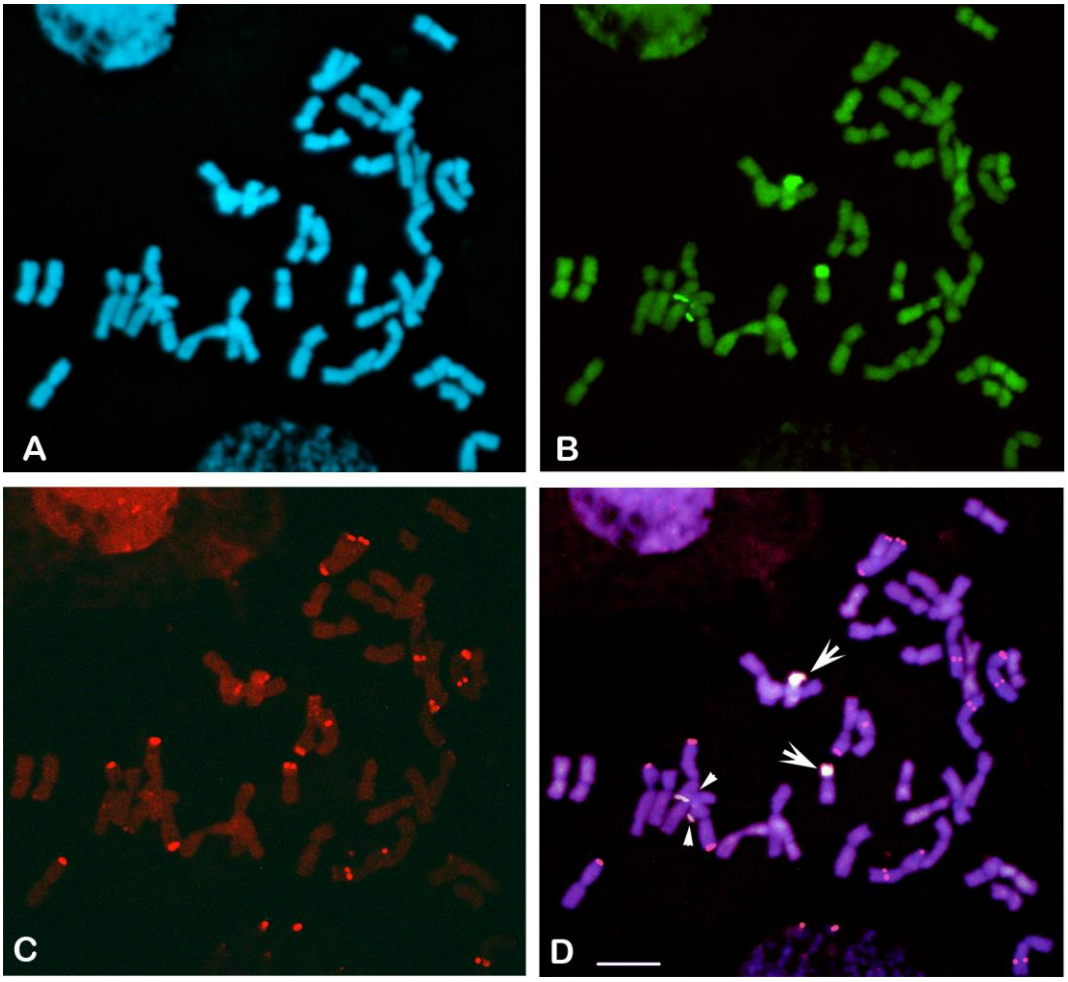


Figure 3.24: Root-tip metaphase chromosomes of the WSMV resistant-line N02Y2016 (2n=42) after fluorescent *in situ* hybridization (FISH). (A) Wheat chromosomes fluoresces blue with DAPI. (B) *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with digoxygenin 11-dUTP (detected in green). The genomic *in situ* hybridization allows the detection of *Th. intermedium*-origin chromosome segments. (C) Hybridization pattern of the pSc119.2 DNA sequence labelled with biotin 16-dUTP (detected in red) hybridizing to B and some A-genome chromosomes. (D) Overlay of A, B and C images, indicating to alien chromosomal arm with telomeric pSc119.2 sites (arrows) and small secondary segments (arrows head). Bar represents 10µm.

3.4.3 Size of the alien fragments

Most resistant lines have *Th. intermedium* chromatin replacing the entire 4DS arm of wheat chromosome (Figure 3.26 and Table 3.1), and the physical BP lies in the centromeric regions. However, small alien fragments of variable sizes are seen in both R and S-lines (Figure 3.20, Figure 3.22). Sometime these fragments are additional to the 4D fragments in R-lines (Figure 3.5). The *in situ* mapping clearly showed in all 1B recombinants, the wheat-alien physical BPs involved the distal region of wheat chromosome (Figure 3.4, Figure 3.5). GISH revealed the sizes of the 1BS *Th. intermedium* segments in line N02Y5018, N02Y5019, N02Y5149, N02Y5156, N02Y5163, and N02Y2016 that span around 22±3.8%, 22.4±4.6%, 22.5±6%, 20±8%, 18.5±2.5%, and 21±2.9% respectively of the recombinant arm length (Table 3.2).

The *Th. intermedium* derived segment in line N02Y5003 is 28.3±4.9% of the recombinant arm. This is the largest 1B fragments found in any R or S-lines (Table 3.2) and is followed by N02Y5149 (22.5±6%). Thus potentially the larger fragment carries more *Th. intermedium* genes. Several dominant and co-dominant PCR markers also reconfirmed the FISH results (Chapter IV). Field resistance screen also show line N02Y5003 is moderately resistant compared to 4D resistant lines. However, line N02Y5149 that carries both 4D and 1B *Th. intermedium* chromatin is highly resistant compared to any other lines that carry 4D or 1B alone (Figure 3.1A&B). Therefore, it is very probable that the resistance in wheat lines N02Y5018, and N02Y2016 may be conditioned by the 4D alien arm alone rather than the 1BS. On the other hand, line N02Y5109 has *Th. intermedium* chromatin substituting around 42.9±2.5% of the 3D long arm. The maximum possible size of the proximal wheat arm was estimated to be 57%. Thus in all recombinant, the wheat-alien chromatin exchange involved the distal ends of wheat chromosomes (Table 3.2).

3.4.4 Confirmation of the recombinant chromosomes

3.4.4.1 Chromosome 1B

GISH revealed small *Th. intermedium* fragments at the distal end of 1BS wheat chromosome in several R and S-lines (Table 3.1). This recombinant wheat chromosome has no dpTa1 sites but showed conspicuous sites of both 5S and 45S-rDNA, proximal to the mapped alien chromatin (Figure 3.25). In addition, two pSc119.2 sites are evident

on the long arm of this chromosome, while the third on the short arm. This recombinant chromosome was confirmed as 1B, and the translocation as 1Ai#1S-1BS.1BL (Fig. 3.25).

3.4.4.2 Chromosome 3D

In situ hybridization was carried out to ascertain the presence of alien chromatin in the WSMV-resistant line N02Y5109. A pair of wheat chromosomes showed *Th. intermedium* chromatin on the long arm distally (Figure 3.17A). This recombinant chromosome was confined to the D-genome of wheat, by labelled *Ae. tauschii* genomic DNA probe (Figure 3.17B). This fragment is larger in size than the fragments of 1B recombinants (Table 3.2). Both 5S and 45S-rDNA do not show hybridization to this chromosome, and a single pSc119.2 site is seen on the small arm in some metaphases. Furthermore, three dpTa1 sites are seen on the small arm of wheat origin, the two distal among these sites are the most prominent among the D-genome chromosomes (Figure 3.17). The observed banding pattern, when compared with the standard karyotype (Mukai *et al.,* 1993) confirms this recombinant chromosome as 3D (Figure 3.25).

3.4.4.3 Chromosome 4D

Dual colour FISH using *Th. intermedium* genomic and other highly repeated DNA probes (section 2.2.9) allowed the identification of a small arm of *Th. intermedium* translocated onto the chromosome 4D of wheat (for example see Figure 3.10). The recombinant chromosomal arm of wheat origin is characterised by its dpTa1 banding pattern, and neither 5S, 45S-rDNA or pSc119.2 show hybridization to the recombinant chromosome. It shows a centromeric Afa or dpTa1 site, and four other dpTa1 sites that are maintained on the long arm of this recombinant chromosome (Figure 3.26). This translocation was confirmed as 4Ai#2S.4DL as mentioned in Friebe *et al.,* (2009) for some other WSMV-resistant lines.

Table 3.2: Mean alien to wheat arm length ratios, calculated from ten randomly selected recombinant chromosomes.

Population	Line	Rec. chromosome	Final rating	% mean alien arm	% estimated wheat arm	Standard deviation
Pop-I	N02Y5018	1B	R	22.0	78.0	3.4
	N02Y5019	1B	S	22.4	77.6	4.6
	N02Y5003	1B	R	28.3	71.7	4.9
Pop-III	N02Y5109	3D	R	42.9	57.1	2.5
Pop-IV	N02Y5149	1B	R	22.5	77.5	6.04
	N02Y5156	1B	S	20.0	80.0	8.08
	N02Y5163	1B	S	18.5	81.5	2.5
	N02Y2016	1B	R	21.0	79.0	2.9

Rec. chromosome = indicating to the recombinant wheat chromosome, where alien fragments of less than the whole arm were observed.
Final rating = field evaluation response of a line to WSMV in trails (Divis *et al.*, 2006).
% mean alien arm = proportion of alien to corresponding wheat chromosomal arm in percent.
% estimated wheat arm = represent the difference of total arm length and the identified alien fragments in percent.

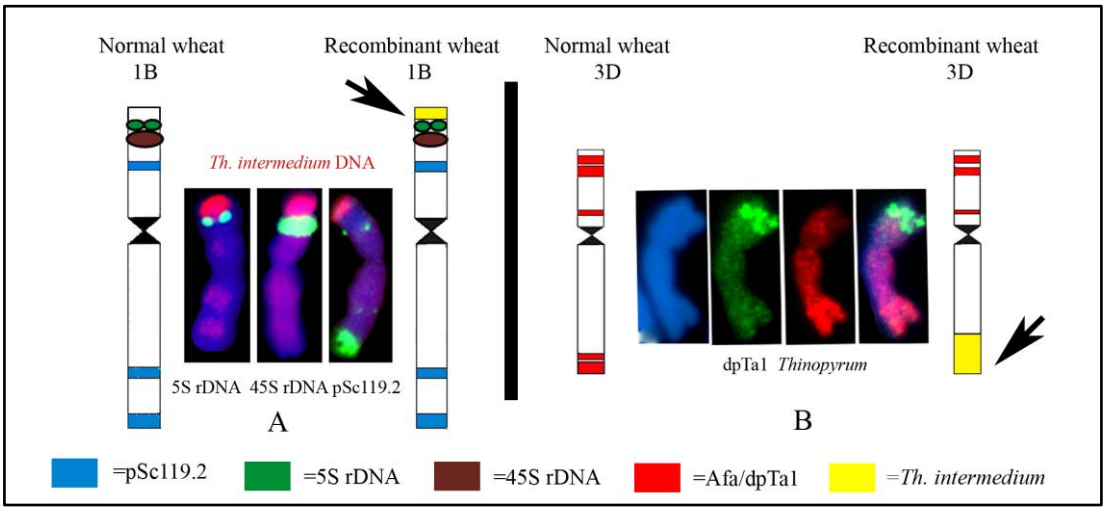


Figure 3.25: Identification and schematic representation of normal and recombinant wheat chromosome 1B and 3D. (A) Sketch of the normal wheat chromosome 1B (left) showing the unique arrangement of 5S (green) and 45S (brown) rDNA sites along with pSc119.2 (blue). The recombinant wheat chromosome 1B (right) shows identical banding pattern of 5S, 45S rDNA and pSc119.2, except the distal wheat region above the 5S and 45S of the small arm is lost due to translocation with *Th. intermedium* chromatin (yellow), indicated by arrow. (B) Sketch of the normal wheat chromosome 3D (left) showing three dpTa1 sites on the small arm and two on the long arm. The recombinant wheat chromosome 3D (right) is identified by genomic DNA from *Ae. tauschii*, the D-genome donor (not shown) and *Th. intermedium* together with dpTa1 sequence. The dpTa1 sites on the short arm of the recombinant 3D are retained, while distal two dpTa1 sites from the long arm are lost and replaced by *Th. intermedium* chromatin (yellow), indicated by arrow.

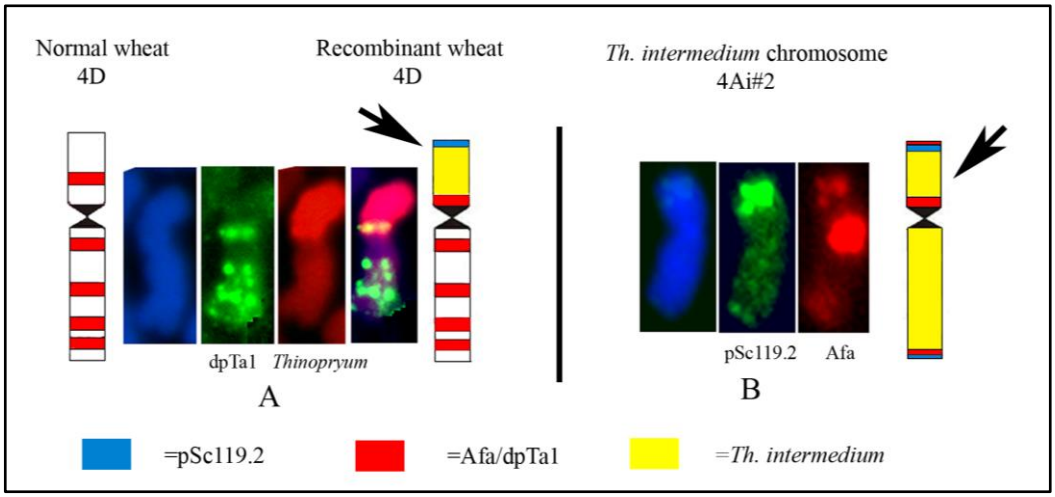


Figure 3.26: Identification and schematic representation of normal and recombinant wheat chromosome 4D and *Th. intermedium* chromosome 4Ai#2. (A) Sketch of the normal wheat chromosome 4D (left) showing a characteristic banding pattern of dpTa1 (red) along the chromosomal arms. The long wheat-origin arm in this recombinant chromosome (right) is characterized by four dpTa1 sites. While the *Th. intermedium*-origin chromosomal arm (yellow) is characterised by genomic DNA from *Th. intermedium* along with a centromeric Afa/dpTa1 (red) and telomeric pSc119.2 site (blue), indicated by arrow. (B) DAPI stained 4Ai#2 chromosome of *Th.* intermedium (left). This chromosome harbours the WSMV resistance gene(s) and the small arm of 4Ai#2 chromosomes is used as a source of resistance (arrow). This chromosome is characterised by a strong centromeric Afa/dpTa1 (red) and terminal pSc119.2 sites (blue) on the small arm. Weak signals of both Afa/dpTa1 and pSc119.2 are also seen on both arms.

3.5 Discussion

3.5.1 Characterization of novel sources for WSMV-resistance

Physical localization of chromosomal BPs along the 1BS, 3DL and 4DS arms in lines carrying the WSMV-resistance genes was investigated by means of GISH combined with highly repeated DNA sequences (section 2.2.9). Previous *in situ* hybridization and C-banding analysis of wheat-*Th. intermedium* hybrid lines mapped the WSMV-resistance gene to *Th. intermedium* chromosome group-7 long arm, $2J^S$ and more recently to 4Ai#2 small arm. Lines incorporating 4Ai#2 segments are currently used in cultivar improvement as the other two sources previously lack the compensating translocation (Friebe al. 1996, Chen *et al.,* 1999a, Friebe al. 2009). However, Liu *et al.,* (2011) have recently reported a Robertsonian translocation in the group-7 lines. The incorporation of alien derived WSMV-resistance in wheat is of utmost importance. However, the potential of alien genes cannot be fully exploited due to different crossing barriers in wide crosses (Mujeeb-Kazi and Hettel, 1995). The results reported here, support the readily crossing nature of *Th. intermedium* with wheat (Table 3.1) and agree with the findings of Li and Wang *et al.,* (2009).

Resistance to WCM alone reduces the incidence of WSMV and prevents losses of wheat yields (Harvey *et al.,* 2003, Harvey *et al.,* 2005, Martin *et al.,* 1984). To date, *Wsm1* and *Wsm2* are the only genes used in bread wheat improvement. However, *Wsm1* is effective against the virus its vector and (Friebe *et al.,* 2009, Li and Wang *et al.,* 2009). Biotypes of WCM are reported to have overcome the resistance conditioned by the host gene (Hein and 2010). Using genomic DNA from *Th. intermedium*, the size of alien chromatin was detected (Table 3.1). These recombinant chromosomes were then targeted with repetitive DNA probes (section 2.2.9). The dpTa1 and pSc119.2 sequences are widely used wheat cytogenetic research due to its high copy number and polymorphic location along the chromosomes (Vershinin *et al.,* 1994, Contento *et al.,* 2005). Both have previously been useful for describing and identifying chromosomes of wheat as they produce multiple hybridization sites on most chromosomes (Rayburn and Gill 1986, Mukai *et al.,* 1993, Castilho *et al.,* 1996, Graybosch *et al.,* 2009, Schwarzacher *et al.,* 2011). Afa and dpTa1 hybridize well to the D-genome chromosomes (Rayburn and Gill, 1986, Anamthawat-Jonsson and Heslop-Harrison, 1993 and Figure 3.10) while pSc119.2 detects the B and some of the A-genome

chromosomes (Contento *et al.,* 2005 and Figure 3.3). The correct identification of recombinant chromosomes was made easy by the use of genomic DNA from *Ae. tauschii* as a probe, which labelled the D-genome (Figure 3.17B). Additional information was gained by simultaneous and sequential use of different probes on the metaphase chromosome spreads. Up to four different probes were visualised on the same metaphase, and has given more information than individual probes in different metaphases. Thus re-probing enhances the usefulness of repetitive DNA, to be used as markers for identifying the recombinant chromosome. The observed banding pattern of dpTa1 and pSc119.2 in the recombinant chromosomes is in general agreement to that of Mukai *et al.,* (1993), Castilho *et al.,* (1996), Pedersen and Langridge (1997) and Biagetti *et al.,* (1999). Hence, it was easy and fast to distinguish the recombinant chromosomes. Here the previously characterised effective source of WSMV-resistance was reconfirmed in the form of 4Ai#2S.4DL translocation (Friebe *et al.,* 2009, Graybosch *et al.,* 2009, Fahim *et al.,* 2011b) and report two novel sources of resistance in the form of recombinant 1B and 3D wheat chromosomes (Table 3.1).

3.5.2 Cytogenetic basis and significance of the diverse sources of WSMV resistance

3.5.2.1 Recombinant chromosome 4D

The identification of 4D recombinants was relatively straight forward, as most of the currently used WSMV-resistant lines carry the 4Ai#2 chromosome in the form of 4Ai#2S.4DL translocation (Seifers *et al.,* 1995, Divis *et al.,* 2006, Graybosch *et al.,* 2009, Fahim *et al.,* 2011). The reference resistant lines also had alien material in the form of 4Ai#2S.4DL translocation (Figures 3.2-3.3 and Table 3.1). The successful transfer of 4Ai#2 small arm of *Th. intermedium* in the form of 4Ai#2S.4DL translocation dates back to Wells and his co-workers (Wells *et al.,* 1973, 1982). Since then several lines with smaller *Th. intermedium* fragments on 4DS have been reported (Friebe *et al.,* 2009). This represents the vastly exploited source of WSMV-resistance which is widely spread across the wheat growing world (Wells *et al.,* 1973, Seifers *et al.,* 1995, Baley *et al.,* 2001, Fedak *et al.,* 2001, Chen *et al.,* 2003a, Cox *et al.,* 2002, Divis *et al.,* 2006, Haber *et al.,* 2007, Friebe *et al.,* 2009, Graybosch *et al.,* 2009).

The banding pattern of dpTa1 along the long arm of 4Ai#2S.4DL recombinant chromosome provides the basis for its cytogenetic characterization (Figure 3.26 and

section 3.4.4.3). Often all the four dpTa1 sites are not distinct (see Figure 3.18). However, several GISH experiments combined with repetitive DNA probes (section 2.2.9) allowed identifying the recombinant chromosome as 4Ai#2S.4DL translocation. The observed banding pattern of the 4DL is identical to that of Mukai *et al.,* (1993) and Pedersen and Langridge (1997). Several published reports describing the significance of this translocation are available in the literature (Friebe *et al.,* 1991, Divis *et al.,* 2006, Graybosch *et al.,* 2009, Schwarzacher *et al.,* 2011). Further, no 4D recombinants with smaller *Th. intermedium* chromosomal segments or heterozygotes were observed.

The presence of centromeric Afa/dpTa1 sites (Figure 3.10 also see insert in Figure 3.11) and telomeric pSc119.2 (Friebe *et al.,* 1991 and Figure 3.20) allowed us to further dissect this alien arm harbouring the *Wsm1* gene and map cytogenetic markers for this arm. The strategy was efficient not only for the detection of physical BP in 4D recombinant lines, but also in identifying the alien arm carrying the *Wsm1* gene in the wild *Th. intermedium* genome (Figure 3.26). Centromeres are specialized regions of the plant chromosomes, composed mainly of satellite repeats and centromeric retrotransposons (Ma *et al.,* 2007, Heslop-Harrison, 2000a, Heslop-Harrison and Schwarzacher, 2011a). They are responsible for sister chromatid cohesion, kinetochore assembly and spindle fibre attachment during cell division (Dong *et al.,* 1998, Miller *et al.,* 1998, Schwarzacher, 2008, Mutti *et al.,* 2010). Large segmental duplications, deletions and rearrangements of centromeric DNA seem common processes governing the evolution of centromeres (Mutti *et al.,* 2010, Heslop-Harrison, 2000a, Ma *et al.,* 2007). The repetitive DNA family, Afa/dpTa1 sites were physically mapped to the centromere of the *Wsm1* carrying arm in both *Th. intermedium* and hybrid wheat lines (Figure 3.10, Figure 3.26). This reveals the active role and involvement of Afa/dpTa1 as a possible hotspot in this recombination. However, no further evidence is available at this time and the need of future research in this regard is encouraged.

3.5.2.2 Recombinant chromosome 1B

Often small alien fragments, additional to the 4Ai#2S.4DL chromatin were observed in the form of 1Ai#1S-1BS.1BL translocation (Table 3.1). The sizes of these fragments vary considerably between R and S-lines (Table 3.2) and their origin is not clear (see below). However, these fragments always involved the distal end of wheat chromosomes in the recombination, although the individual break points are scattered

along the 1BS arm in different wheat lines (Table 3.2). These results suggest the existence of several recombination hot spots in the distal 30% small 1BS of wheat (see Chapter IV).

To date the value of 1BS in relation to WSMV-resistance is unknown. Although among the seven homoeologous groups, the group-1 of wheat are well understood due to important wheat genes (McIntosh *et al.,* 2010, Reddy *et al.,* 2008) including at least 22 genes and QTL on 1B chromosome that confer disease resistance (Peng *et al.,* 2004). The main reason that makes breeding for resistance an attractive approach is, to stack useful genes. The newly characterised WSMV-resistance in line N02Y5003 (Figure 3.9) is representing only 28.3±4.9% of the recombinant 1BS (Table 3.2). It is effective alone, but has the potential for further exploitation. It can be combined with other known 4D (Graybosch *et al.,* 2009) or the newly identified 3D (Figure 3.17 and below) resistances to achieve the desired goals of deploying combinations of effective genes. Nevertheless, its direct utilization as a WSMV-resistant line and breeding with other elite germplasm should improve the durability of WSMV-resistance in commercial wheat cultivars.

With exceptions mainly two lines, KS91H184 and KS91H174 have been used as sources of introducing alien WSMV-resistance for almost 40-years (Wells *et al.,* 1973, also see Table 2.3) and several WSMV-resistant cultivars have been released (Graybosch *et al.,* 2009, Mutti *et al.,* 2011). However, surprisingly the presence of multiple alien fragments in relation to WSMV or its presence on chromosome 1B or 3D has never been reported in literature. These fragments are present in two different populations (see pedigree of lines M&M chapter). The only common elements of the two pedigrees are the cultivar Rio Blanco, and the donor sources of the WSMV resistance, KS91H184 and the related line KS91H174. Rio Blanco was developed by a private firm, and the known pedigree is OK11252A/W76-122. The two parents were experimental breeding lines of which no information is publicly available. Thus, the 1B fragment might be originated from one of these donor varieties. But the precise origin of these two resistant selections, unfortunately, is unknown (Graybosch personal communication).

3.5.2.3 Recombinant chromosome 3D

Field trials rated line N02Y5109 as a consistently resistant line (Graybosch personal communication). The GISH results also revealed the basis of this effective resistance is associated with 42.9±2.5% of the *Th. intermedium* chromatin, replacing the distal end of wheat 3DL (Figure 3.17A&B). Some important genes, including the *R* genes and the stripe rust resistance gene *Yr45* have been mapped on the long arm of 3D (Devos *et al.*, 1992, Li *et al.*, 2011). *R* genes control the red grain colour trait and lie on the long arms of homoeologous group-3. *R* genes are also associated with seed dormancy and have a role in pre-harvest sprouting, which is a serious constraint to grain quality in the temperate wheat-growing world (Devos *et al.*, 1992). At the moment no direct evidence is available, whether any of these genes are lost during translocation. However, this alien chromatin has a smaller size and is represented by 42.9±2.5% of the recombinant wheat arm (Figure 3.17A and Table 3.2). Studies conducted to investigate the negative agronomic and end use quality traits linked with this alien fragment could discover none (Divis *et al.*, 2006). Furthermore, this line germinates as good as any other bread wheat cultivar (personal observation) and therefore, it was assumed that these important genes are being maintained. However, some of these potentials may be attributed to 3Ai#1 segments of *Th. intermedium*. Previously, 4Ai#2s fragments of *Th. intermedium* have been described to provide benefits alone (Divis *et al.*, 2006, Friebe *et al.*, 2009, Graybosch *et al.*, 2009, Schwarzacher *et al.*, 2011). This is also a novel compensating translocation and this resistance can be easily transferred to any other elite germplasm.

Before the *in situ* hybridization, no genetic information was available about the recombinant chromosomes 1B or 3D. Approaches using only molecular markers would have required testing of various dominant and co-dominant markers along the 21 pairs of chromosome arms until polymorphism was seen. However, through *in situ* hybridization, it was easy to target and confirm the recombinant chromosomes. Molecular markers were then applied for the presence of alien material and characterization of BPs along the recombinant 1BS. Thus cytogenetic basis of resistance provided ground for molecular approaches. These results will be discussed in chapter IV.

3.5.3 Negative impacts and chromosomal location of WSMV-resistance genes

Many of the present day WSMV-resistant, wheat cultivars have benefited from *Wsm1* of 4Ai#2 origins. However, lines that initially carried *Wsm1* were associated with undesirable traits, such as yield and bread-making qualities (Baley *et al.,* 2001, Sharp *et al.,* 2002). Wild relatives that often provide diverse sources of resistance, are sometimes associated with some undesirable traits of yield (Seifers *et al.,* 1995, Qi *et al.,* 2010) segregation distortion (Zhang and Dvorak, 1990, Prins *et al.,* 1997, Sibikeeva *et al.,* 2004) and flour colour (Ayala-Navarrete *et al.,* 2007). However, when the undesirable traits are linked to large alien chromatin, it can be successfully shortened by chromosomal engineering while still retaining the genes of interest (Ayala *et al.,* 2007, Qi *et al.,* 2007, Friebe *et al.,* 2009).

Major segregation distortion loci exist on wheat chromosome 4D (Fans *et al.,* 1998). Similarly, pairing anomalies in lines carrying alien chromatin are also not rare (Sibikeeva *et al.,* 2004). Coincidence of the presence of multiple *Th. intermedium* fragments on chromosome 1B and 4D (see Table 3.1 and Figure 3.20) and the gain of terminal pSc119.2 sites by the small *Th. intermedium* chromosomal arm, involved in 4D recombination (Figure 3.3) was ample to assume the existence of a reciprocal translocation between chromosomes 1B and 4D (Table 3.1). However, subsequent control FISH experiments involving *Th. intermedium* and 4D recombinants lines alone, confirmed the smaller alien fragments are derived from *Th. intermedium* chromosome, other than the small arm of 4Ai#2 (compare *Th. intermedium* arms in Figure 3.16 and Figure 3.26B). A similar description of the small *Th. intermedium* arm with terminal pSc119.2 sites in wheat-*Thinopyrum* addition lines is available in Friebe *et al.,* (1991). Furthermore, no pairing imperfections were associated in lines carrying the 4Ai#2S.4DL chromatin of *Th. intermedium* in the current study (see insert in Figure 3.11D and chapter V).

Nothing is known about exact location of the group-4 derived alien *Wsm1* gene (Qi *et al.,* 2007, Fahim *et al.,* 2011). However, Friebe *et al.,* (2009) physically mapped the *Wsm1* gene by reducing the size of 4Ai#2 chromatin into the distal 20% of the 4Ai#2S.4DL arm. In the current study, breeding lines that retained the distal 20% of 1B *Th. intermedium* fragments were rated susceptible and have shown characteristic symptoms of WSMV (Table 3.2, Graybosch personal communication). No known WSMV-resistance genes have been previously mapped to wheat chromosome 1BS or

3DL (see Figure 3.25 and above). Therefore, these are potentially novel WSMV-resistance genes, and these new genes are designated as *Wsm4* and *Wsm5* for the group-1 and group-3 origin fragments of *Th. intermedium* respectively. Different PCR markers were applied and it also supported the different origin of these resistances (see Chapter IV). The results shown here also indicate, these new WSMV-resistance genes would lie at the distal ends of *Th. intermedium* to be translocated (comparing Figures 3.25 and 3.26) and span around 28.3±4.9% and 42.9±2.5 regions of the recombinant arms (Table 3.2). However, it was difficult to estimate the exact size of these fragments after GISH due to the complex nature of the experiment. Strongly labelled *Th. intermedium* DNA may fluoresce much brighter, and may result in overestimation of the fragment size than it may exist in real (Lukaszewski *et al.,* 2005).

CHAPTER IV: MOLECULAR APPROACHES TO DETECT ALIEN CHROMATIN AND MAP THE NOVEL *WSMV*-RESISTANT GENE ON WHEAT CHROMOSOME 1B

4.1 Introduction

Despite the critical role of cultural practices and chemicals in reducing the incidence and severity of *Wheat streak mosaic virus* (Slykhuis, 1955, Thomas and Hein, 2003, De Waard *et al.,* 1993, Coutts *et al.,* 2008) deployment of WSMV-resistant cultivars is the most effective, economical and environmentally safe strategy for controlling the disease (see Graybosch *et al.,* 2009, and section 3.2.1-3.2.3).

In breeding programmes, not only the transfer of alien chromatin but also the accurate identification the desired genes makes the introgressed alien material more attractive and readily available to be transferred into acceptable wheat backgrounds (Gill and Raupp, 1987, King *et al.,* 1993, Forsström *et al.,* 2002, Harper *et al.,* 2011, Schwarzacher *et al.,* 2011).

A number of novel *Th. intermedium* derived WSMV-resistance sources were characterised after a thorough cytogenetic screening using GISH (Chapter III). Conventional exploitation of the introgressed alien material would involve hybrid populations of acceptable wheat lines with the newly identified sources of WSMV-resistance (Table 3.1). Further exposure to suitable stress, screening by phenotype and finally re-confirmation by cytological procedures such as GISH (Schwarzacher *et al.,* 1989, Seifers *et al.,* 1995, Divis *et al.,* 2006, Wang *et al.,* 2010, Heslop-Harrison and Schwarzacher 2011b). Thus, breeding a new wheat variety may take up to 4-8 years, and even then the release of an improved variety cannot be guaranteed (Borlaug 1983). Therefore, conventional breeding and subsequent characterization of an improved germplasm is rather a slow and time-consuming process (Perry, 2004, Carvalho *et al.,* 2009, Mangini *et al.,* 2010). It requires time and resources that are generally beyond the limits of most breeding programs. Thus, the practical utilization and efficacy of the resistant sources is always minimized (Talbert *et al.,* 1996, Reddy *et al.,* 2008, Liu *et al.,* 2011).

On the other hand, phenotype is the interaction of genotype with environment and epidemics of WSMV are strongly influenced by environmental stimuli (Christian and Willis 1993, Coutts *et al.,* 2008 and section 3.1.2). Therefore, selection purely based on phenotypic traits may result in inaccurate selection (Wang *et al.,* 2010, Talbert *et al.,* 1996, Prasad *et al.,* 2000). Strategies involving the deployment of wheat cultivars with multiple or combinations of effective genes "stacked" together are more valued in protecting against diseases (Ayala *et al.,* 2007, Wang *et al.,* 2010, Liu *et al.,* 2011). However, this practice relies on the availability and adequate prior knowledge of a range of resistance genes (Larkin *et al.,* 1995, King *et al.,* 1997a, Singh *et al.,* 2008a, Singh *et al.,* 2008b, Singh *et al.,* 1998, Scholz *et al.,* 2009, McIntosh *et al.,* 2010). Successful deployment of several genes should prolong the resistance and make it more durable, as it greatly reduces the probability of simultaneous mutation in the pathogen and also lessens the selective pressure of using the same resistant gene (Li and Wang, 2009, Li *et al.,* 2011, Qi *et al.,* 2003, Mujeeb-Kazi and Hettel, 1995).

In practice, benefits of desired genes are multiplied by tagging them in a hybrid background (Ayala *et al.,* 2001, Cato *et al.,* 2001, Peng and Lapitan, 2005, Talbert *et al.,* 1996, Wang *et al.,* 2010).Therefore, inexpensive and reliable molecular tagging approaches are required, especially when screening large segregating populations in the early generations (Divis *et al.,* 2006, Graybosch *et al.,* 2009, Mangini *et al.,* 2010). Molecular markers (MMs) particularly, PCR-based markers provide a powerful and diagnostic approach and has renewed optimism among plant breeders (Heslop-Harrison, 2000a, Saeidi *et al.,* 2008, Collard and Mackill, 2008, Chee *et al.,* 2005). PCR markers are numerous in every genome and can be selected to be polymorphic, stable and reproducible (Röder *et al.,* 1998b, Röder *et al.,* 1998a, Talbert *et al.,* 1996, Cato *et al.,* 2001, Korzun, 2002, Todorovska *et al.,* 2005, Todorovska *et al.,* 2001). They are neither affected by the tissue, developmental stages nor by environmental factors (Prasad *et al.,* 2000, Perry, 2004). In addition, the transferable nature and reliability of PCR based MMs in diverse backgrounds, makes them powerful tools for marker-assisted selection (MAS) breeding programs and other screening or mapping studies (Ganal and Röder, 2007, Röder *et al.,* 1998a, Reddy *et al.,* 2008, Gadaleta *et al.,* 2009). Hundreds of genotypes may be assessed and cultivars with desired traits may be selected in minimal time at low costs (Collard *et al.,* 2008, Reddy *et al.,* 2008, Mangini *et al.,* 2010). Therefore, MAS-breeding allows registration of new germplasm and its

subsequent availability to wheat growers in a short period of time (Perry 2004, Divis *et al.,* 2006, Graybosch *et al.,* 2009, Mutti *et al.,* 2011).

The usefulness of various MMs for mapbased cloning and MAS-breeding depends upon their proximity to the target genes (Song *et al.,* 2005). Fortunately, many of the important wheat or alien derived genes in wheat backgrounds have been tracked with closely or completely linked MMs and has radically improved gene pyramiding and MAS breeding approaches (see King *et al.,* 1993, Talbert *et al.,* 1996, Ayala *et al.,* 2001, Somers *et al.,* 2004, Reddy *et al.,* 2008, Li *et al.,* 2010, Wang *et al.,* 2010, Liu *et al.,* 2011, Fahim *et al.,* 2011).

A number of MMs, most of them PCR based including restriction fragment length polymorphism (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphism (AFLPs), simple sequence repeats (SSRs) or microsatellites, expressed sequence tag sites (ESTs), single nucleotide polymorphism (SNPs), resistance gene analog polymorphism (RGAP), retrotransposon-microsatellite amplified polymorphism (REMAPs) and inter-retrotransposon amplified polymorphism (IRAPs) have been developed over the years and are applied to cereal research (see Korzun 2002, Todorovska *et al.,* 2005, Collard *et al.,* 2008). However, because of the higher levels of polymorphism, low cost, known map locations and reliable amplification, ESTs and SSRs are the most frequently used MMs.

ESTs are fragments of cDNA sequence complementary to mRNA and represent parts of expressed genes. Thus, ESTs provide a short cut for new gene discoveries and therefore, are very informative in gene tracking (Adams *et al.,* 1991, Peng *et al.,* 2004). However, because of their conserved nature, genetic mapping with ESTs alone may show low levels of polymorphism (Gao *et al.,* 2004, Qi *et al.,* 2007, Xue *et al.,* 2008, Qi *et al.,* 2004). On the other hand, SSRs or microsatellites are sequences of 1-6bp in length, consisting of tandem repeats. They are co-dominant markers and show Mendelian inheritance (Röder *et al.,* 1998a, Roder *et al.,* 1993, Morgante *et al.,* 2002, Guyomarc'h *et al.,* 2002). Unlike ESTs, they show high levels of polymorphism and therefore, are well suited for mapping studies. They could detect even the low levels of intra specific polymorphism in inbreeding species (Röder *et al.,* 1998a, Sourdille *et al.,* 2004a, Sourdille *et al.,* 2001, Somers *et al.,* 2004).

Although, natural resistance provides an attractive control strategy against WSMV (Friebe *et al.,* 1991, Talbert *et al.,* 1996, Divis *et al.,* 2006, Graybosch *et al.,* 2009, Schwarzacher *et al.,* 2011), the transfer of WSMV-resistance to agronomically

acceptable germplasm has been relatively slow, mainly due to two reasons. First, due to scarcity of diverse and effective WSMV-resistance in wheat backgrounds i.e. wheat lines with effective alien derived resistance (see section 3.2.3). Secondly the laborious screening procedures, that must be carried out after successful introgression to test a line under disease pressure (Borlaug, 1983, Ayala-Navarrete *et al.,* 2009, Divis *et al.,* 2006, Mujeeb-Kazi and Hettel, 1995).

MMs as described above, offer a time and cost effective screening opportunity to monitor the transfer of WSMV-resistance, since it relies only on identified markers linked to the resistant gene. Therefore, it does not require rearing of the pathogen or exposure of wheat lines to disease pressure etc. Breeding lines with potential WSMV-resistance can be screened and selected at seedling stage without difficult pathological tests (Talbert *et al.,* 1996, Chen *et al.,* 1998a, 1998b, 2003, Lu *et al.,* 2011, Fahim *et al.,* 2011). However, most of the known markers are linked to *Wsm1* and detect resistance derived from 4Ai#2 chromosome of *Th. intermedium* only (see below).

Earlier effective WSMV-resistance was associated with two novel wheat-*Th. intermedium* recombinants to the homoeologous group-1 (1BS) and group-3 (3DL) along with other sources carrying the known group-4 resistance (see Chapter III). The aim of this study was to identify potential markers for WSMV-resistance screening, confirm the origin of 1BS and 3DL resistance as novel resistances, and determine the molecular breakpoint (BP) as well as loss of any important genes from the 1BS recombinant lines.

4.2 Materials and Methods

4.2.1 DNA extraction, PCR amplification and gel electrophoresis

Details of the DNA extraction, PCR amplification and gel electrophoresis are given in M&M chapter II.

Polymorphic PCR markers were applied for genetic mapping and monomorphic markers were used to assess the polymorphism. Markers that could amplify DNA from *Th. intermedium* or wheat alone, were considered dominant and those that showed polymorphism between the two were considered co-dominant markers. List of markers (often referred to as primer pairs) applied are given in Tables 4.1, 4.2 and 4.3. Nucleotide sequence of the previously known and newly identified markers detecting

Th. intermedium alien fragments along with their melting temperature, source and polymorphism levels are given as Table 4.1 and Table 4.2, while those for BP mapping of the 1BS recombinants are given in Table 4.3.

All 64-markers (100%) tested in this study successfully amplified one or more loci from wheat and/or *Th. intermedium*. However, some markers that were previously reported polymorphic for *Th. intermedium* and 'Chinese Spring' wheat were not useful for the material used here (see Table 4.1). Most of the markers used here are available in the public domain at GrainGenes database (http://wheat.pw.usda.gov). However, nucleotide sequences for some of the publically unavailable Gatersleben Wheat Microsatellites (GWM) markers were kindly provided by Marion S. Röder (IPK, Gatersleben Germany).

Table 4.1: List of published PCR markers, their melting temperature (T_m) and product sizes applied for detecting *Th. intermedium* chromatin.

Sr#	Marker name	Primer sequences	Tm (°C)	Product size (bp)	Polymorphism information[*1]	Source/reference
1	STS-J15	F: GTAGCAGGGGAAGCTGAAGA R: CCGAGCTCACACGCTAATTT	60	420	Dominant 4D marker	Talbert *et al.*, 1996. (Linked to group-4 of *Th. intermedium)*
2	SCM4	F: GCCCTGCCATTGATCCCAAGCTG R: TGGGCCAGGTCTTTCAGGTGACG	60	1300	No polymorphism	Zhang *et al.*, 2002 (linked to group-2 of *Th. intermedium*)
3	BG263898	F: TGCTCAATAAGAACTGGCAGAACG R: GGAATCACAACTCAGGGGAAACAG	56	310	No polymorphism	Qi *et al.*, 2007. (Linked to group 4 of *Th. intermedium* and was used without restriction enzyme digestion)
4	*Bdv3*	F: CTTAACTTCATTGTTGATCTTA R: CGACGAATTCCCAGCTAAACTAGACT	52	206 & 288	No polymorphism	Kong *et al.*, 2009 (linked to group-7 of *Th. intermedium*)
5	BE404744	F: AGATGGATGGTGCCTGACT R: AACCTCGTCTACTGCTTCG	54	-	No polymorphism	Gao *et al.*, 2009 (linked to Bdv2 group-7 of *Th. intermedium*)
6	P4	F: TGACTCCAGCATTTTATGGGTG R: CAACATGACAAGTGTCGGTTCCT	48	~500	Some polymorphism	Wang *et al.*, 2010. (Linked to group-2 of *Th. intermedium)*
7	P85	F: GCAAACCCTGTATCACTAAAG R: CAATCATGGCTCCAATAAGT	53	-	No polymorphism	Wang *et al.*, 2010. (Linked to group-2 of *Th. intermedium)*
8	P91	F: TGTCATCCAACCATAGCAGAG R: TCGACCAGCACCATCGA	55	-	No polymorphism	Wang *et al.*, 2010. (Linked to group-2 of *Th. intermedium)*
9	P93	F: CCATTGCCAAGGGCTGTA R: TCTTCACGCCGCTTGTTG	58	-	No polymorphism	Wang *et al.*, 2010. (Linked to group-2 of *Th. intermedium)*
10	P31	F: TGGTGAATCTACAGCAGAAAAG R: GTGGCGTGGTTTACCTTCT	54	-	No polymorphism	Wang *et al.*, 2010. (Linked to group-2 of *Th. intermedium)*
11	P36	F: GTCCGCCGTCAATGTCAAG R: GCCCGAACGGAGCAGTAGT	60	-	No polymorphism	Wang *et al.*, 2010. (Linked to group-2 of *Th. intermedium)*
12	P96	F: GGCGAACAACTACTACCGTG R: CAAGTAGCCCAGGGAGGAG	55		No polymorphism	Wang *et al.*, 2010. (Linked to group-2 of *Th. intermedium)*
13	P97	F: ATTGCTGATGACGCTGTTAT R: CTTCTCGTTGTCTTGGGTT	56	~750	No polymorphism	Wang *et al.*, 2010. (Linked to group-2 of *Th. intermedium)*
14	P68	F: TGTGCTAACTGGGCAAAACC R: GAAGGCAAACGAACTCATAAA	55	~500	No polymorphism	Wang *et al.*, 2010. (Linked to group-2 of *Th. intermedium)*
15	P73	F: CGCACCACAGTTCAGCA R: CACATCGCAGGAGCAGA	53	-	No polymorphism	Wang *et al.*, 2010. (Linked to group-2 of *Th. intermedium)*
16	P41	F: AGATAACGGTGGTGAAATG R: TGGAAGTAAAGGTAGGCTC	54	-	No polymorphism	Wang *et al.*, 2010. (Linked to group-2 of *Th. intermedium)*
17	P17	F: CTTAGAAGTAGCCCAGCAACG R: GACTCGCAGCAGGCAAAA	52	-	No polymorphism	Wang *et al.*, 2010. (Linked to group-2 of *Th. intermedium)*

Table 4.1: continued

Sr#	Marker name	Primer sequences	Tm (°C)	Product size (bp)	Polymorphism information[*1]	Source/reference
18	P77	F: AGCCACGAGCAGAAGAGCAC R: GAGGGCGTCGCTGTCCA	60	-	No polymorphism	Wang *et al.*, 2010. (Linked to group-2 of *Th. intermedium)*
19	P79	F: AAAATGAAACATCTCCTCGC R: AGTCAAATAACACAACCAATAAG	54	~520	No polymorphism	Wang *et al.*, 2010. (Linked to group-2 of *Th. intermedium)*
20	P80	F: GCTTCTCCCCCTTCTGTAAT R: GCAGCCAAACGAATAGTCAG	55	-	No polymorphism	Wang *et al.*, 2010. (Linked to group-2 of *Th. intermedium)*
21	WSR2	F: CACAAGGCACAAGCAGAAAA R: GTGAGCAAAGGAAGGACTGC	60	239	No polymorphism	Fahim *et al.*, 2011. (Linked to group-4 of *Th. intermedium)*
22	WSR9	F: GTTTCATGCAGATTGGCCTT R: TGTTAGGTCGTCCGATAGGG	60	~250 ~320	Dominant 4D & 1B marker	Fahim *et al.*, 2011. (Linked to group-2 and 4 of *Th. intermedium)*
23	WSR11	F: TCCCGGTACTTATCGAGGTG R: CCGCAAGTCTTACTGCAACA	60	200	Dominant 4D marker	Fahim *et al.*, 2011. (Linked to group-4 of *Th. intermedium)*
24	WSR17	F: TACCAATGTCTTCAGCTGCG R: ACTGCTCCTCCGTCTCAAAA	60	220	Dominant 4D marker	Fahim *et al.*, 2011. (Linked to group-4 of *Th. intermedium)*
25	WSR65	F: TGTTGTGACCAGTAGTGCTGC R: CCTCAAAAGCTGCTACGACA	60	1300	Dominant 4D marker	Fahim *et al.*, 2011. (Linked to group-4 of *Th. intermedium)*
26	CL167	F: CGGAAGGACTTCATCATCATTTGT R: CCTCTGCTGCTTCTCCTTCTCAG	66	300	No polymorphism	Fahim *et al.*, 2011. (Linked to group-2 and 4 of *Th. intermedium)*

*1 No polymorphism: refers to when obtained PCR products were not correlated to the presence or absence of *Th. intermedium* chromatin as identified by GISH (Chapter III), Dominant 1B or 4D marker: refers to amplified products from specific *Th. intermedium* chromatin present on 1B or 4D only.

Table 4.2: Details of newly identified polymorphic markers that show polymorphism between WSMV-resistant and susceptible lines.

Sr#	Marker name	EST accession/ description	Primer sequences	Tm (°C)	Expected product size (bp)	Chromosome assignment	Source/Definition/ Bin map position
27	Xpsp2530.1	EST[*1]	F: CCTAAACCCTAAACCCTAGAC R: TTCTCACCCAACCACCAGCAGCT	55	~580 (obtained)	4Ai#2S	Mao *et al.*, 1997.
28	UL-Thin-1	EU520257[*2]	F: CTGACCTTTTTAGCAACGCC R: AGGAGTGCTGCTACGTCCAT	60	239	1Ai and 4Ai#2S	*Thinopyrum intermedium* RAPD marker APR5 genomic sequence
29	UL-Thin-2	ACU31172[*2]	F: GGCCGACCCGTCTTTAGTAT R: CGCCCATTCTTGACTCTCTC	58	269	4Ai#2S	*Agropyron cristatum* P genome repetitive DNA sequence
30	UL-Thin-3	BE445831[*2]	F: GAATGGAGGGACACCATTTG R: CCCACAATGCTGTGTTTGTC	58	393 ~550[*4]	4Ai#2S	Wheat etiolated seedling root normalized cDNA (Deletion Bin 4DS2-0.82-1.00)
31	UL-Thin-4	BG604678[*2]	F: ACCCTCCTCCACTGGTCAAT R: GTCTCAAGCACCCGTCATCT	55	334 ~890[*4]	4Ai#2S	Wheat 5-15 DAP spike cDNA library (Deletion Bin 4DS2-0.82-1.00)
32	*Xgwm*1028	SSR[*3]		50	~100bp (obtained)	1Ai and 4Ai#2S	Ganal and Röder 2007.

*1 is described as 1BS specific EST in Mao *et al.*, (1997),
*2 these markers are mentioned in Fahim *et al.*, (2011) supplementary data as non-polymorphic and were named as WSR14, 26, 30 and 50 respectively,
*3 SSR marker for 1BS, mentioned in Ganal and Röder 2007. The nucleotide sequence of this marker was provided by Marion S. Röder (IPK, Gatersleben Germany),
*4 obtained product size differed from the published size.

Table 4.3: List of PCR markers applied for Break point (BP) mapping of the 1BS fragments along melting temperature (T_m) and product sizes.

Sr#	Marker name	Type	Primer sequences	Tm (°C)	Expected product size (bp)	Source
1	*Xpsp3000*	SSR	F: GCAGACCTGTGTCATTGGTC R: GATATAGTGGCAGCAGGATACG	55	252-286	Bryan *et al.*, 1997*Gli*-1 locus polymorphic
2	*Xwmc49*	SSR	F: CTCATGAGTATATCACCGCACA R: GACGCGAAACGAATATTCAAGT	60	206	Somers *et al.*, 2004
3	*Xwmc500*	SSR	F: ATAGCATGTTGGAACAGAGCAC R: CTTAGATGCAACTCTATGCGGT	60	185	Somers *et al.*, 2004
4	*Xfc618*	SSR	F: TCTACATACGGACTGAAATGGATAC R: CCTGATTGAGACTCTGGTTACATAAGACTACTC	60	250	Reddy *et al.*, 2008.
5	XBF293222	RFLP	F: GGTTTGCTTTTGCCAATTGTTCTTG R: TATATGTTGGATGGGAGCAAAATCC	50	~400[*1]	Reddy *et al.*, 2008.
6	XBF474204	EST	F: AATCACACGACCCAGTAAGTTCTC R: CTCAAGTACCTCTGCTTCAACTTC	52	~480[*1]	Reddy *et al.*, 2008.
7	Xpsp2530.1	EST	F: CCTAAACCCTAAACCCTAGAC R: TTCTCACCCAACCACCAGCAGCT	55	~200[*1]	Mao *et al.*, 1997.
8	*XksuD14a*	RFLP	F: CCAAAGAGCATCCATGGTGT R: CGCTTTTACCGAGATTGGTC	50	~550[*1]	Talbert *et al.*, 1994.
9	*Xwmc85*	SSR	F: GGAGTAAGAGAAACATGCCGAA R: GTGCATGCATGAGAATAGGAAC	61	228	Somers *et al.*, 2004
10	*Xgwm0550*	SSR	See *2	55	150 ~300[*3]	Ganal and Röder 2007.
11	*Xgwm0911*	SSR	See *2	55	272	Ganal and Röder 2007.
12	*Xgwm1028*	SSR	See *2	50	116	Ganal and Röder 2007.
13	*Xgwm1078*	SSR	See *2	55	144	Ganal and Röder 2007.
14	*Xgwm1130*	SSR	See *2	60	116	Ganal and Röder 2007.
15	*Xgwm1100*	SSR	See *2	50	227	Ganal and Röder 2007.
16	*Xgwm3035*	SSR	See *2	60	225	Ganal and Röder 2007.
17	*Xgwm4144*	SSR	See *2	60	191	Ganal and Röder 2007.
18	*Xgwm4435*	SSR	See *2	60	214	Ganal and Röder 2007.

Table 4.3: continued

Sr#	Marker name	Type	Primer sequences	Tm (°C)	Expected product size (bp)	Source
19	*Xwmc230*	SSR	F: AGAAGCGAGCAGGTGTGTTTGA R: CTGCTTCCTCCCACAACAGATG	60	213 ~230[*3]	Somers *et al.*, 2004.
20	*Xbarc119*	SSR	F: CACCCGATGATGAAAAT R: GATGGCACAAGAAATGAT	55	208	Developed by P. Cregan and Q. Song (available at http://wheat.pw.usda.gov)
21	*Xgpw1170*	SSR	F: AGATCGTTCATCCGATCTGC R: CAATCTCAGTTTGATGTCCTTCAG	60	166	Sourdille *et al.*, 2004.
22	*Xgpw363*	SSR	F: GTGTGTGGTTGGAGGGAACT R: ATAAGAACATCGAGCGACCG	60	242	Sourdille *et al.*, 2004.
23	*Xbarc194*	SSR	F:CGCAATCATGTTCCTAAGAATATTTGTCCA R: CGCATGTCCCGCTAACCAATAGTCT	50	166	Developed by P. Cregan and Q. Song (available at http://wheat.pw.usda.gov)
24	*Xgwm264*	SSR	F: GAGAAACATGCCGAACAACA R: GCATGCATGAGAATAGGAACTG	60	160	Röder *et al.*, 1998
25	*Xucr_6*	EST	F: TCGAAGGAGAATACGCTGGT R: GCCCATAAGATTTTGCAACG	60	1100	Sharma *et al.*, 2009.
26	*Xucr_8*	SSR	F: CCTGCTCTGCCATTACTTGG R: TGCACCTCCATCTCCTTCTT	60	165	Sharma *et al.*, 2009.
27	*Xgpw1143*	SSR	F: CTGTTGTGGGGTGTGCATGT R: CCCCAGCAGCATGAATAAGT	60	206	Sourdille *et al.*, 2004.
28	*Xwmc329*	SSR	F: ACAAAGGTGCATTCGTAGA R: AACACGCATCAGTTTCAGT	54	118	Somers and Isaac 2004
29	*Xwmc406*	SSR	F: TATGAGGGTCGGATCAATACAA R: CGAGTTTACTGCAAACAAATGG	60	217	Somers and Isaac 2004
30	*Xgpw7059*	SSR	F: AACACCAATGACCTGATCGC R: TCCTCAACAGCTCCAGTGC	60	~220 (obtained)	Sourdille 2009.
31	*Xgwm374*	SSR	ATAGTGTGTTGCATGCTGTGTG TCTAATTAGCGTTGGCTGCC	60	180	Röder *et al.*, 1998.
32	*Xbarc128*	SSR	GCGGGTAGCATTTATGTTGA CAAACCAGGCAAGAGTCTGA	60	250	Developed by P. Cregan and Q. Song (available at http://wheat.pw.usda.gov)

*1 size was estimated from the result here, as authors have not given the expected product size,
*2 unpublished oligos see note Table 4.2,
*3 obtained product size differed from the published size.

4.3 Results

4.3.1 Assignment of the MMs to recombinant wheat chromosomes

A total of 32 PCR markers were applied to correlate the presence of alien chromatin as identified by GISH (see Chapter III) with WSMV-resistance screen of field trials (Divis *et al.*, 2006, also see Figure 3.1A&B) in lines given in Table 4.4. These markers included 6 newly identified (Table 4.2) and 26 markers, previously reported polymorphic for 'Chinese Spring' and *Th. intermedium* (Table 4.1). The initial PCR marker screen for correlation with WSMV-resistance was accomplished using DNA from *Th. intermedium*, KS96HW10-1, N02Y5003, N02Y5109 and 'Chinese Spring' wheat. However, final screening included thirty lines (Table 4.4). *Th. intermedium*, KS95H102 and KS96HW10-1 were used as control resistant lines, while 'Chinese Spring', CS N4B T4D and CS N4D T4B were used as control susceptible lines.

Among the 32-markers screened for polymorphism between WSMV-resistant and susceptible lines, 21-markers (65.6%) amplified one or several monomorphic loci from both wheat as well as *Th. intermedium* chromosomes (Table 4.4). In some instances a polymorphic band was seen but it could not be correlated to presence of the 1B, 3D or 4D recombinant chromosomes (Appendix 4.1) and therefore, could not be assigned to a specific wheat or *Th. intermedium* chromosomes (see Table 4.1). However, 11-markers (34.4%) were informative for correlation with WSMV-resistance and were assigned to the homoeologous group-1 or group-4 of *Th. intermedium* chromosomes (Table 4.4 and below).

Table 4.4: Results of polymorphic PCR markers used for correlation of *Th. intermedium* fragments and WSMV-resistance.

Line	GISH analysis[*1]			Published polymorphic markers[*2]									New polymorphic markers[*3]				
	Rec. 4D	Rec. 1B	Rec. 3D	STS J15[*4]	WSR2	WSR9		WSR 11	WSR 65	WSR 17	*Xpsp3000 (Gli-1)*	P4	UL-Thin-1	UL-Thin-2	UL-Thin-3	UL-Thin-4	Xpsp2 530.1	*Xgwm 1028*
KS95H102	+/+	-/-	-/-	1	1	1	0	1	1	1	1	1	1	1	1	1	#	#
KS96HW10-1	+/+	-/-	-/-	1	1	1	0	1	1	1	1	1	0	1	1	1	1	0
Millennium	-/-	-/-	-/-	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
N02Y5018	+/+	+/+	-/-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
N02Y5019	-/-	+/+	-/-	0	1	0	1	0	1	0	1	1	1	1	0	0	0	1
N02Y5021	-/-	-/-	-/-	0	1	0	0	0	0	0	1	1	1	0	0	0	#	#
N02Y5025	+/+	-/-	-/-	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
N02Y5003	-/-	+/+	-/-	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0
N02Y5057	+/+	-/-	-/-	1	1	1	0	1	1	1	1	1	0	1	1	1	#	#
N02Y5075	+/+	-/-	-/-	1	1	1	0	1	1	1	1	1	0	1	1	1	#	#
N02Y5078	+/+	-/-	-/-	1	1	1	0	1	1	1	1	1	0	1	1	1	#	#
N02Y5082	-/-	-/-	-/-	0	1	0	0	0	0	0	1	1	0	0	0	0	#	#
N02Y5096	-/-	-/-	-/-	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0
N02Y5105	-/-	-/-	-/-	0	1	0	0	0	0	0	1	1	0	0	0	0	#	#
N02Y5106	+/+	-/-	-/-	1	1	1	0	1	1	1	1	1	0	1	1	1	#	#
N02Y5109	-/-	-/-	+/+	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0
N02Y5117	+/+	-/-	-/-	1	1	1	0	1	1	1	1	1	0	1	1	1	1	0
N02Y5121	-/-	-/-	-/-	0	1	0	0	1	0	0	1	1	0	0	0	0	#	#
N02Y5149	+/+	+/+	-/-	1	1	1	0	0	1	1	0	1	1	1	1	1	1	0
N02Y5154	+/+	-/-	-/-	1	1	1	0	1	1	1	1	0	1	1	1	1	#	#
N02Y5156	-/-	+/+	-/-	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0
N02Y5163	-/-	+/+	-/-	0	1	0	1	0	1	0	1	0	1	?	0	0	0	0
N02Y2016	+/+	+/+	-/-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
Chinese spring	-/-	-/-	-/-	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
CS N4AT4D	-/-	-/-	-/-	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
Manaska				1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
Beef maker				1	1	1	1	1	1	1	0	1	1	1	1	1	#	#
Hay maker				1	1	1	1	1	1	1	0	1	1	1	1	1	#	#
Reliant				1	1	1	1	1	1	1	0	1	1	1	1	1	#	#
N4DT4B	-/-	-/-	-/-	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0

*1 presence or absence of *Th. intermedium* fragments revealed by GISH (see section 3.4 &Table 3.1), +/+ alien fragments of similar size seen (homozygous), -/- when no alien fragments seen, *2 polymorphic markers from previous studies, *3 newly identified polymorphic markers (see Table 4.2 for details), 0 absence of the marker allele, 1 presence of the marker allele, # not tested .

4.3.2 MMs for breakpoint mapping of 1BS recombinants

Since a novel WSMV-resistance gene was mapped to the telomeric region of recombinant wheat chromosome 1B (see Figure 3.25 chapter III). For BP mapping of the 1BS recombinants, 32 published PCR markers were applied (Table 4.3). These markers are mainly from three deletion bins 1BS.sat18-0.50-1.00, 1BS.sat19-0.31-0.50 and 1BS.sat.-0.31, located above the NOR region of 1BS arm (http://wheat.pw.usda.gov/wEST/binmaps/). Initial marker screening was carried out with *Th. intermedium* land race Manaska, 'Beaver' (1RS.1BL wheat-rye translocation variety), N02Y5003, N02Y5025, *T. aestivum* cv. 'Chinese Spring' and CS N1B T1A lines. However, final mapping involved nineteen lines (Table 4.5). Millennium, N02Y5096 and 'Chinese Spring' wheat were used as positive control, while CS N1B T1A and Beaver were used as negative control. Manaska, N02Y5109, N02Y5117, CS N4A T4D and CS N4D T4B lines were included to see if of the applied markers could amplify homoeologous group-3 or group-4 origin fragments from *Th. intermedium.*

Among the 32 PCR markers applied, 13 markers (40.6%) amplified one or multiple alleles from the recombinant 1BS lines as well as from the nulli-1B line and could not be scored for 1B (Table 4.5). However, 19-markers (59.4%) were polymorphic and produced characteristic loci from chromosome 1BS of wheat. Most of the polymorphic markers amplified PCR products that were comparable to the expected size (Table 4.3 and Appendix 4.1) and were assigned to the 1BS arm of wheat (Table 4.6). Few polymorphic PCR markers amplified multiple loci (Figure 4.3, 4.4), however only the bands for the expected product size were scored (Table 4.6). The breakpoint between wheat and *Th. intermedium* chromatin was identified by the appearance of one or several wheat markers on the recombinant 1BS arm of wheat after taking into account its presence-absence from the control lines ('Chinese Spring' and nulli-1b line).

Table 4.5: PCR markers used for breakpoint (BP) mapping of the recombinant1BS. Yellow highlighted markers are the polymorphic markers informative in detecting BPs and given in Table 4.6 below. Experimental lines with recombinant 1BS are highlighted.

Line	GISH analysis[*1]			*Xpsp3000*	*Xwmc49*	*Xwmc500*	*Xfc618*	XBF293222	XBF474204	Xpsp2530.1	*XksuD14a*	*Xwmc85*	*Xgwm0550*	*Xgwm0911*	*Xgwm1028*	*Xgwm1078*	*Xgwm1130*	*Xgwm1100*	*Xgwm3035*
	Rec. 4D	Rec. 1B	Rec. 3D																
KS96HW10-1	+/+	-/-	-/-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Millennium	-/-	-/-	-/-	1	1?	1	1	1	1	1	1	1	1	1	1	1	1	1	1
N02Y5018	+/+	+/+	-/-	1	1	1	0	1	1	1	0	1	1	0	1	1	1	1	1
N02Y5019	-/-	+/+	-/-	1	1	1	0	1	1	1	1	1	1	0	1	0	1	1	1
N02Y5025	+/+	-/-	-/-	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1?	1
N02Y5003	-/-	+/+	-/-	0	0	1	0	1	0	1	0	1	1	0	1	0	0	1?	1
N02Y5096	-/-	-/-	-/-	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1
N02Y5109	-/-	-/-	+/+	1	0	1	1	1	0	1	0	1	1	1	1	1	1	1	1
N02Y5117	+/+	-/-	-/-	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
N02Y5149	+/+	+/+	-/-	0	0	1	0	1	0	1	1	1	1	0	1	0	0	1?	1
N02Y5156	-/-	+/+	-/-	0	0	1	0	1	0	1	1	1	1	0	1	1?	0	1	1
N02Y5163	-/-	+/+	-/-	1	1	1	0	1	0	1	1	1	1	0	1	1	1	1	1
N02Y2016	+/+	+/+	-/-	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1
Beaver (1RS.1BL)	-/-		-/-	0	0	1	0	1	0	1	1	1	1	0	1	0	0	0	1
Manaska	+/+	+/+	+/+	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0
CS N4A T4D	-/-	-/-	-/-	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1
CS N4D T4B	-/-	-/-	-/-	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1
Chinese spring	-/-	-/-	-/-	1	1	1	1	1	?	1	0	1	1	1	1	1	1	1	1
CS N1B T1A	-/-	-/-	-/-	0	#	#	0	#	0	#	#	1	#	0	#	0	0	0	1

*1 presence or absence of *Th. intermedium* fragments revealed by GISH (see section 3.4&Table 3.1), +/+ alien fragments of similar size seen (homozygous), -/- when no alien fragments seen, 1 presence of the marker allele, 0 absence of the marker allele, 1? most probably present, # when DNA was not available for PCR.

Table 4.5: continued

Line	GISH analysis[*1]			*Xgwm 4144*	*Xgwm 4435*	*Xwmc 230*	*Xbarc 119*	*Xgpw 1170*	*Xgpw 363*	*Xbarc 194*	*Xgwm 264*	*Xucr_ 6*	*Xucr_ 8*	*Xgpw 1143*	*Xwmc 329*	*Xwmc 406*	*Xgpw 7059*	*Xgwm 374*	*Xbrac 128*
	Chr. (4D)	Chr. (1B)	Chr. (3D)																
KS96HW10-1	+/+	-/-	-/-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Millennium	-/-	-/-	-/-	1?	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
N02Y5018	+/+	+/+	-/-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
N02Y5019	-/-	+/+	-/-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
N02Y5025	+/+	-/-	-/-	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
N02Y5003	-/-	+/+	-/-	0	1?	0	1?	1	0	0	1	0	1	0?	1	0	0	1	1
N02Y5096	-/-	-/-	-/-	1?	1	1	1	1	0	1	1	1	1	1	¢	1	1	1	1
N02Y5109	-/-	-/-	+/+	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
N02Y5117	+/+	-/-	-/-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
N02Y5149	+/+	+/+	-/-	1	1	0	1	1	0	0	1	0	1	1?	1	0	0	1	1
N02Y5156	-/-	+/+	-/-	1?	1	0	1	1	0	0	1	0	1	1	1	0	0	1	1
N02Y5163	-/-	+/+	-/-	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1
N02Y2016	+/+	+/+	-/-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Beaver (1RS.1BL)	-/-		-/-	0	0	0	1	1	0	0	1	0	1	1	1	0	0	1	1
Manaska	+/+	+/+	+/+	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1
CS N4A T4D	-/-	-/-	-/-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CS N4D T4B	-/-	-/-	-/-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Chinese spring	-/-	-/-	-/-	1	1	1	1?	1	1	1	1	1	1	1	1	1	1	1	1
CS N1B T1A	-/-	-/-	-/-	0	0	0	1	1	0	0	1	0	1	0?	1	0	0	1	1

*1 presence or absence of *Th. intermedium* fragments revealed by GISH (see section 3.8.2&Table 3.1), +/+ alien fragments of similar size seen (homozygous), -/- when no alien fragments seen, 1 presence of the marker allele, 0 absence of the marker allele, 1? most probably present, 0? most probably absent, # when DNA was not available for PCR, ¢ PCR not successful for technical reasons.

Table 4.6: List of the polymorphic 1BS markers applied in breakpoint mapping arranged in their most probable order. Markers were grouped into BP (breakpoints) I-IV and were ordered by the appearance of a wheat locus along the recombinant arm.

	BP-I					**BP-II**							**BP-III**	**BP-IV**			**BP-V**		
Line	*Xfc 618*	*Xgwm 0911*	*Xbarc 194*	*Xgpw 7059*	XBF4 74204	*Xgwm 1130*	*Xpsp3 000*	*Xwmc 230*	*Xgpw 363*	*Xwmc 406*	*Xwmc 49*	*Xucr _6*	*Xgwm1078*	*XksuD 14a*[*2]	*Xgwm 4144*	*Xgpw 1143*	*Xgwm 1100*[*1]	*Xgwm1 028*[*1]	*Xgwm 4435*[*1]
N02Y5018	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
N02Y5019	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
N02Y5003	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-?	+?	+	-?
N02Y5149	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+?	+	+
N02Y5156	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+?	+	+	+	+
N02Y5163	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
N02Y2016	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chinese spring	+	+	+	+	+?	+	+	+	+	+	+	+	+	-	+	+	+	+	+
CS N1B T1A	-	-	-	-	-	-	-	-	-	-	#	-	-	#	-	-?	-	#	-
	2- markers lost (N02Y5018, N02Y5019 and N02Y2016)					5-markers lost N02Y5163							12-markers lost N02Y5156	13-markers lost N02Y5149			16-markers lost N02Y5003		

+ presence of a marker allele, - absence of a marker allele, +? most probably present, -? most probably absent, # when DNA was not used for amplification, *1 proximal markers selected from Ganal and Röder (2007) map that delimited the resistant gene identified on the 1BS arm of wheat (see also Figure 4.10A), *2 the most likely position of *KsuD14a* is between *Xgwm*1078 and *Xgwm*4144, marker lost (bottom layer) refer to the absence of 1BS markers from a recombinant line/group, that are present in the control 'Chinese Spring' wheat.

Based upon the size of *Th. intermedium* chromatin detected with MMs, the 1BS recombinants were divided into 5 BP groups (Table 4.6 also see Figures 4.10&4.11). Line N02Y5018, N02Y2016 and N02Y5019 involved the smallest while N02Y50003 has incorporated the largest alien chromatin (Table 4.6 and Figure 4.11). Lines in BP-I (N02Y5018, N02Y2016 and N02Y5019) have lost only the two distal markers (Table 4.6, Figure 4.11). Size of the lost 1BS arm in N02Y5163 is also small as it has retained most of the distal markers like *Xgwm1130* and *Xpsp3000* (Figure 4.10) and is placed in BP-II group here (Table 4.6, Figure 4.11). It was clear from the GISH results (Chapter III) that the lost 1BS segment in N02Y5156 (BP-III) was larger than N02Y5163 (BP-II). The SSR-marker *Xgwm1078* also validated the GISH results (see Chapter III and Figure 4.11). All markers that identify BP-II are missing in N02Y5156 (Table 4.6). The applied MMs have shown that the size of *Th. intermedium* chromatin on the 1BS arm of N02Y5149 (BP-IV) is the second largest after N02Y5003 (Table 4.6, Figure 4.11).

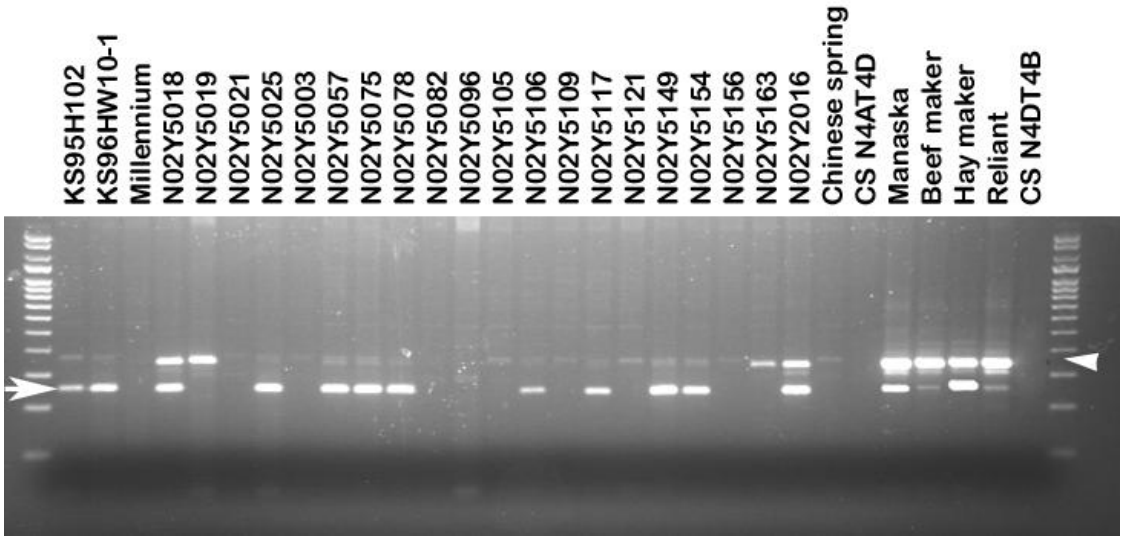


Figure 4.1: PCR amplification pattern of the WSR9 marker from wheat-*Th. intermedium* hybrid lines (Table 4.4). Arrow indicates the ~250bp amplicons produced by *Th. intermedium* and the WSMV-resistant lines with 4Ai#2S chromosomal translocation. This marker could also amplify ~350bp product (arrow head) from *Th. intermedium* and wheat lines with recombinant 1BS arm (Table 4.4). On either side of the agarose gel (2%) is a DNA length marker Q-Step 2.

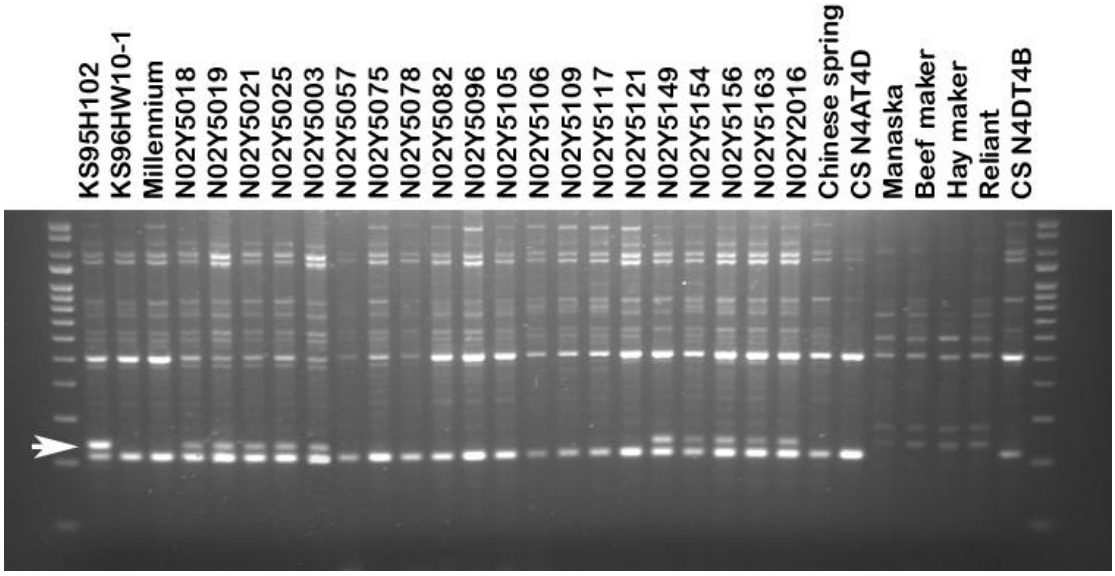


Figure 4.2: PCR amplification pattern of the UL-Thin-1 marker from wheat-*Th. intermedium* hybrid lines (Table 4.4). Arrow indicates the 239bp amplicons produced by *Th. intermedium*, two resistant lines with 4Ai#2S chromosomal translocation and all lines with 1BS recombinant chromosome (Table 4.4). On either side of the agarose gel (3%) is a DNA length marker Q-Step 2.

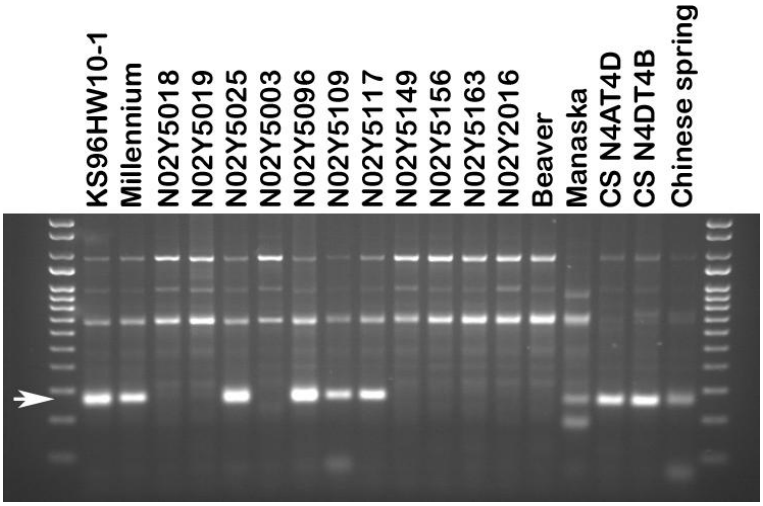


Figure 4.3: PCR amplification pattern of the *Xfc618* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates the 250bp amplicons produced by lines with no 1BS recombinant chromosome. The results indicated it is one of the most distal SSR located on 1BS of wheat (Table 4.6). On either side of the agarose gel (1.5%) is a DNA length marker Q-Step 2.

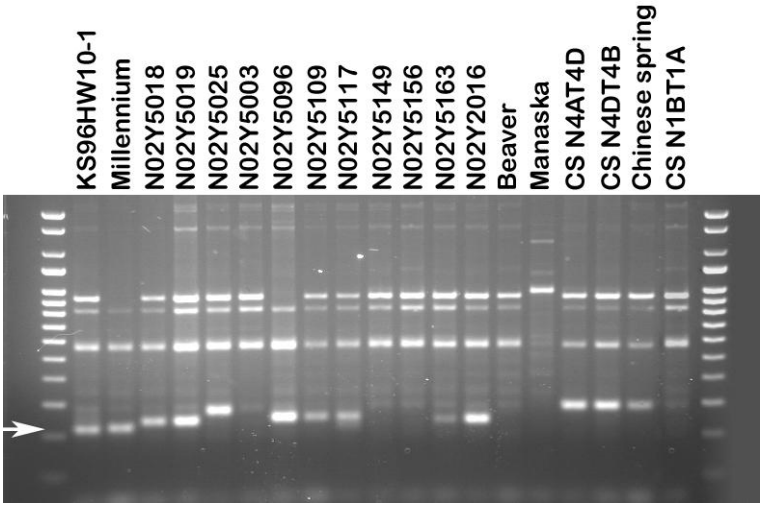


Figure 4.4: PCR amplification pattern of the *Xpsp3000* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates the 252-286bp amplicons produced by lines with normal 1BS and lost by few lines with recombinant 1BS (Table 4.6). On either side of the agarose gel (3%) is a DNA length marker Q-Step 2.

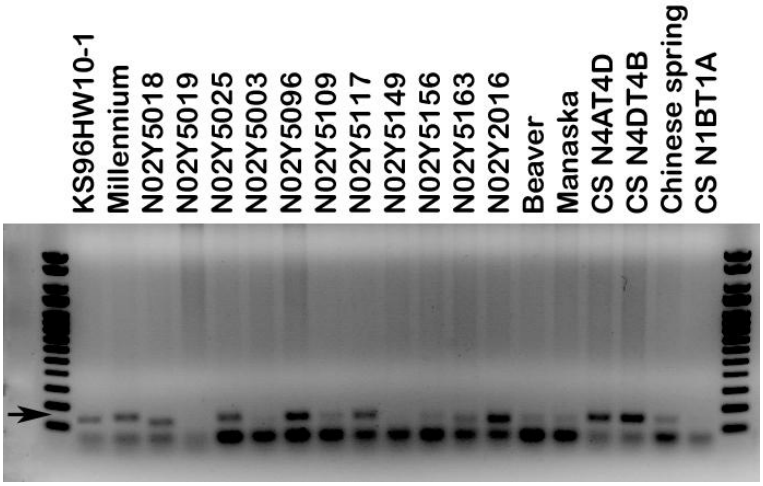


Figure 4.5: PCR amplification pattern of the *Xgwm1078* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates the 144bp amplicons produced by lines with no or a small 1BS recombinant chromosome (Table 4.6). On either side of the agarose gel (3%) is a DNA length marker Q-Step 2.

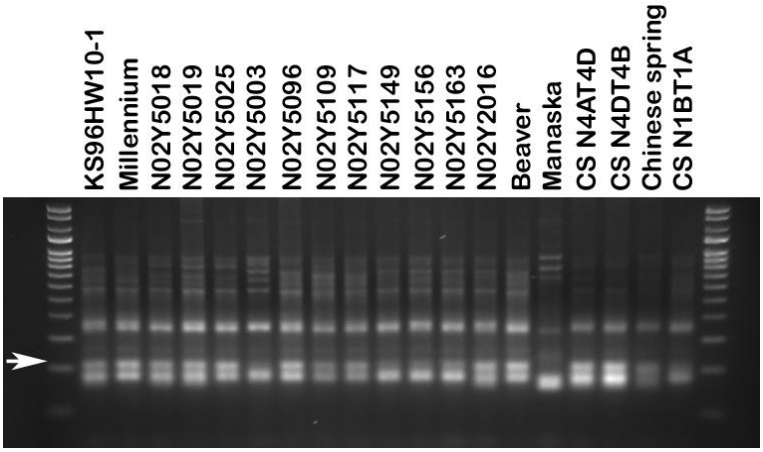


Figure 4.6: PCR amplification pattern of the *Xgpw1143* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates the 206bp amplicons produced by all lines the recombinant 1BS chromosome except N02Y5003 (Table 4.6). On either side of the agarose gel (3%) is a DNA length marker Q-Step 2.

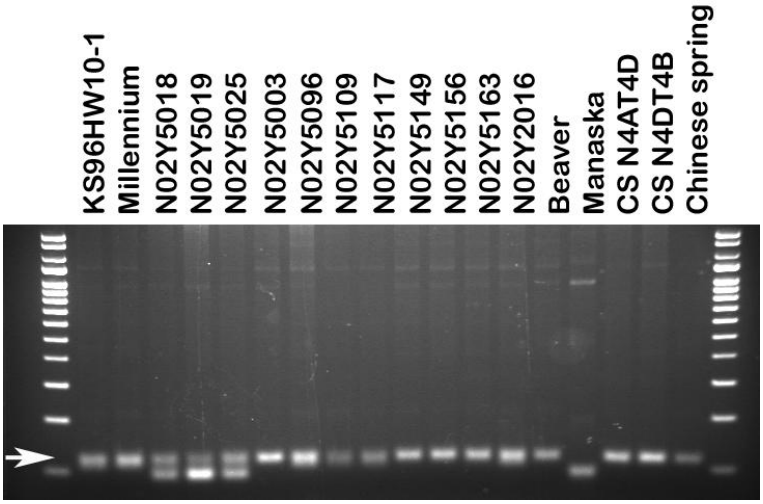


Figure 4.7: PCR amplification pattern of the *Xgwm1028* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates the 144bp amplicons produced by all lines with 1BS recombinant chromosome delimiting the resistant gene (Table 4.6). This marker could also amplify a fragment of ~100bp from *Th. intermedium* group-1 derived chromosomal segment (Table 4.4). On either side of the agarose gel (3%) is a DNA length marker Q-Step 2.

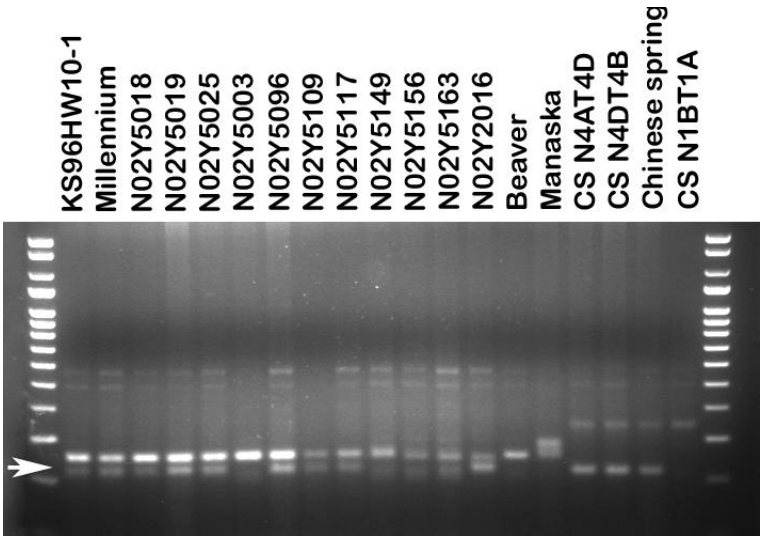


Figure 4.8: PCR amplification pattern of the *Xgwm4435* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates the 214bp amplicons produced by all lines with 1BS recombinant chromosome (Table 4.6). However, the amplification in line N02Y5005 is not very clear. This is the last (proximal) marker in Ganal and Röder *et al.,* (2007) map delimiting the resistant gene. On either side of the agarose gel (3%) is a DNA length marker Q-Step 2.

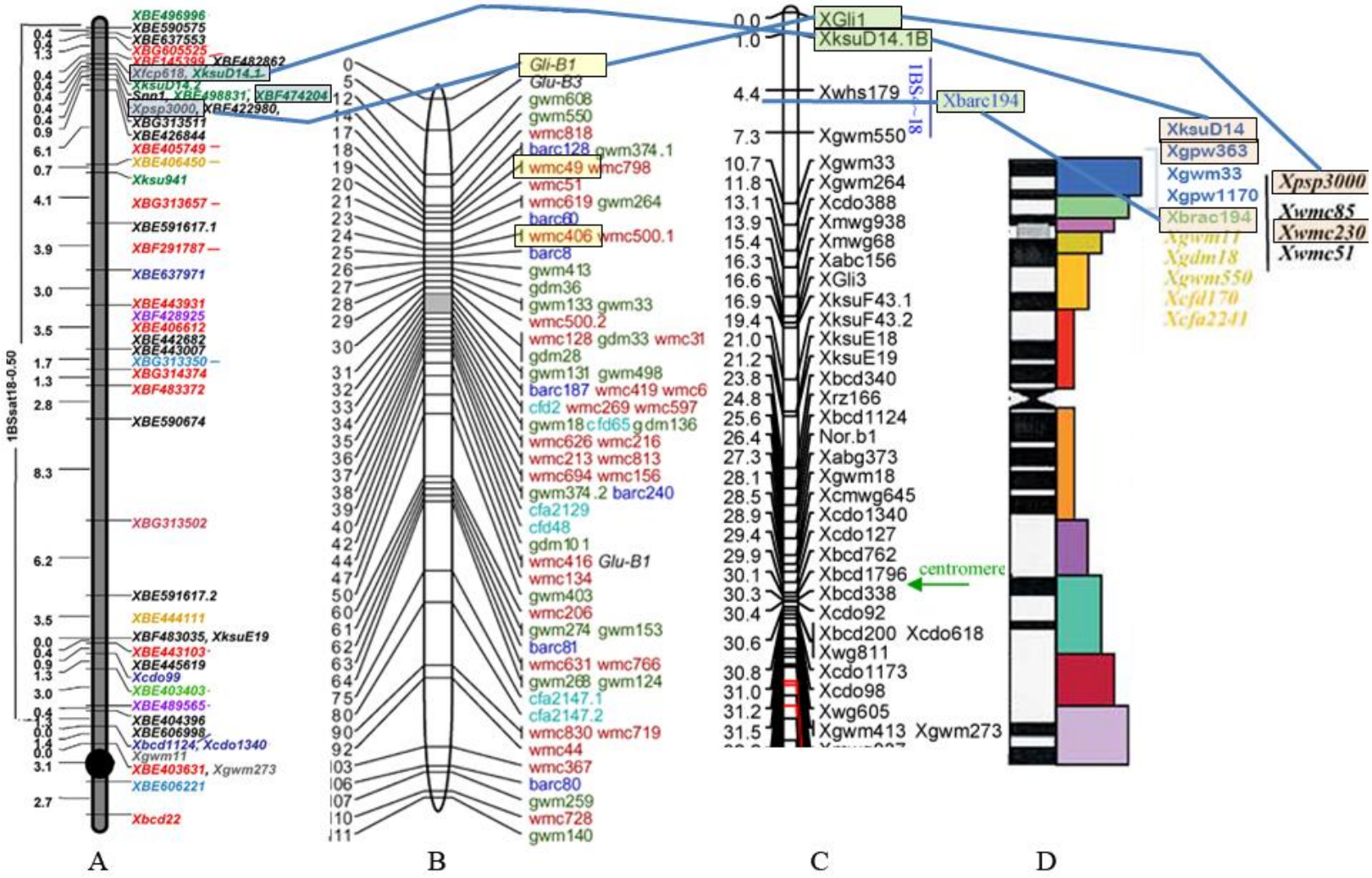


Figure 4.9: Genetic and deletion bin maps of wheat chromosome 1B modified from Reddy *et al.*, 2008 (A), Somers *et al.*, 2004 (B), Song *et al.*, 2005 (C) and Sourdille *et al.*, 2004 (D). Few of the 1BS polymorphic markers were selected from these maps (highlighted in boxes). Relative position of the same marker (connected by solid blue lines) varies among these maps (compare *Xpsp3000=Gli-1B* and *Ksud14)*.

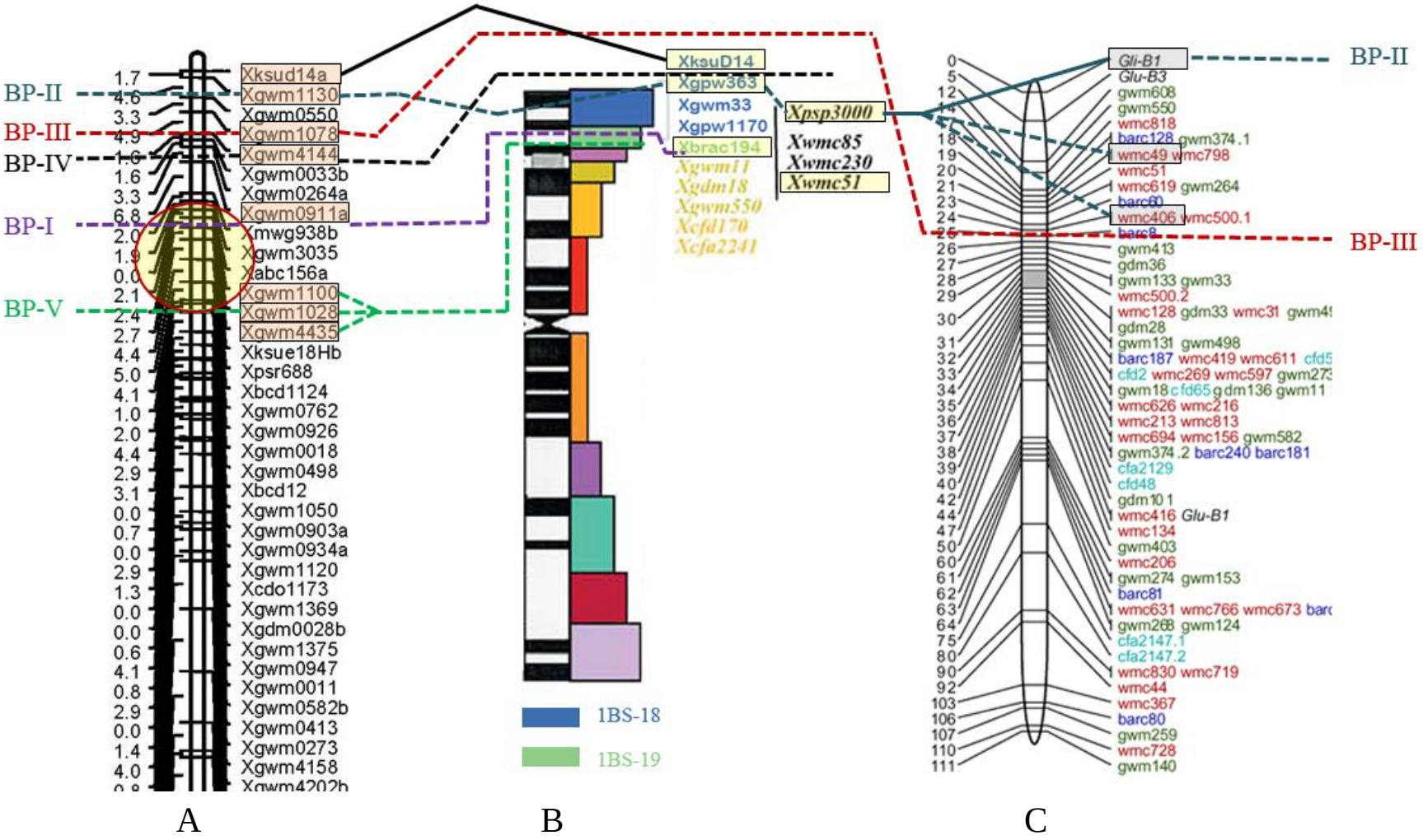


Figure 4.10: Identification of BPs (colour dash lines) and mapbased position of WSMV-resistant gene (red circle in A) identified with MMs on the 1BS arm of wheat. Position of the same marker in these maps is connected with a solid line. Maps of Ganal and Röder 2007 (A) Sourdille *et al.*, 2004 with first two deletion bins (blue and green) highlighted (B) and Somers *et al.*, 2004 (C). See section 4.5.3 and 4.5.4 above for detail.

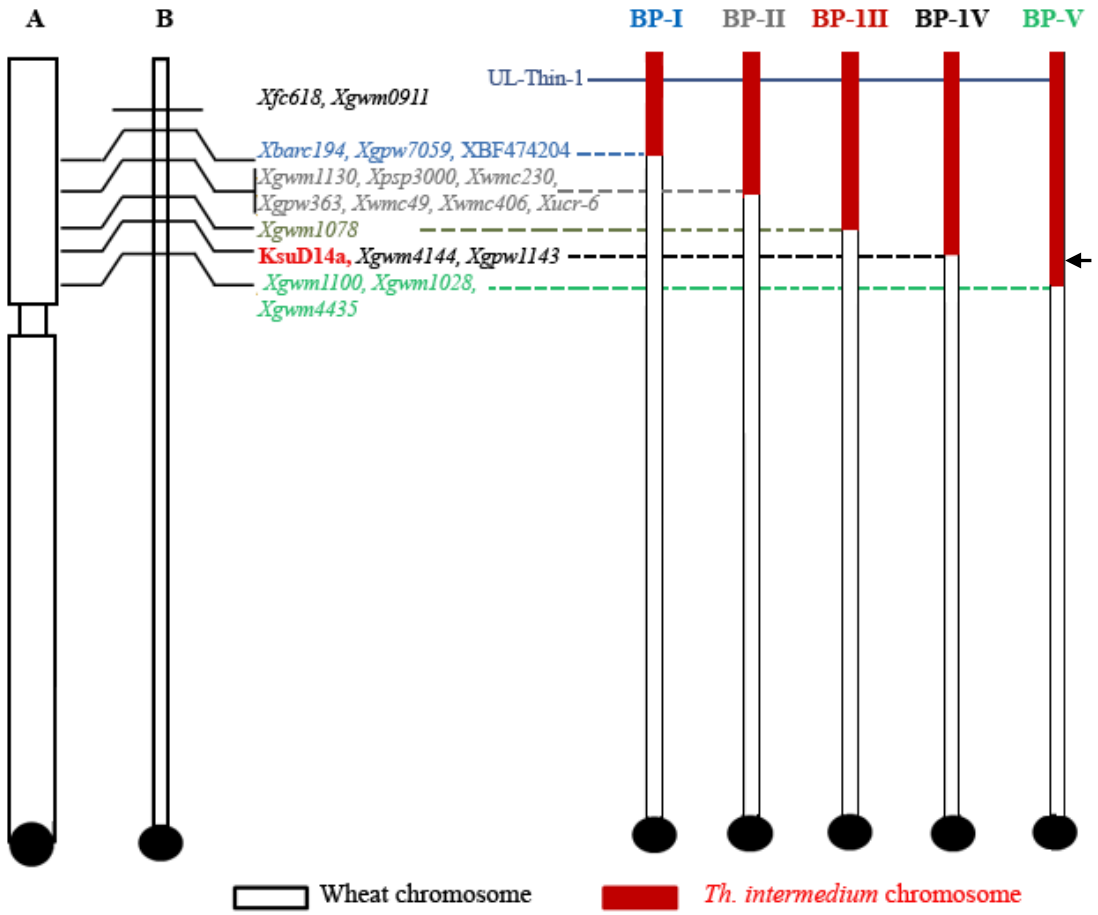


Figure 4.11: Genetic and physical map showing the BPs along the recombinant 1BS in wheat-*Th. intermedium* hybrid lines detected with MMs. White bars representing wheat 1BS and red bars represent *Th. intermedium* chromosomal segments, wheat centromeres are represented by dark circles. **(A)** Physical map of the wheat 1BS, indicating the physical BPs detected by MMs in the genetic map **(B)** and represented as BP-I, BP-II, BP-III, BP-IV and BP-V respectively. Physical length of *Th. intermedium* chromatin in BP-I may not be the same but this BP is identified by the same markers (see Table 4.6). Note only polymorphic markers are used to construct the map. Order and location of the markers along the wheat chromosome is based upon comparative analysis given in published maps (Figure 4.9) and represent the most probable order detected here. The *Th. intermedium* group-1 specific marker UL-Thin-1 co-linearity in different lines or position is unknown but is amplified from all 1BS recombinant lines and is most probably present in the distal region of recombinant 1BS (dark blue). Arrow indicates the position of WSMV-resistance gene.

4.4 Discussion

4.4.1 Efficiency of the previously known polymorphic markers and their significance in detecting WSMV-resistance

To date, *Wsm1* is the only alien derived gene used in bread wheat improvement against WSMV (Friebe *et al.,* 2009). Moreover, this gene is derived from a Robertsonian translocation of *Th. intermedium* to wheat in the form of 4Ai#2S.4DL translocation (Wells *et al.,* 1973, Friebe *et al.,* 2009). Novel WSMV-resistance was associated with recombinant wheat chromosomes 1BS and 3DL in addition to the already known 4DS (Chapter III). Therefore, to identify closely linked MMs applicable across a wide range of wheat germplasm selection and WSMV-resistance, and to analyse the *Th. intermedium* fragments of 1BS and 3DL in more detail, 26 PCR markers were tested, for their efficiency to detect *Th. intermedium* segments (Table 4.1). These markers were previously reported to have shown polymorphism between 'Chinese Spring' wheat and *Th. intermedium* (Qi *et al.*, 2007, Zhang *et al.,* 2002, Gao *et al.,* 2009, Kong *et al.,* 2009, Wang *et al.,* 2010, Fahim *et al.,* 2011). Recently, it was shown that dominant group-4 markers of *Th. intermedium* can amplify *Th. intermedium* chromatin from the group-2 and vice versa (Fahim *et al.,* 2011). Therefore, the applied MMs (Table 4.1) included those linked to WSMV-resistance originated from group-4 (Talbert *et al.,* 1996, Qi *et al.,* 2007, Fahim *et al.,* 2011), BYDV-resistance derived from group-2 (Zhang *et al.,* 2002, Wang *et al.,* 2010) and group-7 of *Th. intermedium* (Kong *et al.,* 2009, Gao *et al.,* 2009). However, neither the group-2 or group-7 markers could reveal any useful polymorphism linked to alien chromatin or WSMV-resistance in lines applied here (Table 4.1, Appendix 4.1). The P4 marker of Wang *et al.,* (2010) showed polymorphism between 'Chinese Spring' wheat and *Th. intermedium* (appendix 4.1). But, it also could not be correlated to WSMV-resistance (see Table 4.4).

The EST-marker BG263898 was described to amplify *Th. intermedium* DNA from the 4D recombinant lines (Qi *et al.,* 2007). Similarly, WSR2 was mentioned informative in 4A translocation lines and CL167 for detecting *Th. intermedium* chromatin from both 4A as well as 4D recombinant lines (Fahim *et al.,* 2011). However, none of the three markers (BG263898, WSR2 and CL16) could detect polymorphism between the WSMV-resistant and susceptible lines in the initial screening here and therefore, were not applied in further screening and WSMV-resistance.

Overall STS-J15, WSR9, WSR11, SWR17 and WSR65 markers proved to be the most promising among the known markers (Table 4.4, Appendix 4.1). The results revealed here confirm the findings of Talbert *et al.,* (1996) who assigned STS-J15 marker to *Wsm1* or 4Ai#2 of *Th. intermedium* and those of Fahim *et al.,* (2011) for WSR7, WSR11, WSR17 and WSR65 for group-4 recombinants (Table 4.1). These markers detected polymorphism among the resistant and susceptible lines (Table 4.4) and are linked to the *Wsm1* gene or 4Ai#2S arm of *Th. intermedium,* present as 4Ai#2S.4DL translocation in most of the WSMV-resistance lines studied here. None of these markers except WSR9 could detect alien chromatin other than 4Ai#2S (Figure 4.1). WSR9 marker amplified a PCR product of around 250bp from all the resistant lines with 4Ai#2S arm of *Th. intermedium* (arrow in Figure 4.1). It also amplified an additional fragment of around ~320bp from the 1BS recombinants including susceptible lines N02Y5019, N02Y5163 and resistant lines N02Y5018, N02Y2016 (arrow head in Figure 4.1). All tested markers were unable to detect the new WSMV-resistance genes identified here *i.e.* the *Wsm4* and *Wsm5* (see Table 4.4). It was therefore concluded these fragments have not derived from the known 4Ai#2S but, have most probably originated most from the homoeologous group-1 and group-3 of *Th. intermedium* and is present in wheat lines N02Y5003 and N02Y5109 respectively (see Table 4.4, Chapter III and below).

The WSR9 marker was previously reported informative for 4D and group-2 addition lines of *Th. intermedium* (Fahim *et al.,* 2011). In the current study WSR9 could identify alien chromatin present on 1B and 4D (Figure 4.1 and Chapter III). However, it could neither amplify the same locus from all 1BS recombinants nor could identify the 1BS resistance (*Wsm4)* in N02Y5003 (Table 4.4). The sequenced PCR product of WSR9 from both 4D and 1B recombinants, when BLASTN searched revealed it was a DNA sequence of *Pseudoroegneria stipifolia* from where the primers were originally designed by Fahim *et al.,* (2011). Further investigation of the sequence revealed it a repetitive DNA element (Appendix 4.1) with a potentially genome wide distribution, and hence was not suitable for determining chromosomal origin.

4.4.2 Identification of new MMs linked to WSMV-resistance

In the current study five new potential ESTs and one SSR marker were identified useful in detecting *Th. intermedium* chromatin (Table 4.2). These markers were able to show polymorphism between the WSMV-resistant and susceptible lines (Table 4.4). Four of these markers (Xpsp2530.1, UL-Thin-2, UL-Thin-3 and UL-Thin-4) are dominant group-4 markers and amplify 4Ai#2S DNA of *Th. intermedium* linked to *Wsm1* gene (Table 4.2 and Appendix 4.1). Only the UL-Thin-1 amplified a PCR product of 239bp from all 1BS recombinant lines (Figure 4.2). This marker also amplified fragments from two resistant lines KS95H102 and N02Y5154 with only a visible 4D recombinant chromosomes. Surprisingly, this marker has amplified a product of the same size from WSMV-susceptible line N02Y5021 (Table 4.4). Though the sequence-based polymorphism cannot be rule out that was not detected. However, earlier GISH results (Chapter III) did not reveal any *Th. intermedium* chromatin in line N02Y5021.

Beside ESTs, the 1BS SSR-marker *Xgwm1028* (Ganal and Röder 2007) also amplified a dominant *Th. intermedium* locus of ~100bp. This fragment was amplified only from *Th. intermedium* and three recombinant wheat lines N02Y5018, N02Y5019 and N02Y5025 (Figure 4.7). Line N02Y5018 and N02Y5019 have the recombinant chromosome 1BS. However, in line N02Y5025, *Th. intermedium* chromatin in the form of 4Ai#2S.4DL translocation was detected only (Chapter III and Table 4.4). It is noticeable, the newly identified markers also did not detect the group-3 derived resistance (*Wsm5)* identified in line N02Y5109 (Table 4.4).

The new polymorphic markers are given along the original description (see Table 4.2). None of them have been described as useful markers for detecting *Th. intermedium* chromatin before. These markers were not selected at random, but rather a thorough approach based on combining the results revealed in published papers as well as from the sequencing (Appendix 4.1) and cytogenetics results of this study (Chapter III) were applied. For example, the non-polymorphic markers from supplementary data of Fahim *et al.,* (2011) were employed to assess alien fragments of *Th. intermedium*. The basis for testing the two ESTs (UL-Thin-3 and UL-Thin-4) that proved polymorphic 4D markers, resided in the fact that these ESTs map in the distal deletion bin 4DS2-0.82-1.00 (http://wheat.pw.usda.gov). Recently, the *Wsm1* gene was physically mapped to the distal 20% of the recombinant 4DS (Friebe *et al.,* 2009), the said deletion bin is located within this 20% chromosomal territory. Similarly, the UL-

Thin-1 and UL-Thin-2 markers were tested as they are described *Th. intermedium* RAPD marker and *Agropyron cristatum* repetitive DNA sequence respectively. Earlier, the sequencing results of the known WSR9 marker revealed it, as a repetitive DNA sequence (Appendix 4.1). Recently, some repetitive DNA sequences of *Th. intermedium* were reported as highly informative PCR-markers in tracking WSMV-resistance (Fahim *et al.,* 2011). The FISH results obtained here, also revealed repetitive DNA often occupied critical chromosomal positions and provide a fingerprint for identifying the alien arm harbouring the *Wsm1* gene (see Figure 3.26). Thus the importance of combining cytogenetic and molecular data (association mapping) in developing closely or completely linked markers for desirable traits is recommended.

4.4.3 Breakpoint mapping of the 1BS recombinants

Pre-screening of the selected markers (Table 4.3) with DNA from six lines (section 4.3.1) showed polymorphism for 19 PCR markers (59.4%) that were applied for BP mapping (see Figure 4.11). The order of the markers and molecular breakpoints along the 1BS recombinants is based on comparative map analysis (Figure 4.9&4.10). Since, the Ganal and Röder (2007) markers delimited the resistant gene in this study (Table 4.6), mapbased position of the resistant gene (Figure 4.10A) is also based on their map, that was previously constructed with 70 recombinant inbred lines derived from the cross of Opata x W-7984. All the results described below are based on the polymorphic 1BS PCR markers (Table 4.6).

Eleven polymorphic markers from different sources were identified and added to the map of Ganal and Röder (2007) in the region flanked by *Ksud14a* and *Xgwm4435* (see Figure 4.10A and Table 4.6). By and large, the results revealed good agreement with the original sources for marker size and relative positions in the distal part of 1BS (see Figure 4.9 and below). Although, there is some discrepancy in the order and location of markers as well as few markers have given negative PCR results in the 'Chinese Spring' wheat (for example see *XksuD14a* in Table 4.6). However, this discrepancy is not rare in genetic maps constructed with different reference lines (see Figure 4.9). Similarly negative PCR amplifications could be argued as modification in the primer region that may alter primer binding sites (Rosato *et al.,* 2012). Furthermore, the BP-classes (BP-I to BP-V) identified with PCR markers for the 7-recombinant lines

here are not in the same order as revealed in published papers (compare Figure 4.10 and Figure 4.11).

The RFLP marker *Ksud14a* of Talbert *et al.,* (1994), which was mapped to the distal end of 1BS in Ganal and Röder (2007) detected multiple loci (Appendix 4.1). Two of them were polymorphic as previously described by Reddy *et al.,* (2008). However, neither of the two could be mapped to the distal end of 1BS. The results revealed here, suggest the most likely position for *XksuD14a* is somewhere between *Xgwm*1078 and *Xgwm*4144 (Table 4.6, Figure 4.11). The relative position of the same marker also varies in different published maps (see Figure 4.9). Similarly, the order of Ganal and Röder (2007) markers *Xgwm1130, Xgwm1078, Xgm0911* (Figure 4.10A) was slightly changed and were found as *Xgm0911, Xgwm1130 and Xgwm1078* respectively (Table 4.6). The *Xfc618, Xpsp3000* and XBF474204 markers were selected as they were described distal (Figure 4.9A) and polymorphic 1BS markers (Reddy *et al.,* 2008). Their location within the distal bin and order has remained perfectly the same (compare Figure 4.9A and Figure 4.11).

The SSR markers *Xfc618* and *Xgwm0911* are mapped here as distal 1BS markers (Figure 4.3 and 4.9). These results validated the position of *Xfc618*, which is the most distal SSR marker in the 1BS map of Reddy *et al.,* (2008). Surprisingly, some of the 1BS markers were also mapped on 1RS of Beaver (Table 4.5 and Figure 4.7). Indicating the low polymorphism between wheat 1BS and rye 1RS. This high degree of conservation could possibly be attributed to the high genic content of small arms (Peng *et al.,* 2004, Peng and Lapitan 2005, Sharma *et al.,* (2009). Likewise, the SSR markers *Xgpw363* and *Xbarc194* were physically mapped to 'Chinese Spring' wheat deletion bins 1BS.sat18-0.50-1.00 and 1BS.sat19-0.31-0.50 respectively (Sourdille *et al.,* 2004). However, Song *et al.,* (2005) mapped the *Xbarc194* to the deletion bins 1BS.sat18-0.50-1.00 (Figure 4.9). The mapping results revealed here go parallel with those of Song *et al.,* (2005), and suggests *Xbarc194* is located distal to *Xgpw363* (Table 4.6 and Figure 4.11).

Physical map data of *Xgpw7059, Xgpw363, Xwmc49* and *Xwmc406* has recently become available from GÉNOPLANTE given by Sourdille *et al.,* (2004) and available at (http://wheat.pw.usda.gov/ggpages/ssrclub/geneticphysical/). These SSR markers have been assigned to deletion bin 1BS.sat18-0.50-1.00. The results obtained here also go parallel to those of GÉNOPLANTE and not only the location of makers but also its orders correlate (Table 4.6 and Figure 4.11). Similarly, the EST-marker *Xucr_6,* was

mapped within the distal 40% region of wheat 1BS (Sharma *et al.,* 2009). It is a dominant wheat 1BS marker (Appendix 4.1) and the current study reveal the most likely position of this marker is within the deletion bin 1BS.sat18-0.50-1.00 (Table 4.6).

No physical map data is available for *Xgwm1078 and Xgwm4144*. However, the data obtained here suggests these markers lie proximal to the markers identifying BP-II (Table 4.6) and most likely occupy the same deletion bin. The GÉNOPLANTE SSR data reveal the physical map position of *Xgpw1143* within the deletion bin 1BS.sat18-0.50-1.00. Since the deletion stock of 1B with known fraction length (FL) values (Endo and Gill 1996) were not applied, therefore they could not be assigned exactly to a deletion bin, but these markers were mapped proximal to the markers detecting the BP-III (Table 4.6). Furthermore, Ganal and Röder (2007) markers *Xgwm1100, Xgwm1028* and *Xgwm4435* are the most proximal markers in the current study that delimited the WSMV-resistance gene identified on 1BS in line N02Y5003 (Figure 4.10A and Figure 4.11). The obtained results here confirm the order of these markers, but their exact physical map position was not determined. Based upon the location of markers in this preliminary data (Table 4.6), the most likely position of these markers could be the proximal 1BS.sat18-0.50-1.00 or distal 1BS.sat19-0.31-0.50 bin (compare translocation sizes chapter III and Table 4.6) and the resistant gene is predicted to be localized here (see Figure 4.10 and Figure 4.11).

4.4.4 Molecular breakpoint detection of the recombinant 1BS

The applied polymorphic markers (see Table 4.6) classified the seven 1BS recombinants into five BP categories (often referred to as BP classes, see also Figure 4.11). These markers are non-randomly distributed in these BPs and clusters are observed that are interspersed by regions of low marker density (see Table 4.6 and Figure 4.11). By and large, the order and location of these markers within the distal 1BS remained as described in the original sources (Table 4.3). However, all markers could not be mapped to 1BS nor the order and position of all markers remained the same (Table 4.5). This discrepancy could be because of the readily and reticulate crossing and back-crossing involved in modern wheat cultivars. Whereby, re-arrangements and shuffling of marker positions or even loss of it is not a rare phenomenon (see Nelson *et al.,* 1995 and pedigree Table 2.3). Other reason for these differences could be

previously undetected interstitial deletions (Qi *et al.,* 2003) or mutation in the priming sites (Rosato *et al.,* 2012).

The GISH results given in chapter III revealed the sizes of alien chromatin in line N02Y5018, N02Y5019 and N02Y2016 to be 22%, 22.4% and 21% respectively of the recombinant 1BS. The PCR markers also revealed similar results, and placed these lines in BP-I missing only the two distal markers (Table 4.6 and Figure 4.11). Further, the BP-I is identified by *Xbarc194, Xgpw7059* and XBF474204 markers. Song *et al.,* (2005) mapped *Xbarc194* to the deletion bin 1BS4-18 (Figure 4.9C) present in 2 deletion stocks TA4524 L9 and TA4512 L4 (Endo and Gill 1996). In these deletion stocks the distal 48% of the satellite is missing (Qi *et al.,* 2005). Similarly, the physical BPs in line N02Y5156 and N02Y5163 was calculated to be 20% and 18.5% respectively of the 1BS arm (Table 3.2). PCR markers analysis also supported the estimated size of line N02Y5156 to be larger than N02Y5163 (see Chapter III, Table 4.6 and Figure 4.11). However, the size of these two translocations (BP-II and BP-III) was revealed to be smaller compared to the 3-lines in BP-I in GISH analysis (compare Table 3.2 of Chapter III and Table 4.6). One reason for this discrepancy in size could be the strong labelling of *Th. intermedium* DNA, as brighter fluoresce may result in overestimation of fragment sizes than they exist in real (Lukaszewski *et al.,* 2005).

It was interesting to see that most of the markers clustered within a small 1.5% region of the physical map (compare Table 3.2 Chapter III and Table 4.6) present in line N02Y5163 or BP-II and absent in line N02Y5156 or BP-III (Table 4.6). Previously such clusters of markers or marker-rich regions along the chromosomes were related to regions with predominant deletions (Gill *et al.,* 1996). Markers *Xgpw7059, Xgpw363, Xwmc406* and *Xwmc49* identify BP-II (Table 4.6). These are the proximal 1BS markers in the current analysis for which physical map position is known. These SSR markers have been assigned to deletion bin 1BS.sat18-0.50-1.00 (Sourdille *et al.,* 2004). Most of the selected markers within this region were linked to important agronomic genes (section 6.4). These results are consistent with the results obtained for the corresponding region by Qi *et al.,* (2005). They mapped 55% of the EST loci within deletion bin 1BS.sat18–0.50–1.00 indicating it as a gene rich region of the 1BS.

As mentioned in chapter III, the estimated sizes of *Th. intermedium* chromatin in N02Y5003 was the largest followed by N02Y5149, which is 28.3% and 22.5% respectively of the recombinant 1BS arm. The MMs results also go parallel to the GISH results and showed that line N02Y5003 (BP-V) has lost most wheat markers (Table 4.6).

Wheat markers *XksuD14a* and *Xgwm4144* are missing in line N02Y5003 but present in N02Y5149 or BP-IV (Table 4.6 and Figure 4.11). It is certain, the WSMV-resistance in line N02Y5003 (BP-V) is from the *Wsm4* gene, that has most likely originated from the homoeologous goup-1 of *Th. intermedium* (Chapter III, see also Figure 4.10 and Figure 4.11). However, line N02Y5149 has got the *Wsm1* gene on the recombinant 4DS arm (see Chapter III) and the size of *Th. intermedium* chromatin on the 1BS arm is second large after N02Y5003 (see Table 3.2). Further, field resistance of N02Y5149 to WSMV is superior than any other line having the 4D or 1B fragments alone (see Figures 3.1A&B). Thus it was concluded, line N02Y5149 has either both the *Wsm4* gene (1BS) pyramided together with *Wsm1* gene (4DS), or the introgressed 1BS alien chromatin in line N02Y5149 has an enhancer gene and therefore, condition superior resistance than other experimental lines with 4D resistance alone (Figure 3.1A&B and Graybosch personal communication). Nevertheless, the other smaller 1BS fragments of Th. *intermedium* in the WSMV-resistant and susceptible lines are still worthy for further screening. As these alien fragments may be potential carriers of some other quality or resistant traits present on the group-1 of *Th. intermedium* (Hu *et al.*, 2011, also see Chapter III&VI).

The Ganal and Röder (2007) markers *Xgwm1100*, *Xgwm1028* and *Xgwm4435* delimited the resistant gene in line N02Y5003 or PB-V (Table 4.6). Thus taking the order of markers in the current analysis, WSMV-resistance gene may be located between *Xgwm4144* and *Xgwm1100* markers of the recombinant 1BS (arrow in Figure 4.11).

4.4.5 Application of molecular markers in breeding programmes and for identification of resistance genes

There are no chemicals available to combat viral infections and hence genetic resistance offer the only means of disease control (Hull, 2004). In this study a number of potential PCR markers were identified and tested, that could be applied in WSMV-resistance breeding and improvement programmes. Now PCR based screening will allow robust marker assisted selection for WMSV-resistance genes from the alien *Th. intermedium* and the 4Ai#2S chromosomal arm in particular. Two novel WSMV-resistance genes from the homoeologous group-1 and 3 of *Th. intermedium* were identified and designated as *Wsm4* and *Wsm5* translocated to wheat chromosome 1B and 3D

respectively. Interestingly, the 1B recombinant chromosomes in lines N02Y5018, N02Y5019, N02Y5003, N02Y5149, N02Y5156, N02Y5163 and N02Y2016 carried different amounts of alien material, and these breakpoints were identified with PCR markers (Figure 4.11). The *Wsm4* resistance gene was pinpointed to a 6% interstitial region of the *Th. intermedium* chromatin translocated to wheat chromosome 1BS. The current study provides basis of isolating the WSMV-resistance gene(s) from *Th. intermedium* and to conduct a more detailed analysis of the orthologous region on chromosome 1BS, using results from the whole genome sequencing project of wheat (http://www.wheatgenome.org/). For a more detailed discussion of the applicability of the results to breeding programmes and the isolation of novel resistance genes see Chapter VI General Discussion.

CHAPTER V: ORGANIZATION AND DIVERSITY OF THE TWO HIGHLY REPETITIVE DNA FAMILIES & DNA METHYLATION STUDIES OF THE DIPLOID AND POLYPLOID *TRITICEAE*

5.1 Introduction

5.1.1 Organization and diversity of two highly repetitive DNA sequences in *Triticeae*

A major fraction of eukaryotic nuclear DNA is comprised of repetitive non-coding DNA sequences that exist in the form of interspersed or tandem repeats and is present ubiquitously throughout the genomes (see Feng *et al.,* 2010 and Chapter I). The *Triticeae,* a heterogeneous group of some 400-500 diploid and polyploid species (Melderis, 1980), are characterized by large genome sizes ranging from 5500-17000Mb (Flavell *et al.,* 1974b, Bennett and Leitch, 1995b). Repetitive DNA accounts for more than 70-80% of their genomes while single copy DNA may account for less than 1% of the their genomes (Smith and Flavell, 1975, Heslop-Harrison, 2000a, Charles *et al.,* 2008). Thus the *Triticeae* members have two to five fold of repetitive DNA compared to the human genome, with similar or even higher degree of complexity and content of repetitive DNA (Stein, 2007).

The genome sizes in plants are remarkably diverse, with a 2350-fold variation among the angiosperms alone (Bennett and Leitch, 1995a, Bennett and Leitch, 2011). In flowering plants, total number of genes (~28000 per haploid genome) is relatively constant, while the repetitive DNA varies greatly (Caldwell *et al.,* 2004, Devos, 2010) and contributes mainly to the variation in genome sizes analysed (Wicker *et al.,* 2003). These repetitive DNA sequences may occur as satDNAs, concentrated at one or more distinct positions in the genome (Anamthawat-Jónsson and Heslop-Harrison, 1993, Vershinin *et al.,* 1994, Contento *et al.,* 2005) or with a widespread and disperse distribution throughout the genome in the form of transposable elements (Wicker *et al.,* 2009, Kuhn *et al.,* 2010, also see chapter I).

The vast majority of the cereal repeat elements are derived from LTR-retrotranspon (Bennetzen *et al.,* 1998) capable of rapid genomic turn over (Bennetzen *et al.,* 1998, Heitkam and Schmidt, 2009). The process seems to be irrevocable, as there is

no efficient mechanism to reverse colonize or delete the repeats (Bennetzen *et al.,* 1998, Devos, 2010) and thus the percentage of a genome comprised by repetitive DNA, reflects the evolutionary history of that species (Langdon *et al.,* 2000). Thus these elements have a major role in the interspecific divergence of species since their origin from a common ancestor (Schwarzacher, 2003b, Charlesworth *et al.,* 1994, Schmidt and Heslop-Harrison, 1998b, Shapiro, 2005) and understanding their role and nature are of great importance. Repetitive DNA, which was once considered as "junk", is now revisited as major player of genome evolution and understanding phylogenetic relationships (Vershinin *et al.,* 1995, Kejnovsky *et al.,* 2009, Kuhn *et al.,* 2010).

However, in spite of the recognized importance and the well-characterised role of few repetitive sequences their overall biological significance still remains obscure (Kuhn *et al.,* 2007, Kuhn and Sene, 2005, Shapiro, 2005, Nagaki *et al.,* 1998a, Nagaki *et al.,* 1998b). For example, some repetitive DNAs are involved in chromatin and chromosomal packaging (Vershinin *et al.,* 1995, Heslop-Harrison and Schwarzacher *et al.,* 2011a) while others are found at the centromeres and telomeres of chromosomes, having a significant role in replication and stabilization of telomeres (Moyzis *et al.,* 1988, Ma *et al.,* 2007, Schwarzacher 2003a). Repetitive DNA is abundant in eukaryotic centromeres, and comprises mainly large arrays of centromeric satellites interspersed with retrotransposons (Ma *et al.,* 2007, Mutti *et al.,* 2010, Heslop-Harrison and Schwarzacher, 2011a) although they vary in abundance and arrangement both within and among species (Wu *et al.,* 1991, Wu *et al.,* 2004, Orgel and Crick, 1980). In addition, some repetitive DNAs play an important role in pairing and recombination during meiosis, resulting in chromosomal rearrangements and are involved in regulation of gene expression (Kubota *et al.,* 1997, Schwarzacher and Heslop-Harrison, 1991, Schmidt and Heslop-Harrison, 1998b, Heslop-Harrison, 2000a, Schwarzacher, 2003). Some repeats are transcribed efficiently and few such as, ribosomal RNA genes have well understood function (Gerlach and Bedbrook, 1979, Wu *et al.,* 1994, Alexandrov *et al.,* 2001). With the availability of genomic sequence data, it is becoming more evident that certain repeats (such as e,g, the *CACTA* DNA transposon elements) are integral parts of important agronomic genes and are valuable sources of genetic diversity (Studer *et al.,* 2011).

Repetitive DNA sequences vary in length from a few to tens of thousands of base pair units tandemly arranged to form large arrays of up to several thousand kilobases (Charlesworth *et al.,* 1994, Henikoff, 2000, Jin *et al.,* 2004, Kuhn and Heslop-

Harrison, 2011, Schwarzacher, 2003b). Often long arrays of different repeat units coexist in the same genome referred to as library of satDNAs (Meštrović *et al.,* 1998). Several arrays of unrelated satDNAs may be present and mainly concentrate in the heterochromatic regions, either around the centromere, at interstitial or subtelomeric regions (Mutti *et al.,* 2010, Kuhn *et al.,* 2007, Kuhn *et al.,* 2009).

In cereals, tandemly repeated DNA sequences were first described in rye (Bedbrook *et al.,* 1980). Since then, several members of tandemly repeated DNA families have been described in other *Triticeae* members (Rayburn and Gill, 1986, McIntyre *et al.,* 1990, Anamthawat-Jónsson and Heslop-Harrison, 1993, Hagras *et al.,* 2005, Kishii *et al.,* 2001, Kishii and Tsujimoto, 2002, Nagaki *et al.,* 1998b, Tsujimoto *et al.,* 1997, Contento *et al.,* 2005). Some of the families are so-called species or genome specific (Vershinin *et al.,* 1994, Anamthawat-Jónsson and Heslop-Harrison, 1993, Nagaki *et al.,* 1995) others have a much wider distribution indicating that they evolved before the split of the various *Triticeae* species (Contento *et al.,* 2005, Bodvarsdottir and Anamthawat-Jonsson, 2003, Tang *et al.,* 2011).

5.1.2 Afa and pSc119.2 sequences in *Triticeae*

Afa and pSc119.2 are two highly abundant, tandemly repeated DNA sequence families in the *Triticeae* (Rayburn and Gill, 1986, McIntyre *et al.,* 1990, Anamthawat-Jonsson and Heslop-Harrison, 1993, Nagaki *et al.,* 1999, Contento *et al.,* 2005, see also Figure 1.5 chapter I). Afa, the so-called D-genome specific repeat was first cloned from the diploid D-genome as pAs1 (Rayburn and Gill, 1986). Later, the same sequence was isolated from other *Triticeae* members, with different names such as pHcKB6 (Anamthawat-Jonsson and Heslop-Harrison, 1993), dpTa1 (Vershinin *et al.,* 1994) and Afa, isolated from a variety of *Triticeae* members (Nagaki *et al.,* 1995, 1998a, 1998b, Tsujimoto *et al.,* 1997).

Nagaki *et al.,* (1995) assigned the name "Afa" to a family of the *Triticeae* repetitive DNA sequences, with an average length of ~340bp and *AfaI* restriction site (GTAC) at 150th bp position of every monomer. However, different *Triticeae* species analysed, revealed conserved size of the monomer unit, but the copy numbers and restriction sites varied among different genomes (Nagaki *et al.,* 1998). For example, the D-genome contains 100-fold more copies of Afa sequence than the B-genome of wheat (Tsujimoto *et al.,* 1997). Afa-family is extensively applied in wheat cytogenetics

research, as it produces unique *in situ* hybridization signals on sub-telomeric and interstitial regions of all D-genome chromosomes (Rayburn and Gill 1986, Mukai *et al.*, 1993, Vershinin *et al.*, 1994, Schwarzacher *et al.*, 2011, see also Figure 1.5 Chapter I) and can be used to identify recipient wheat chromosomes and alien chromatin transfers (see chapter III). To date, 96 Afa-sequences from 14-diploid and polyploid *Triticeae* members have been isolated (http://www.ncbi.nlm.nih.gov/nuccore/?term=afa). However, no Afa sequences from *Th. intermedium* have been described or submitted to the GenBank database so far (NCBI search result of 01-03-2012).

The repetitive DNA sequence of rye origin, pSc119.2, makes another highly important family of satellite DNA in *Triticeae* (McIntyre *et al.*, 1990). It is a family of 120bp repeat units, present in the major heterochromatic blocks of wheat and rye chromosomes (Taketa *et al.*, 2000, Contento *et al.*, 2005). This sequence was originally cloned form rye as pSc119 and was the first cereal repetitive DNA sequence to be cloned (Bedbrook *et al.*, 1980). It was sub cloned as pSc119.1, pSc119.2 and pSc119.3 (McIntyre *et al.*, 1990) and sequence analysis revealed only the pSc119.2 contains the 120bp repeat unit sequence (McIntyre *et al.*, 1990, Vershinin *et al.*, 1995). Later *in situ* and Southern hybridization results, accompanied with PCR and sequencing showed the presence of these repeat units in different diploid and polyploid *Triticeae* members (Mukai *et al.*, 1993, Schwarzacher *et al.*, 1995, Castilho and Heslop-Harrison, 1995, Contento *et al.*, 2005 and below). Similar to Afa-family, this sequence is also applied in wheat cytogenetic research, as this sequence is part of the large sub-telomeric and intercalary heterochromatic blocks in hexaploid wheat (see Figure 1.5 and chapter III). Around 90 members of the pSc119.2 repeat units have been isolated form 16-diploid and polyploid *Triticeae* species. However, no pSc119.2 sequence has been isolated from *Th. intermedium* (NCBI BLAST search result of 01-03-2012).

The aim of this study was to characterize and isolate two major repetitive DNA sequence families (Afa and pSc119.2) from 'Chinese Spring' and *Th. intermedium*. The amplified sequences were cloned and their sequence diversity and phylogenetic analysis carried out in order to follow the evolutionary history of these sequences through events like speciation and polyploidization. In addition both repeat types were applied as probes in FISH to investigate their chromosomal distribution and physical mapping of an alien chromosomal segments incorporating resistance gene.

5.1.3 Methylation patterns of repetitive DNA sequences in *Triticeae*

The lower C values in *Arabidopsis* and significantly higher values among the grasses is revealed by the fact that they contain 20-30% to more than 70% of their genomes made of repetitive DNA (see Flavell *et al.*, 1974, Taketa *et al.*, 2000, Salina *et al.*, 2011, and Chapter I). Most of such DNAs, as described above have little to no direct function for the host genome. Thus the bulk of plant nuclear DNA exists as genomic parasites (Nagaki *et al.*, 1998a, Schmidt and Heslop-Harrison, 1998, Heitkam and Schmidt, 2009) and thus, is epigenetically silenced through cytosine methylation, to protect the host genomes from their possible deleterious effects (Finnegan, 1989, Finnegan *et al.*, 1998, Suzuki and Bird, 2008, Law and Jacobsen, 2010, Lisch, 2009). Cytosine or DNA methylation is a stable epigenetic mark, found in the genomes of both prokaryotes and eukaryotes (Finnegan and Kovac, 2000a, Waterhouse *et al.*, 2001, Bender, 2004) and plays a significant role in genome organization and in regulating gene expression (Lisch, 2009, also see chapter I and below).

Bread wheat, a recent allopolyploid originated some 10,000YA through intergeneric hybridization (Sears, 1966, Feldman and Levy, 2009, Eckardt, 2010, Sakuma *et al.*, 2011). Being a polyploid, it can tolerate genomic changes that are not attainable at the diploid level (Sears, 1966, Feldman and Kislev, 2007). Further, the great success of polyploids is owed to their ability to select the finest possible combinations of genes that controls a trait from their donors (Dubcovsky and Dvorak, 2007, Kashkush *et al.*, 2002, Levy and Feldman, 2004). However, presence of two or more genomes within one nucleus exerts a considerable stress or "genomic shock" on the newly formed species (McClintock, 1984, Josefsson *et al.*, 2006, Gaeta *et al.*, 2007, Yaakov and Kashkush, 2010). Evidence of a wide range of genetic and epigenetic alterations including deletion events such as elimination of non-coding, low copy and high copy DNA sequences, gene conversion events and changes in the rDNA loci, have been well documented in both natural and synthetic polyploids (see Gaeta *et al.*, 2007). The natural and synthetic allopolyploids contain 2-10% less the amount of DNA than the sum of their diploid progenitors, indicating that DNA elimination occurs soon after allopolyploidization events (see Feldman and Levy, 2009). Further, it has been demonstrated that stress or unusual environmental stimuli like hybridization and tissue culture may cause heritable changes of cytosine methylation (Feldman and Levy, 2005b, Matzke *et al.*, 2009, Slotkin *et al.*, 2009). In plants, hybridization followed by the

accumulation and rise in the activities of transposable elements (TEs) is well documented (Comai *et al.,* 2003, Josefsson *et al.,* 2006, Tsukahara *et al.,* 2009). It is believed, hybridization introduces novel TEs into a host, lacking effective silencing mechanisms and thus results in increased TEs activity and other disruptions (Ågren and Wright, 2011) and below.

5.1.4 Methods of assessing DNA methylation

A number of techniques are available for detecting genomic content and patterns of 5-mC distribution and a number of others are rapidly evolving (Singal and Ginder, 1999, Jeltsch, 2002, Yang *et al.,* 2004). Early techniques for assessing total genomic 5-mC levels relied on digesting DNA into single nucleotides and then quantifying them either with high performance liquid chromatography (Wagner and Capesius, 1981), thin-layer chromatography (Bestor and Verdine, 1994) or liquid chromatography (Friso *et al.,* 2002). Later, global methylation patterns were quantified using restriction fragment length polymorphism with methylation-sensitive restriction enzymes combined with Southern hybridization (Bird and Southern, 1978, Kubis *et al.,* 2003a). Over the recent years, several other techniques such as the use of chloracetaldehyde that detect DNA methylation levels in a fluorescent assay, bisulfite sequencing and immunostaining with anti-methylcytosine antibody etc. have been developed (see Yang *et al.,* 2004, Singal and Ginder, 1999).

Several protocols based on bisulfite deamination reaction have been developed and used to detect 5-mC content. Most of these protocols use sodium bisulfite in a chemical reaction, which can selectively deaminate cytosine but not 5-mC to uracil (Frommer *et al.,* 1992). The resulted primary sequence change in the DNA then allows differentiation of cytosine from 5-mC. Once the reaction has completed, the sequence differences between the methylated and unmethylated bases can be exploited by direct sequencing (see Yang *et al.,* 2004). Methods based on bisulfite deamination are valuable, require a smaller amount of DNA are not labour intensive. However, these methods are usually limited as they can only study a single gene or locus at a time and sequencing is still out of reach in many of the developing countries.

The aim of this study was to exploit Southern hybridization with methylation sensitive and insensitive restriction enzymes and immunostaining with anti-5mC antibodies to investigate possible genomic disruptions that may arise from alien gene

transfers in these wheat-*Thinopyrum* hybrid lines. Though, Southern hybridization has the disadvantage of being laborious and need large amounts of high quality DNA, but it reveals comparative assessment of the DNA methylation patterns across different genomes. Here, both these techniques were employed so that DNA methylation levels may be assessed and compared at the global level as well as to investigate possible changes in methylation patterns of the volatile component of *Triticeae* genomes (TEs and satellites).

5.2 Materials and Methods

5.2.1 DNA extraction, restriction enzyme digestion and gel electrophoresis

Total genomic DNA was extracted from the *Triticeae* species as described in M&M chapter II. The DNA was digested with restriction endonucleases, including isoschizomers *Msp*I-*Hpa*II and *Bst*NI-*Scr*FI as well as *Mcr*BC (New England BioLabs). A summary of the restriction sites for each enzyme is given below (Table 5.2). Restriction digestion and gel electrophoresis conditions were as described earlier in M&M chapter II.

Table 5.1: List of *Triticeae* species used in the study.

Species	**Line/variety/ land race**	**Genome**	**Remarks**
T. durum		AABB	Durum wheat or macaroni wheat
Ae. Tauschii		DD	Jointed goatgrass
Th. intermedium	Manaska	$JJJ^{S}J^{S}SS$	Intermediate-wheat grass, source of WSMV-resistance
T. aestivum	Chinese Spring	AABBDD	Wheat cultivar
T. aestivum	Millennium	AABBDD	WSMV-susceptible wheat cultivar
T. aestivum	KS96HW10-1	AABBDD	WSMV-resistant line with recombinant 4D chromosome
T. aestivum	N02Y5003	AABBDD	WSMV-resistant line with recombinant 1B chromosome
T. aestivum	N02Y5117 (MACE)	AABBDD	WSMV-resistant line with recombinant 4D chromosome
T. aestivum	N02Y5109	AABBDD	WSMV-resistant line with recombinant 3D chromosome
T. aestivum	N02Y5163	AABBDD	WSMV-susceptible line with recombinant 1B chromosome

Table 5.2: Summary of the restriction site(s) and methylation sensitiveness of *Msp*I, *Hpa*II, *Bst*NI, *Scr*FI and *Mcr*BC endonucleases.

Enzyme	Restriction site	Site(s) cut	Site(s) not cut
*Msp*I	5'-CCGG-3'	CCGG C^{m}CGG	^{hm}CCGG ^{m}CCGG ^{m}C^{m}CGG
*Hpa*II	5'-CCGG-3'	CCGG ^{hm}CCGG	^{m}CCGG C^{m}CGG ^{m}C^{m}CGG
*Bst*NI	5'-CCNGG-3'	CCNGG ^{m}CCNGG C^{m}CNGG ^{m}C^{m}CNGG	
*Scr*FI	5'-CCNGG-3'	CCNGG ^{m}CCNGG	C^{m}CNGG ^{m}C^{m}CNGG
*Mcr*BC	5'-Pu^{m}C ($N_{40\text{-}3000}$) Pu^{m}C-3'	Pu^{m}C ($N_{40\text{-}3000}$) Pu^{m}C Pu^{hm}C ($N_{40\text{-}3000}$) Pu^{m}C Pu^{m}C ($N_{40\text{-}3000}$) Pu^{hm}C Pu^{hm}C ($N_{40\text{-}3000}$) Pu^{hm}C	PuC ($N_{40\text{-}3000}$) Pu^{m}C Pu^{m}C ($N_{40\text{-}3000}$) PuC Pu^{hm}C ($N_{40\text{-}3000}$) PuC PuC ($N_{40\text{-}3000}$) Pu^{hm}C

^{m}C: methylated cytosine, ^{hm}C: hemi-methylated cytosine, Pu: purine bases (A or G)

Sources: Yoder *et al.,* 1997, Jeddeloh *et al.,* 1998, Liu *et al.,*1998, Stewart and Raleigh 1998, Kubis *et al.,* 2003, Han *et al.,* 2003, Xu *et al.,* 2009, Yaakov and Kashkush 2010, Cohen-Karni *et al.,* 2011 and Mette *et al.,* 2002.

5.2.2 Primer design, PCR amplification and amplicons purification

New primers were developed and applied to amplify at least one complete repeat unit of Afa and pSc119.2 sequences (Table 5.3). The primers design and PCR conditions were the same as described in M&M chapter II.

5.2.3 Cloning and sequence analysis of the Afa and pSc119.2 sequences

Eluted PCR products of Afa1, F25/R147 and pSc119.2-AF/AR from 'Chinese Spring' and *Th. intermedium* were cloned and their sequences were analysed as described in M&M chapter II (section 2.2.7-2.2.8). For Afa-family, 20 clones of *Th. intermedium* and 'Chinese Spring' were sequenced, while for pSc119.2 sequence, 24 clones from each were sequenced.

5.2.4 *In situ* hybridization and Immunostaining

For *in situ* hybridization and immunostaining see M&M chapter II (section 2.2.11-2.2.12).

5.2.5 Southern hybridization

For Southern hybridization see M&M chapter II (section 2.2.15).

5.2.5.1 Probes for Southern hybridization

For probes detail see M&M chapter II (section 2.2.9).

Table 5.3: List of PCR primers used to amplify repetitive DNA sequences.

Sr#	Primer name	Sequence	Tm (°C)	Source
1	F25 R147	GTGCTGATGACCGASACG GCACTCGCAGTTTTGGCCG	60	Contento *et al.*, 2005
2	F106 R208	CGGTGAGTGATAGTCCACG GGGGTCCCGGAGTGATTTCC	60	Contento *et al.*, 2005
3	R42	CCCCGGGGTGCGTTTACG	60	Contento *et al.*, 2005
4	AS-A AS-B	GATGATGTGGCTTGAATGG GCATTTCAAATGAACTCTGA	58	Nagaki *et al.*, 1995
5	pSc119.2-A	F: GGATTGCAAGGCCAGAATCG R: GTGCGTTTACGTGTCGGTC	60	
6	pSc119.2-B	F: AGGTAATCTTCCAACAGGTG R: AAATCACCCCGGTACCC	60	
7	pSc119.2-C	F: CTTCCAACAGGTGCATGGT R: AATCCCCGGATCAGCATAG	60	
8	pSc119.2-D	F: AGGATCCTTGGCTATGCTGA R: ATCTGGATTGAAGACACACCTC	60	
9	Afa1	F: GATGATGTGGCTTT**T**GAATGG R: TCGGAATTCATTTGTAGTGC	58	
10	Cas2probe	F: TCATTGTCTTCCATCATAACC R: GTCGTCCTACATAAACCCTTC	55	Sergeeva *et al.*, 2010
11	LTR6150	CTGGTTCGGCCCATGTCTATGTATCCA CACATGTA	53	Teo *et al.*, 2005

Note: the underlined T is indicating to the singly bp difference between AS-A of Nagaki *et al.*, (1995) and the here designed Afa1-F primer.

5.3 Results

5.3.1 PCR amplification of Afa and pSc119.2 sequences

Both sets of Afa primers (Nagaki *et al.,* 1995 and Afa1) were applied to amplify Afa-family sequences from 'Chinese Spring' and *Th. intermedium*. However, primers and conditions described in Nagaki *et al.,* (1995) amplified the incomplete repeat units of 260bp only (Appendix 5.1). Complete Afa repeat units of 340bp were amplified with Afa1 primers developed in this study by aligning dpTa1 (Vershinin *et al.,* 1994) and another related sequence of pHcKB6 (Anamthawat-Jonsson and Heslop-Harrison, 1993). Forward primer (Afa1-F) was designed within dpTa1 and reverse (Afa1-R) from pHcKB6. The comparison revealed, sequence of the Afa1-F primer varied from Nagaki *et al.,* (1995) AS-A by only one base pair (see Table 5.3).

The positions of forward and reverse Afa1 primers within the long array Afa units are indicated (Figure 5.2A). As expected, Afa1 primers amplified the tandem arrays of Afa-family repeats from both 'Chinese Spring' and *Th. intermedium*. The amplicons were comprised of monomer, dimers and multimers (Figure 5.1A) in the form of a ladder which is characteristic of the repetitive DNA sequences (Vershinin *et al.,* 1994, Kubis *et al.,* 2003, Kuhn *et al.,* 2005). Smears are usually seen in repetitive DNA amplification however, it was successfully reduced by manipulating the extension time in PCR reaction. These smeared fragments are presumably representing some of the incomplete repeat units present in the long arrays of the Afa-family sequences, as well as dispersed single or double units throughout the genome (Nagaki *et al.,* 1995, Contento *et al.,* 2005). In some instances when higher amounts of DNA were used smears re-appeared (personal observation). The monomers, dimers and trimers obtained with Afa1 primers pair, corresponded in size to around 260bp, 400bp and 600bp products respectively (Figure 5.1A and Figure 5.2B). These amplicons were compared with the 1015bp sequence map of pAs1 (Rayburn and Gill 1986) given in Nagaki *et al.,* (1995). The results were parallel to those given in the map for product size. Thus, prominent bands from both 'Chinese Spring' and *Th. intermedium* were cloned (arrow in Figure 5.1A) with enough confidence of the target satellite sequence. The Southern hybridization results has revealed fewer copies of Afa-family in *Th. intermedium* genome than 'Chinese Spring' (see below) and PCR results confirmed these findings too (compare higher PCR bands in Figure 5.1A).

Four newly developed primers were applied in conjunction with the published F25-R147, F106-R42 and F106-R208 (Table 5.3) to amplify pSc119.2 sequences from 'Chinese Spring' and *Th. intermedium*. New primers were designed according to their anchoring position within the original pSc119.2 sequence (McIntyre *et al.*, 1990) and are distributed throughout the unit. The positions of pSc119.2-AF/AR are shown along the complete pSc119.2 sequence only (Figure 5.3A) as the amplicons of pSc119.2-AF/AR and F25/R147 (Figure 5.3B) were cloned and analysed (see below). The new primer pairs (Table 5.3) proved very robust and amplified the tandem array of pSc119.2 sequence in the form of a ladder from both *Th. intermedium* and 'Chinese Spring' (Figure 5.1B&C).

For investigating the higher order repeat structure, various combinations of the primers were used that included different combinations to the original design (compare lanes A, B, C and D with E, F, G, H, I, J, K, L, M, N and O in Figure 5.1B&C). All primer combinations except three amplified PCR products and the complex banding patterns indicate that repeat units are present in all sorts of combinations of head-head, head-tail and tail-tail with possible degeneration in their long range organization. It was interesting to see that different combination resulted in polymorphic PCR products that varied significantly between 'Chinese Spring' and *Th. intermedium* (see Figure 5.1B&C). A possible explanation could be the indels of different elements within the long arrays of the sequence, although is no sequence data is available to prove this.

Primer combination pSc119.2-CF/CR and pSc119.2-BF/CR (lane C and I in Figure 5.1B) resulted in almost identical amplification (Figure 5.1B). Reverse primer pSc119.2-CR was common between the two lanes, indicating the products in both lanes are amplified most probably by pSc119.2-CR alone, giving further evidence of inverted repeat units. Further, the FISH and Southern hybridization results have shown the existence of relatively low copy number of pSc119.2 in *Th. intermedium* genome (Figure 5.9 and below). However, PCR showed almost identical pattern of amplification form both 'Chinese Spring' and *Th. intermedium* with some primer combinations (see lanes A, G, Q, R and S in Figure 5.1B and 5.1C).

5.3.2 Sequence analysis of Afa and pSc119.2 repeats

Prominent bands of Afa1 (arrow in Figure 5.1A), pSc119.2-AF/AR and F25/R147 (dimers, trimers and tetramers from lane A^* and R^* in Figure 5.1B and 5.1C) were cloned and sequenced from 'Chinese Spring' and *Th. intermedium*. Out of 40-Afa clones 33 were sequenced successfully with M13F and M13R and were 99% or above identical. Out of 48 pSc119.2 clones, 46 clones including monomers, dimers or even complete trimer were sequenced successfully. Only 2 clones were short corresponding to incomplete repeat units. Overall 9 clones were not suitable and were omitted from further sequence analysis.

Sequencing of Afa1 products (arrow in Figure 5.1A) resulted in a sequence of around ~590bp long (Figure 5.2B). Primer sequences were deleted and the remaining sequence of 551bp long, comprised of one and a half repeat unit (338bp and 213bp) was further evaluated (Figure 5.2B). The repeat units of both Afa (331-340bp) and pSc119.2 (116-119bp) family sequences were arranged in head-to-tail organization (Figure 5.2A&5.3A). Start of both monomer units was taken arbitrarily and was just after the Afa1-F for Afa-family sequences (Figure 5.2A&B) and within pSc119.2-AF primer sequence for pSc119.2 monomers (Figure 5.3A&B). Monomers of both Afa and pSc119.2 families were aligned (see multiple sequence alignment files Appendix 5.1) and generated consensus sequences (339bp and 118bp) were used for homology search and database mining.

A total of 248 BLAST hits (within 68 genomic and 20 BAC clone sequences) showing 85-100% identity were obtained for Afa sequences (see Appendix 5.1) while for pSc119.2 a total of 177 BLAST hits (within 80 genomic sequences) displaying 72-98% homology were obtained. Out of the 80 pSc119.2 homologous sequences, 73 sequences were already described as *Triticeae* satellites and repeats while the remaining 7 sequences are published as RAPD or genomic AFLP sequences (see Appendix 5.1) and are reported here as pSc119.2 related sequences. However, neither all Afa nor pSc119.2 hit sequences could be used in this analysis as most published Afa sequences are 260bp long only. Furthermore, the start point of some published Afa and pSc119.2 sequences was in the middle of sequences isolated here.

For phylogenetic analysis, sequences that showed ~90% coverage of the query files were used (see homology search tables Appendix 5.1). A total of 141 Afa-family sequences (including 33 sequenced units and 109 hits from NCBI) homologous to the original pAs1 monomers, and 127 pSc119.2 units (including 83 sequenced and 44 hits

from NCBI) were analysed. Afa is an AT rich (64%) sequence while pSc119.2 is a GC rich (53.4%) sequence. Afa contains both direct and indirect repeats along with one or two *AfaI* sites (GTAC). The longest inverted repeated region is 29bp (Figure 5.4A). The length of complete repeat unit in all sequenced clones was from 331-340bp (see multiple sequence alignment file Appendix 5.1). However, the position of *AfaI* site was variable. Thus the present results are consistent to those previously obtained (Tsujimoto *et al.*, 1997, Nagaki *et al.*, 1998). Few of the Afa units isolated here were internally polymorphic and contained *Eco*RI- restriction sites (Appendix 5.1). Similarly, pSc119.2 sequence has two TaqII (GACCGA) restriction sites, a 13bp inverted repeat region and a 14bp direct repeat region within the 118bp unit (Figure 5.4B).

5.3.3 Phylogenetic analysis

Phylogenetic analysis of the Afa and pSc119.2 families was carried out by Maximum Likelihood (ML) method in MEGA5 (Tamura *et al.*, 2011). The analysis involved 141 and 127 nucleotide sequences of Afa and psc119.2 respectively. Afa-family sequences showed strong sequence grouping that was evident from the deeply branched phylogenetic tree, with high bootstrap support values (Figure 5.5). Unlike Afa-family, pSc119.2 sequences did not show strong sequence grouping. Further, the tree was not deeply branched and nodes were with low bootstrap values (Figure 5.6). Comparison of the evolutionary history of the both repeat families (Figure 5.5&5.6) revealed some insights of the *Triticeae* genomes. Homogenization and amplification events were inferred to have involved both new and ancestral repeat units. A model, leading to the homogenization of both repeat units in *Triticeae* genomes was proposed (Figure 5.7, for further detail see discussion).

Overall, Afa sequences clustered into three large clades A, B and C (Figure 5.5). Clade-A comprised of 50 sequences exclusively of *H. vulgare* origin, and were subdivided into two sub-clades (sub-clade A1&A2). The sub-clade A1 contains sequences predominantly of *H. vulgare* chromosome-3 origin, while sub-clade A2 contains mostly sequences from chromosome-5 (clade A Figure 5.5). Clade B included 69 sequences, and was further divided into three sub-clades (B1, B2 and B3). By and large, clade B was dominated by sequences of wheat origin (TA= *T. aestivum*, CS= Chinese Spring). However, it also included sequences of *Ae. tauschii* (AE), *H. vulgare* (HV), *Th. intermedium* (Thin), *T. turgidum* (TT) as well as *T. urartu* (TU). The clade-C

included 22 sequences, including those of *T. turgidum* and *T. urartu* (sub-clade C1) and *T. aestivum* (sub-clade C2). The Afa-family sequences from *T. aestivum* (TA or CS) and *H. vulgare* (HV) and *Ae. tauschii* (AE) clusters significantly (see clades A&B Figure 5.5) but those of *Th. intermedium* (Thin), *T. turgidum* (TT) and *T. urartu* (TU) did not and are dispersed throughout the tree (Figure 5.5). The results indicated clustering of Afa sequences heavily relied on copy number of the repeat present. These results are consistent with the results obtained for the same family sequence by Tsujimoto *et al.,* (1997) and Nagaki *et al.,* (1998).

For convenience, clusters of the pSc119.2 sequences were divided into four main clades D, E, F and G (Figure 5.6), although clades E and F are not very clearly separated. Clade D included 34 sequences, and can be divided into three sub-clades, D1, D2 and D3. Clade E is comprised of 46 sequences and was subdivided into five sub-clades, E1-E5. Similarly, clade F included 29 sequences and clade G is comprised of 18 sequences exclusively of *Hordeum vulgare* origin. Except *Th. intermedium* (sub-clade D1) and *Hordeum vulgare* sequences (clade G) and few *T. aestivum* sequences (sub-clade E5), no significant sequence clusters were evident in all analysed pSc119.2 sequences (Figure 5.6). All *Th. intermedium* sequences were isolated during this study and were 95% identical, while *Hordeum vulgare* sequences of clade G were identified in BACs (Appendix 5.1) and were present in head-to-tail organization. Therefore, these sequences showed low diversity and significant grouping. The original pSc119.2 repeats (McIntyre *et al.,* 1990) were grouped at the middle of the tree together with *Th. intermedium* sequences isolated here (sub-clade E3, Figure 5.6). Similarly, other pSc119.2 sequences that made the sub-clades and positioned close to one another mostly (but not always) belonged to the same species or cloned as dimers, trimers or tetramers (compare sub-clades C1 & D2, Figure 5.6) or were amplified with the same primers (for example sub-clade D1&D2 Figure 5.6 are amplified by pSc119.2-AF/AR) or arranged side by side in the sub-clades (sub-clades D1-E1 Figure 5.6). It was evident that sequences from all species are distributed throughout the tree with no effect of the ploidy level or domestication. Unlike Afa-family sequence, variation was independent of the copy number in a genome. These results are similar to those obtained previously in species such as *T. monococcum*, *T. tauschii* or *L. mollis* where the pSc119.2 sequences are not abundant (Mukai *et al.,* 1993, Cuadrado *et al.,* 1995, Contento *et al.,* 2005).

5.3.4 *In situ* hybridization (ISH)

ISH with Afa, dpTa1 and pSc119.2 sequences revealed distinct fingerprints for the chromosomal arm (4Ai#2S) that carries the WSMV-resistance gene in hybrid wheat lines (see chapter III). Therefore, not only were these repetitive sequences were isolated from 'Chinese Spring' and *Th. intermedium* (see above), but also applied to karyotype *Th. intermedium* and identify the origin of chromatin transferred in both hybrid wheat lines as well as in the wild *Th. intermedium* genome (Figure 5.8, see also chapter III). The Afa-family sequence when used in conjunction with pSc119.2 sequence (Figure 5.9) confirmed the alien arm that carries *Wsm1* gene, identified by strong centromeric Afa and terminal pSc119.2 sites (arrow in Figure 5.9).The karyotype of *Th. intermedium* shows at least two pairs of chromosomes carrying centromeric dpTa1 sites (Figure 5.8) and prominent bands chromosomal arms representing interstitial heterochromatic blocks. However, weak hybridization signals along the euchromatin on few chromosomes was also seen, suggesting the presence of complete or degenerate repeats dispersed throughout the chromosomes (see Figure 5.8,5.9 and below).

5.3.5 DNA methylation

Genome wide DNA methylation patterns of diploid and polyploid *Triticeae* species (Table 5.1) was assessed by immunostaining with anti-5-mC and Southern hybridization. Immunostaining was combined with *in situ* hybridization using Afa/dpTa1 and pSc119.2 repeat units or with the total genomic DNA of *Ae. tauschii* and *Th. intermedium* probes. Southern hybridization was carried out with Afa, pSc119.2, LTR-probe and Cas2 probes (see below).

5.3.5.1 Immunostaining with anti-5-mC antibody

The only diploid species analysed here by immunostaining with anti-5-mC antibody, was *Ae. tauschii* (2n=14). Almost all analysed metaphases (95%) of *Ae. tauschii* chromosomes showed uniform methylation along all fourteen chromosomes (Figure 5.10). However, the centromeric regions of most and telomeric regions of the long arm chromosomes including some, but not all, dpTa1 sites showed low levels of methylation (see Figure 5.10). On the other hand, the methylation pattern of the hexaploid wheat *T. aestivum* 'Millennium', N02Y5075, N02Y2016 (ABD, 2n=6x=42) and *Th. intermedium*

(JJ^SS, 2n=6x=42) was observed to be uneven (Figures 5.11-5.14). The vast majority (~90%) of analysed metaphases (at least 10 for every line) showed uneven and patchy distribution of the methylated cytosine with many centromeres showing low DNA methylation (see Figures 5.11-5.14). Regardless of the presence or absence of *Th. intermedium* chromatin, all analysed hexaploid wheat chromosomes showed some minor differences in the intensity of methylation signals between them (e.g. compare Figures 5.11, 5.13 and 5.14). Most chromosomes show heavy methylation in their sub-telomeric and pericentromeric regions containing the heterochromatic blocks (see Figures 5.10-5.14). The effects of polyploidization were evident in the immunostaining results. For example, the D genome chromosomes, that showed uniform distribution of methylated cytosine as diploid *Ae. tauschii* (Figure 5.10) showed a much more uneven distribution of DNA methylation in the context of hexaploid wheat *T. aestivum* 'Millennium', presenting chromosomal regions with high and low DNA methylation signal (compare Figure 5.10 and Figure 5.11).

Methylation levels and pattern of *Th. intermedium* was also uneven and comparable to those of hexaploid wheat lines (compare Figure 5.11 and Figure 5.12). Though, some regions of *Th. intermedium* chromosomes also show much higher methylation signals than others (Figure 5.12). To associate any modification in the global DNA methylation level (if any) in the lines used in this study, the methylation levels of wheat line 'Millennium' lacking alien chromatin was compared with N02Y5075 and N02Y2016 having 4Ai#2S chromosomal arm (see Chapter III and IV). In meiotic prophase, the DNA methylation levels of the *Th. intermedium* arm were less or similar to the average wheat chromatin (arrows in Figure 5.13). However, the translocated *Th. intermedium* arms show low levels of methylation in mitotic metaphase (arrows in Figure 5.14). The 'Millennium' has no alien chromatin, and most of its D-genome chromosomes showed DNA methylation levels similar to the other wheat chromosomes (Figure 5.11).

5.3.5.2 Southern hybridization

Cytosine methylation was also analyzed by comparative hybridization to genomic DNA restricted with endonucleases (Table 5.2). The isoschizomers *Msp*I and *Hpa*II recognize the same restriction site (CCGG), but differ in their sensitivity to cytosine methylation. *Hpa*II is sensitive to methylation of either cytosine (except when the external cytosine is hemi-methylated i.e. methylation is on one strand of DNA), whereas *Msp*I cannot

cleave when the external cytosine is fully or hemi-methylated (Yoder *et al.*, 1997, Liu *et al.*, 2001, Han *et al.*, 2003, Xu *et al.*, 2009, Yaakov and Kashkush, 2010). The *Bst*NI and *Scr*FI identify CCNGG as restriction site (N=A or T), but *Bst*NI is insensitive to methylation (Cohen-Karni *et al.*, 2011), whereas *Scr*FI is sensitive to methylation and cannot digest the DNA if the internal C is methylated (Mette *et al.*, 2002, Fulneček *et al.*, 2009). *Mcr*BC was applied to assess asymmetrical CpHpH methylation (H, may be any nucleotide but G), it recognizes and cleaves DNA at 5'-Pu^{m}C ($N_{40\text{-}3000}$) Pu^{m}C-3' sites (Pu=purine bases) containing at least two half sites of the form (G/A)^{m}C (Stewart and Raleigh, 1998). These recognition sites can be separated by 40bp-3kp, however optimal separation is 55-103bp (Stewart *et al.*, 2000, Kubis *et al.*, 2003b). *Mcr*BC does not act upon unmethylated DNA, each of the half sites must contain at least one ^{m}C in for *Mcr*BC to cut, and that may be on the same or opposite strand. Therefore, if the DNA is hemi methylated it should still be cleaved by *Mcr*BC (Gowher *et al.*, 2000, Irizarry *et al.*, 2008). *Mcr*BC cuts between each pair of half-sites, the cutting positions are distributed over several base pairs approximately 30bp from the methylated base (Stewart and Raleigh 1998) therefore when multiple *Mcr*BC sites are present in DNA, the cleavage sites may overlap and result in a smear rather than sharp banding pattern (Panne *et al.*, 2001, Panne *et al.*, 1999).

a). Whole-genome DNA methylation patterns of the Afa-family sequence in diploid and polyploid *Triticeae*

There are no CpG or CpNpG sites, within the consensus Afa sequence generated with 33 clones isolated here (see multiple sequence alignment of Afa-family Appendix 5.1). However, in 7 clones (out of 33) a CCGG site and in one a CCNGG site was observed. *Msp*I digestion confirmed the presence of the CCGG site in Afa and produced a ladder pattern after Southern hybridization (2nd lane for each DNA of Figures 5.15 and 5.16 right). The existence of smears and ladder pattern organization of DNA in lanes restricted with *Scr*FI and *Bst*NI and hybridized with Afa-family (4th and 5th lanes for each DNA Figure 5.15&5.16 right respectively) indicates more frequent CCNGG sites. The expected fragments sizes after digestion, in presence-absence of methylated cytosines at these symmetrical CpG and CpNpG sites are shown (Figure 5.2C). The presence of at least one CpG or CpNpG site in the tandem array of Afa-family results in

a variety of fragment multiples of 340bp, the smallest one is 340bp itself (Figure 5.2A&5.2C).

Regardless of the ploidy level or alien introgression, in the diploid D-genome (*Ae. tauschii*), tetraploid AB-genome (*T. turgidum*), hexaploid $JJ^{S}S$-genome (*Th. intermedium*) and various ABD-genomes (*T. aestivum*) the *Msp*I and *Bst*NI restrictions (2^{nd} and 5^{th} lanes for each DNA in Figures 5.15&5.16 right) generated a ladder like banding pattern in all analysed lines except *T. turgidum* that contain less copies of Afa sequences (B, Figures 5.15 right). However, *Hpa*II has digested the DNA to a little extent. In all lines high molecular weight DNA (above 10kb) with a little smear is visible (Figures 5.15&5.16 left). This suggests heavy levels of methylation at the overall CpG sites and of the Afa-family specifically, as evident after Southern hybridization (Figures 5.15&5.16 right). *Scr*FI which cleaves the unmethylated CCNGG sites (4^{th} lanes for each DNA in Figure 5.15&5.16) showed overall more digestion indicating less methylation at CpNpG than at CpG sites (Figure 5.15&5.16 left) in *Th. intermedium* and other wheats. In all lines a smear is seen after Southern hybridization with Afa sequences, however in *Th. intermedium* a weak ladder like banding pattern was observed (see star in H, Figure 5.16 right) indicating the CCNGG sites of Afa are less methylated in *Th. intermedium* genomes compared to other diploid and polyploid *Triticeae* members (see 4^{th} lane for each DNA in Figure 5.15&5.16 right).

The weak smeared signal with *Mcr*BC indicates the small proportion of asymmetric methylation present in all *Triticeae* genomes (6^{th} lanes for each DNA in Figure 5.15&5.16 left and right). Further, Afa-family is abundant in the D-genome, while scarce in the AB-genome of wheat (Vershinin *et al.,* 1994, Nagaki *et al.,* 1999) and the present Southern hybridization results reconfirm the previous findings (compare B and C in Figure 5.15 right).

b). Whole-genome DNA methylation patterns of the pSc119.2 sequence in diploid and polyploid *Triticeae*

The consensus 118bp pSc119.2 sequence of both 'Chinese Spring' and *Th. intermedium* has two CCGG sites at 53-56bp and 81-84bp (arrows in Figure 5.3C). However, in some clones both sites are modified to CCNGG by a single nucleotide insertion (see multiple sequence alignment of pSc119.2 sequence Appendix 5.1). The expected fragments sizes after digestion, in presence-absence of methylated cytosines at these

symmetrical CpG and CpNpG sites are shown (Figure 5.3D). In case, where both these sites are digested in the monomers, fragments corresponding to 28bp and 90bp are generated, while fragments of 118bp if only one of the two corresponding sites are digested in adjacent monomers (a dimer), and longer fragments of 145bp if only two sites are digested (e.g. at 53 and 199bp) within a dimer (Figure 5.3D). By considering three adjacent monomers (a trimer) fragments corresponding to 208bp, 236bp and 264bp are resulted by the different combinations of cutting sites within each monomer. Thus if four adjacent monomers are taken into account, the variety of cutting sites will produce various fragments including mentioned above, and larger fragments of 324bp, 354bp and 382bp respectively and so on (see also Figure 5.3D).

Overall genomic digestion (Figures 5.17&5.18 left) are the same as for previous experiment (Figures 5.15&5.16 left). *Msp*I and *Bst*NI restrictions (2^{nd} and 5^{th} lanes for each DNA in Figures 5.17&5.18 right) generated a ladder like banding pattern in all analysed lines except *Ae. tauschii* (C, Figures 5.17 right). This is because pSc119.2 repeat family is abundant in the B-genome rather than the D-genome (Rayburn and Gill 1986, Vershinin *et al.,* 1994, Tsujimoto *et al.,* 1997, Taketa *et al.,* 2000). *Msp*I targets CCGG sites and is sensitive to external cytosine methylation (^{m}CCGG or ^{hm}CCGG) while *Bst*NI targets CCNGG sites and cleave the target in any context (Table 5.2). Therefore difference in the intensity of restriction fragments of pSc119.2 was evident (compare bands of 200-1000bp in Figure 5.18 right). The intensity of observed *Msp*I and *Bst*NI restriction fragments in 'Chinese Spring' and *T. turgidum* was more or less uniform (A and B Figure 5.17 right).

Similar to Afa-family restriction pattern (Figures 5.15&5.16 right left) both *Hpa*II and *Mcr*BC have restricted DNA to a very small extent in all species (see 3^{rd} and 6^{th} lanes for each DNA in Figures 5.17&5.18 left). *Hpa*II is sensitive to methylation but digest CCGG sites when the external cytosine is hemimethylated (Jeffrey 1996, Liu *et al.,*1998) and *Mcr*BC can digest asymmetric methylation sites even if they are not on the same strand (Table 5.2). Both *Hpa*II and *Mcr*BC show almost no restriction of pSc119.2 sites in case of 'Chinese Spring' and *T. turgidum* (see A and B in Figure 5.18) but show a little smear in wheat-*Th. intermedium* hybrid lines (3^{rd} and 6^{th} lanes for each DNA in Figures 5.17&5.18).

The FISH results indicated pSc119.2 sequence is one of the major components of the *Triticeae* heterochromatin (see Chapter III), where most of the TEs and satDNA reside and are epigenetically silenced (see Chapter I and above). The Southern

hybridization results with *Hpa*II and *Scr*FI also go parallel, as *Hpa*II and *Scr*FI could generate only low levels of smears and not the ladder pattern (3rd and 4th lanes for each DNA in Figures 5.17&5.18) that *Hpa*II and *Scr*FI could have generated otherwise, if the pSc119.2 sequence was not heavily methylated (see Table 5.2 and 3rd, 4th lanes Figures 5.17&5.18). Further, these results also revealed that pSc119.2 has predominant symmetric methylation as *Hpa*II and *Scr*FI showed minimal digestion in their CCGG and CCNGG recognition sequences (3rd and 4th lanes for each DNA in Figures 5.17&5.18).

c). Whole-genome DNA methylation patterns of the LTR-probe in diploid and polyploid *Triticeae*

Afa-family sequences were recognised as part of, or flanked by a variety of TEs in several wheat and barley BACs (see Figure 5.4C). Similar TEs between 'Chinese Spring' and *Th. intermedium* were amplified and isolated with Afa1-F and the TE-LTR specific primer, LTR6150 (Teo *et al.,* 2005) combination. A distinct PCR product of around 500bp (Appendix 5.1) amplified was sequenced from both 'Chinese Spring' and *Th. intermedium* each. Although only four clones (two from each) were sequenced, still they showed 78.9% homology between those of 'Chinese Spring' and *Th. intermedium* (see multiple sequence alignment Appendix 5.1). The *Th. intermedium* sequenced clones displayed maximum homology (95.2%) between them. BLASTN search of the consensus sequence (488bp) hit many TEs (both class-I&II) of wheats including those of *Ae. tauschii*, *T. durum* and *T. aestivum* (GenBank accession numbers AY534123, EF560592-91 and AB061329) with 95%, 93% and 91% sequence identity and 100% query coverage respectively. Therefore, the *Th. intermedium* origin clone was named as LTR-probe and applied in hybridization, to assess possible changes in the TEs component of wheat-*Th. intermedium* hybrid lines in response to intergeneric hybridization.

No CCGG or CCNGG sites were observed in the consensus sequence (see multiple sequence alignment Appendix 5.1). But being part of a TE, still much diversity in terms of sequence and methylation context was expected in the genomes of both wheat and *Th. intermedium*. Comparative genomic restriction resulted in a quite similar pattern of digestion among different genomes (e.g. compare Figures 5.19&5.20 left with Figures 5.17&5.18 left). Both *Msp*I and *Bst*NI produced degenerate ladder pattern in the

analysed lines indicating some adjacent and interspersed TE copies (2nd and 5th lanes for each DNA in Figure 5.19&5.20 right). However, it was interesting to see the methylation sensitive *Hpa*II produced a smear across the whole length of the gel, indicating demethylation and the presence of overlapping restriction sites that result in smaller fragments and not a ladder pattern (3rd lanes for each DNA in Figure 5.20&5.20 right). The *Msp*I enzyme which tolerates the internal cytosine methylation only (Table 5.2) revealed difference between 'Millennium', lacking alien fragments and other wheat-*Th. intermedium* hybrid lines (compare 2nd lanes for each DNA in Figure 5.19&5.20 right). The lower bands of 200-600bp are absent in 'Millennium' but present in wheat-*Th. intermedium* hybrid lines (see F with G, H, I and J in Figure 5.20 right). This may be indicative of the possible genomic shuffling resulted from demethylation or reawakening of the TEs. However, no visible difference was evident in the *Msp*I digestion patterns of 'Chinese Spring' and 'Mace' (compare 2nd lanes in A and E in Figure 5.19 right). The *Scr*FI, which cleaves unmethylated CCNGG sites, produced a smear in all lines including 'Chinese Spring' and 'Millennium' (4th lane for each DNA in Figures 5.19&5.20 right). Suggesting all CpNpG sites are not demethylated (compare *Scr*FI and *Bst*NI 4th and 5th lanes for each DNA in Figures 5.19&5.20 right). The asymmetric methylation is insignificant across the *Triticeae* (Fulnecek *et al.,* 2002) and so was evident, by the low levels of smears in the *Mcr*BC restriction (6th lanes Figure 5.20&5.20 right).

d). Whole-genome DNA methylation patterns of the Cas2-probe in diploid and polyploid *Triticeae*

By and large, the hybridization pattern of a CACTA DNA transposon Cas2-probe (Sergeeva *et al.,* 2010) did not reveal any major differences in relation to the presence of alien fragments (see below). The comparative restriction pattern of total genomic DNA was more or less identical to those seen in other gels (e.g. compare Figures 5.21&5.22 left with Figures 5.15&5.16 left). Similar to the LTR probe (mentioned above), degenerate and uneven ladder like patterns were obtained in lanes restricted with *Msp*I and *Bst*NI (see 2nd and 5th lanes for each DNA in Figure 5.21&5.22 right) indicating dispersed, but also some adjacent interspersed CACTA elements . However, the differences in the intensity of bands resulting from *Msp*I digestion alone were apparent in different genomes suggesting copy number variation (compare ABD, AD,

D, JJ^{S}J genomes in A, B, C and D of Figure 5.21 right). Higher bands of 2.5kb and above are seen in wheat-*Th. intermedium* hybrid lines but not in the 'Chinese Spring' or 'Millennium' (compare 2nd lanes of A and F with E, G, H, I and J in Figures 5.21&5.22 right). Similarly, the bands obtained from *Bst*NI restriction are not very clear and comparable in all analysed lines (5th lanes for each DNA in Figures 5.21&5.22). Smear, running across the whole length of gel, is seen in lanes restricted with *Scr*FI (4th lanes for each DNA in Figures 5.21&5.22) indicating the lack of methylated cytosines in CCNGG sequence context. However it is present in all lanes and could not be correlated to the presence of alien chromatin. *Hpa*II which is sensitive to the methylation of both cytosines in CCGG sequence, unless external cytosine is hemimethylated also showed a smear (3rd lanes for each DNA in Figures 5.21&5.22 right). Furthermore, the smears resulting from *Mcr*BC restriction was also seen in all lines irrespective of alien introgression or different genomes restricted (6th lanes for each DNA in Figure 5.21&5.22), which confirms the low levels of asymmetric methylation across the grass genomes (Fulnecek *et al.*, 2002).

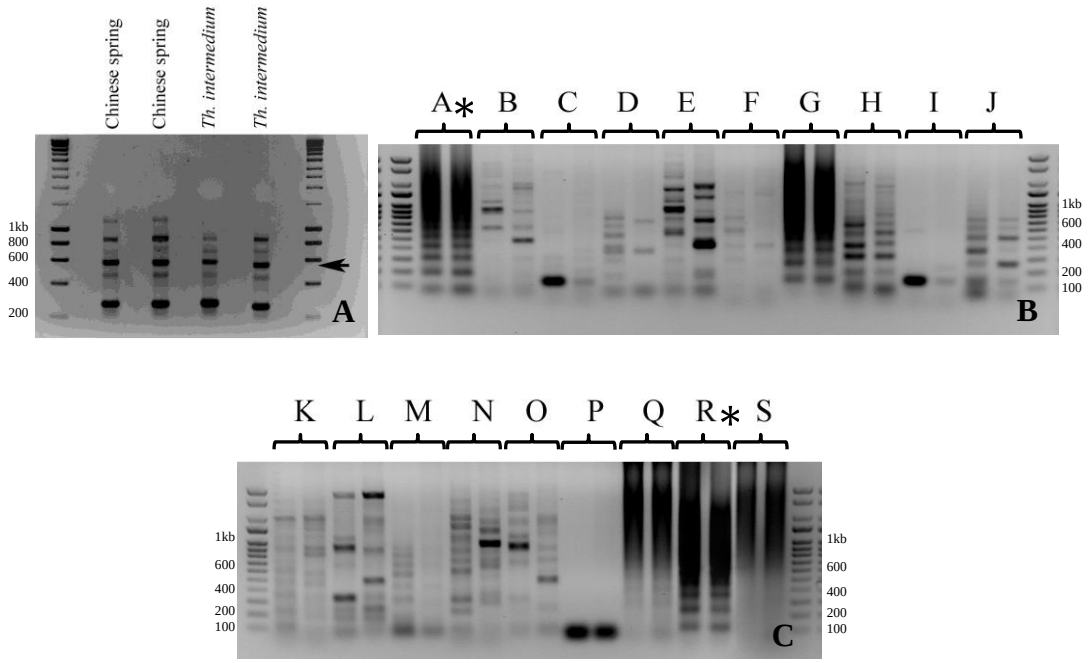


Figure 5.1: Agarose gel electrophoresis, images are inverted to show the faint bands present in some lanes. **(A)** PCR amplification pattern of Afa-family from 'Chinese Spring' and *Th. intermedium*. Arrow indicates the PCR product cloned and sequenced. **(B & C)** PCR amplification pattern of pSc119.2 sequences in 'Chinese Spring' and *Th. intermedium*. Every first well (from A-S) contains 'Chinese Spring' and the second *Th. intermedium* DNA, respectively. Dimers, trimers and tetramers from A^* and R^* were sequenced and used in phylogenetic analysis.

(B) Lanes A: Primer pair pSc119.2-AF/AR, B: pSc119.2-BF/BR, C: pSc119.2-CF/CR, D: pSc119.2-DF/DR, E: pSc119.2-AF/BR, F: pSc119.2-AF/CR, G: pSc119.2-AF/DR, H: pSc119.2-BF/AR, I: pSc119.2-BF/CR, J: pSc119.2-BF/DR.

(C) Lanes K: pSc119.2-CF/AR, L: pSc119.2-CF/BR, M: pSc119.2-CF/DR, N: pSc119.2-DF/AR, O: pSc119.2-DF/BR, P: pSc119.2-DF/CR, Q: F106/R208, R: F25/R147, S: F106/R42. Three primer sets including two of Contento *et al.,* (2005) and one described here failed to amplify PCR products (lane P, Q and S). On either side of the agarose gel (1.5%) is a DNA length marker HyperLadder (A) or Q-Step 2 **(B & C).**

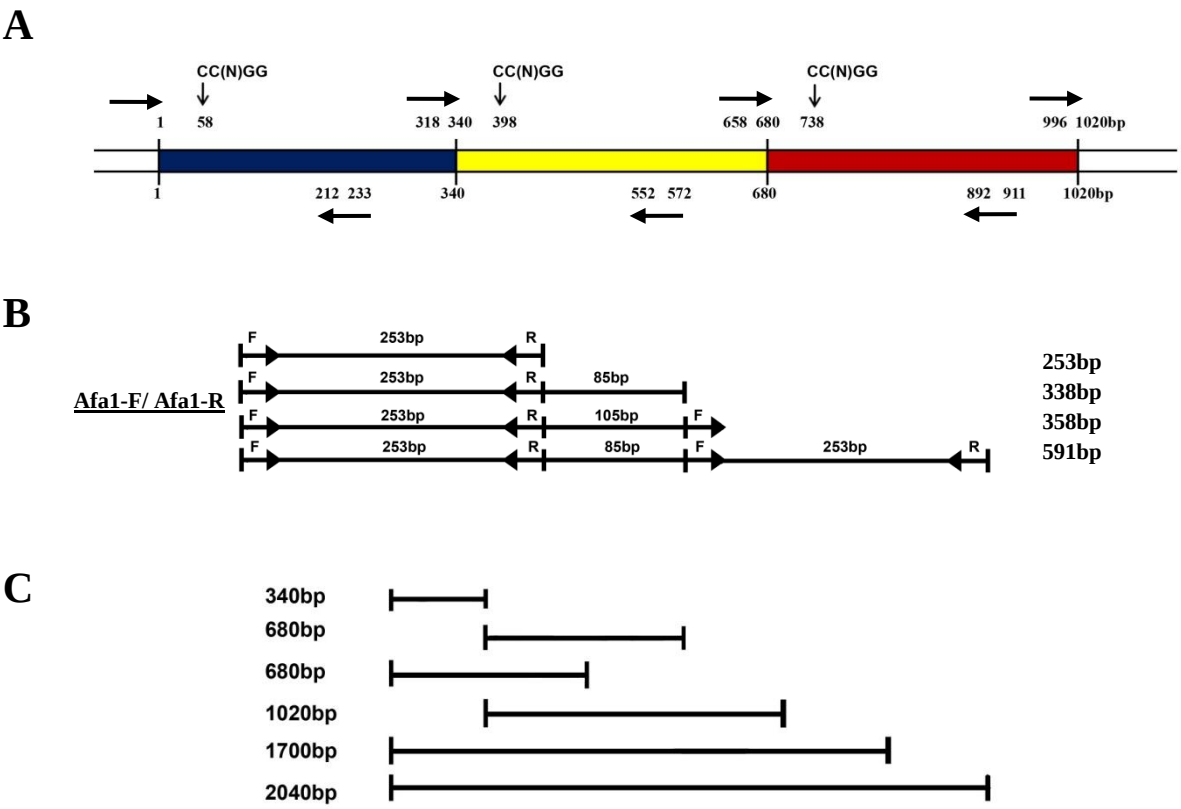
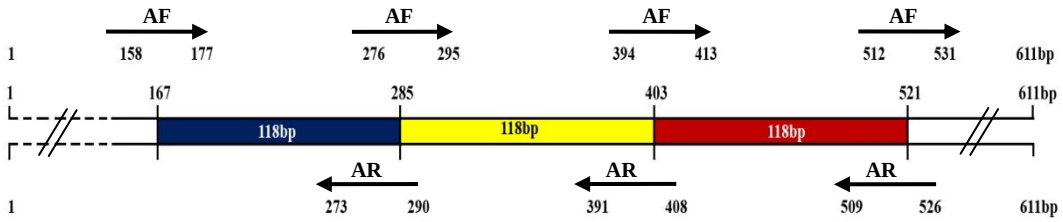


Figure 5.2: Organization of the Afa repeats monomer within 'Chinese Spring' and *Th. intermedium* genomes. **(A)** Head-to-tail organization of the 340bp Afa repeat units is represented by the coloured bars. Numbers along the bars represent nucleotide positions. Only three monomers of the tandem array are shown. Positions and orientations of the Afa primers are indicated with arrows. **(B)** Primer pair used to amplify the complete 340bp repeat unit. Lengths of fragments correspond to actual PCR products confirmed with sequencing. **(C)** The sizes of few expected fragments after digestion in presence or absence of methylated cytosines at CpG and CpNpG sites in the Afa sequence. Right angle arrows (in Figure A) pointing to the target sequence of CCGG and CC(N)GG for methylation-sensitive and insensitive restriction enzymes.

Note: The position of priming sites and orientation of primers is based on consensus sequence. No CCGG or CCNGG site exists within the consensus sequence. However, in the 33 sequenced clones of 'Chinese Spring' and *Th. intermedium* 7 clones contained a CCGG sites while in 1 clone a CCNGG site was observed. The CCNGG site are shown based on the results obtained with *Bst*NI and *Scr*FI enzymes here (see 4th and 5th lanes for each DNA in Figure 5.15&5.16).

Figure 5.3: Organization of the repeat monomers within the complete pSc119.2 sequence (McIntyre *et al.,* 1990). **(A)** Head-to-tail organization of the tandem repeat units is represented by coloured bars. Numbers inside bars indicate the length of monomer units and along the sides represents nucleotide position. Dashed lines (1-166bp) represent a relatively unrelated sequence present in the pSc119.2 sequence (McIntyre *et al.,* 1990). Arrows represent the position and orientation of pSc119.2-AF and pSc119.2-AR primers only. **(B)** Primer pairs used to amplify the 118bp repeat units. Lengths of fragments correspond to actual PCR products confirmed with sequencing. **(C)** Restriction map of pSc119.2 sequence isolated form wheat and *Th. intermedium.* The tandem array of 118bp repeat units is represented by solid coloured bars. Arrows are pointing to the target CCGG and CCNGG sites for *Msp*I-*Hpa*II and *Bst*NI-*Scr*FI restriction enzymes. Numbers above the repeat units (coloured bars) indicate the position of CCGG or CC(N)GG sites found within the consensus sequence. **(D)** The size of few expected fragments after digestion in presence or absence of methylated cytosines at CpG and CpNpG sites in the 118bp repeat unit family.

A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AF
AF
AF
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276
295
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531
611bp
1
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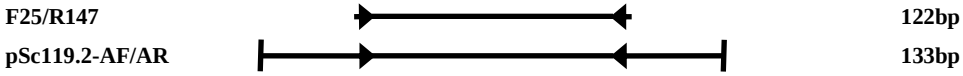
611bp

B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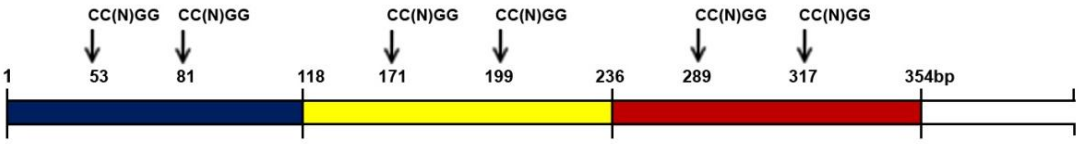
pSc119.2-AF/AR
133bp

C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CC(N)GG
CC(N)GG
CC(N)GG
CC(N)GG
CC(N)GG
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53
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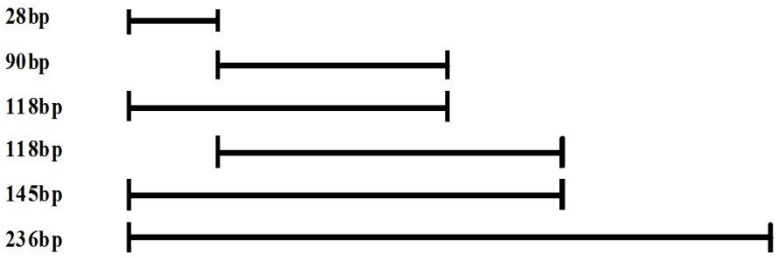
199
236
289
317
354bp

D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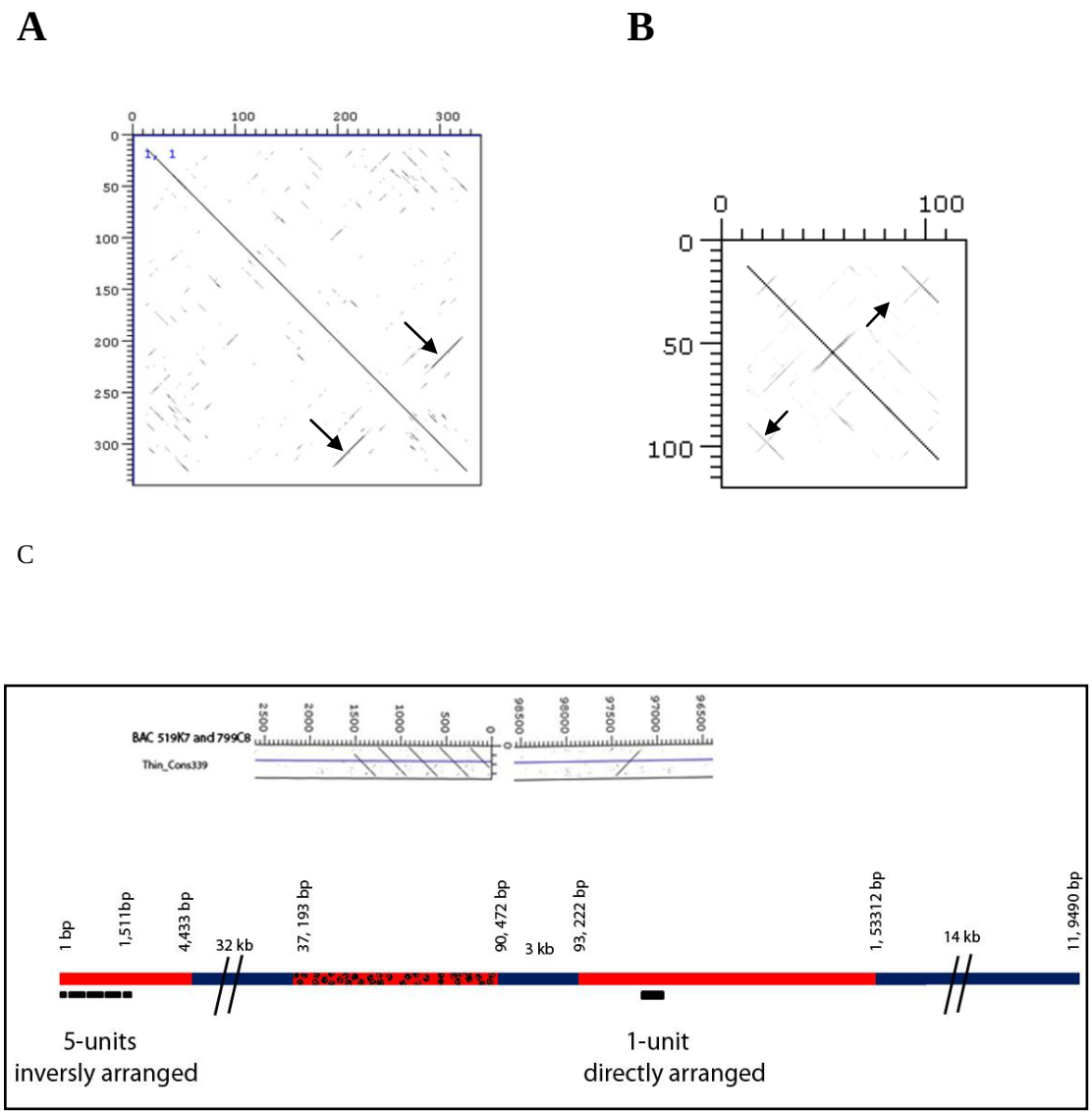
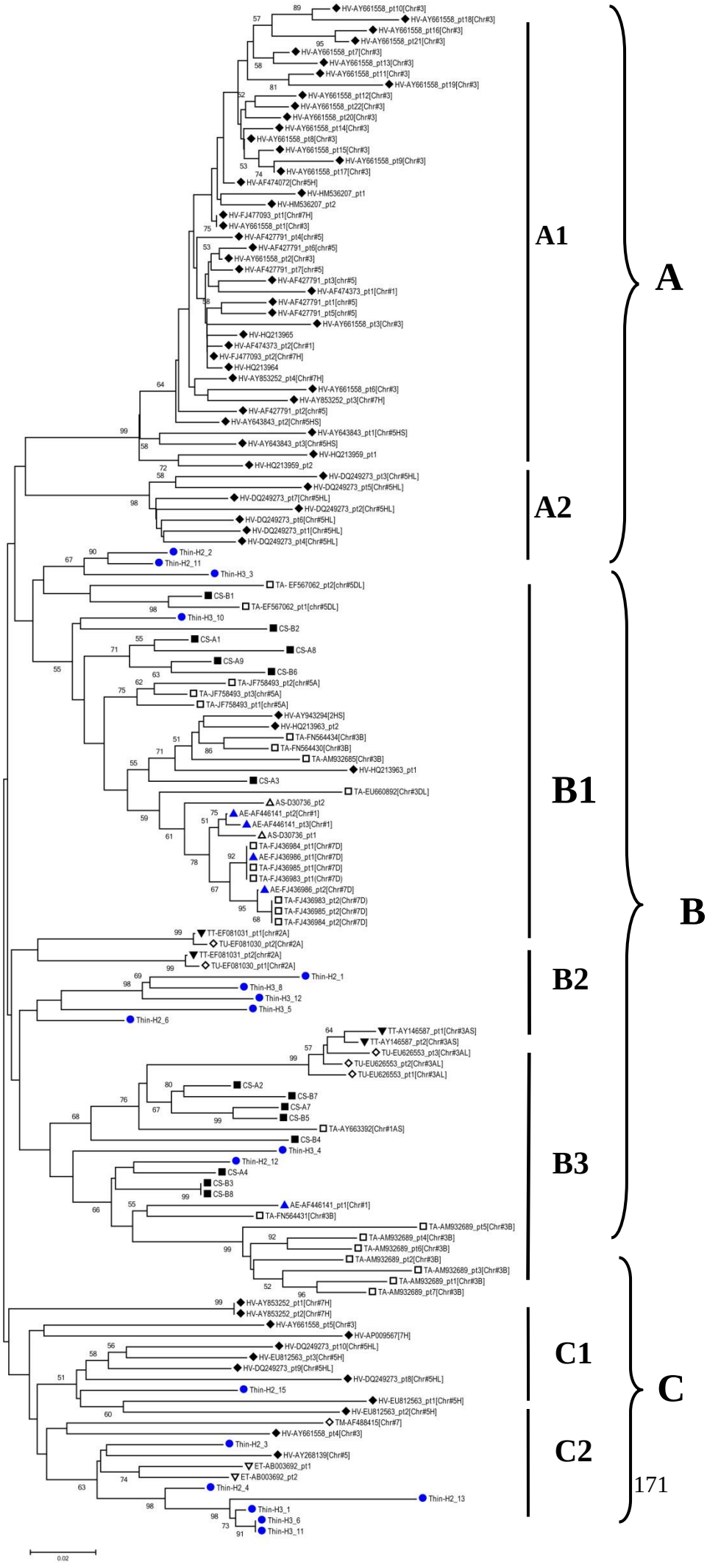


Figure 5.4: Sequence dotplot analysis of the consensus Afa **(A)** and pSc119.2 **(B)** sequences. Arrows pointing to the 29bp inverted and 14bp direct repeat region found in Afa and pSc119.2 sequences respectively. **(C)** Organization of Afa-family sequences in a BAC clone (GenBank number AY643843) of *H. vulgare*. The consensus Afa sequence is plotted (vertical) against the BAC sequence (horizontal). The diagonal lines (above) indicate the inversely and directly arrange 6 Afa units in the BAC, represented by solid blue bar (1bp -119490bp). Three CACTA elements (red) were found in the BAC, the one in the middle (37193-90472bp) had other mobile elements nested in it (represented with black dots). The flanking regions to the CACTA elements also contain other mobile elements (copia, gypsy, LINEs etc.). Solid black bars below the BAC representing the relative position of Afa units.

Figure 5.5: Molecular phylogenetic analysis of Afa-family sequences by Maximum Likelihood method. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Branches without numbers received bootstrap values smaller than 50%. The analysis involved 141 nucleotide sequences either isolated here or from GenBank. All positions containing gaps and missing data were eliminated. Clone names are composed of an abbreviated species name, plus the clone serial number. In case of published sequences abbreviated species name is followed by GenBank number, plus clone serial number. Solid black diamond represents *H. vulgare* sequences, open diamond represents *T. urartu* and *T. monococcum* sequences, solid black squares represent *T. aestivum* sequences isolated here, while open squares represent *T. aestivum* sequences from the database, all *Th. intermedium* sequences are represented by solid blue circles and were isolated here, solid blue triangles represents *Ae. tauschii*, open triangles represents *Ae. speltoides*, solid inverted triangles represents *T. turgidum* and open inverted triangles represent *Elymus trachycaulus* sequences.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HV-DQ249273_pt5[Chr#5HL]
HV-DQ249273_pt7[Chr#5HL]
HV-DQ249273_pt2[Chr#5HL]
HV-DQ249273_pt6[Chr#5HL]
HV-DQ249273_pt1[Chr#5HL]
HV-DQ249273_pt4[Chr#5HL]
Thin-H2_2
Thin-H2_11
Thin-H3_3
TA- EF567062_pt2[chr#5DL]
CS-B1
TA-EF567062_pt1[chr#5DL]
Thin-H3_10
CS-B2
CS-A1
CS-A8
CS-A9
CS-B6
TA-JF758493_pt2[chr#5A]
TA-JF758493_pt3[chr#5A]
TA-JF758493_pt1[chr#5A]
HV-AY943294[2HS]
HV-HQ213963_pt2
TA-FN564434[Chr#3B]
TA-FN564430[Chr#3B]
TA-AM932685[Chr#3B]
HV-HQ213963_pt1
CS-A3
TA-EU660892[Chr#3DL]
AS-D30736_pt2
AE-AF446141_pt2[Chr#1]
AE-AF446141_pt3[Chr#1]
AS-D30736_pt1
TA-FJ436984_pt1[Chr#7D]
AE-FJ436986_pt1[Chr#7D]
TA-FJ436985_pt1[Chr#7D]
TA-FJ436983_pt1(Chr#7D)
AE-FJ436986_pt2[Chr#7D]
TA-FJ436983_pt2[Chr#7D]
TA-FJ436985_pt2[Chr#7D]
TA-FJ436984_pt2[Chr#7D]
TT-EF081031_pt1[chr#2A]
TU-EF081030_pt2[Chr#2A]
TT-EF081031_pt2[chr#2A]
TU-EF081030_pt1[Chr#2A]
Thin-H2_1
Thin-H3_8
Thin-H3_12
Thin-H3_5
Thin-H2_6
TT-AY146587_pt1[Chr#3AS]
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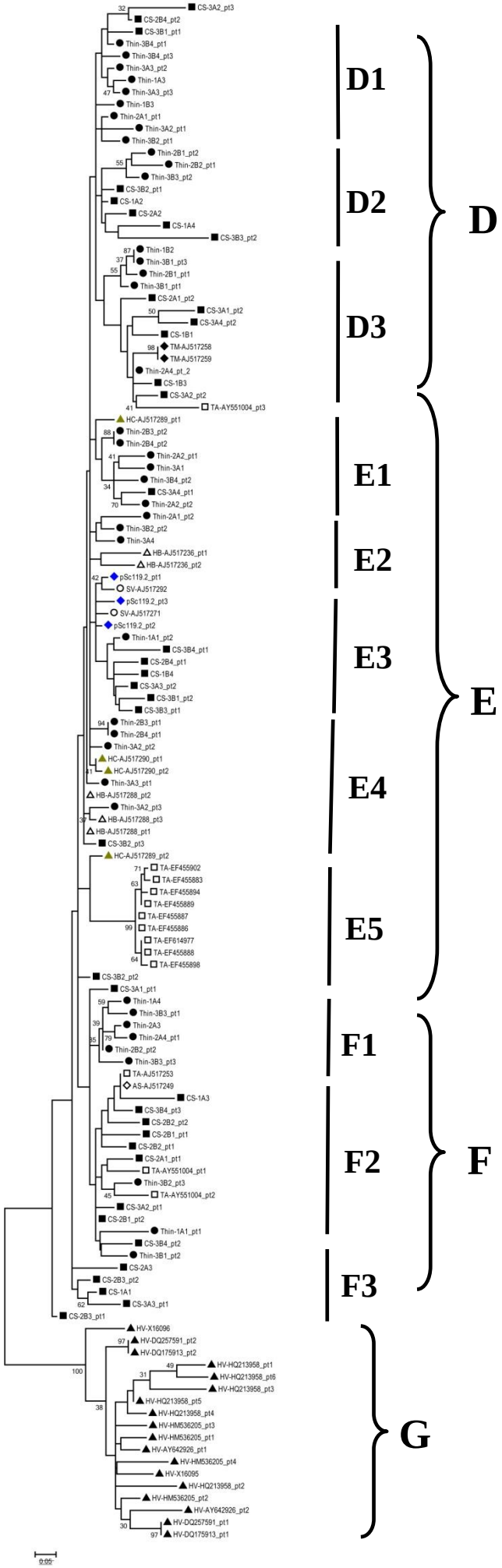
TU-EU626553_pt3[Chr#3AL]
TU-EU626553_pt2[Chr#3AL]
TU-EU626553_pt1[Chr#3AL]
CS-A2
CS-B7
CS-A7
CS-B5
TA-AY663392[Chr#1AS]
CS-B4
Thin-H3_4
Thin-H2_12
CS-A4
CS-B3
CS-B8
AE-AF446141_pt1[Chr#1]
TA-FN564431[Chr#3B]
TA-AM932689_pt5[Chr#3B]
TA-AM932689_pt4[Chr#3B]
TA-AM932689_pt6[Chr#3B]
TA-AM932689_pt2[Chr#3B]
TA-AM932689_pt3[Chr#3B]
TA-AM932689_pt1[Chr#3B]
TA-AM932689_pt7[Chr#3B]
HV-AY853252_pt1[Chr#7H]
HV-AY853252_pt2[Chr#7H]
HV-AY661558_pt5[Chr#3]
HV-AP009567[7H]
HV-DQ249273_pt10[Chr#5HL]
HV-EU812563_pt3[Chr#5H]
HV-DQ249273_pt9[Chr#5HL]
HV-DQ249273_pt8[Chr#5HL]
Thin-H2_15
HV-EU812563_pt1[Chr#5H]
HV-EU812563_pt2[Chr#5H]
TM-AF488415[Chr#7]
HV-AY661558_pt4[Chr#3]
Thin-H2_3
HV-AY268139[Chr#5]
ET-AB003692_pt1
ET-AB003692_pt2
Thin-H2_4
Thin-H2_13
Thin-H3_1
Thin-H3_6
Thin-H3_11
0.02
A
A1
A2
B
B1
B2
B3
C
C1
C2

Figure 5.6: Molecular phylogenetic analysis of pSc119.2 sequences by Maximum Likelihood method. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Branches without numbers received bootstrap values smaller than 30%. The analysis involved 127 nucleotide sequenceseither isolated here or from GenBank.All positions containing gaps and missing data were eliminated. Clone names are composed of an abbreviated species name, plus the clone serial number. In case of published sequences abbreviated species name is followed by GenBank number, plus clone serial number. Solid squares represent *T. aestivum* sequences isolated here, while open squares represent *T. aestivum* sequences from the database, solid circles represents *Th. intermedium* sequences isolated here, open circles represents *Secale vavilovii,* solid black diamond represents *T. monococcum*, solid blue diamond represents the pSc11.2 sequences of McIntyre *et al.*, (1990), open diamonds represents *Ae. speltoides*, solid black triangles represents *H. vulgare,* solid green triangles represents *H. chilense* while open triangles represent *H. bulbosum* sequences.


D1
D2
D3
D
E1
E2
E3
E4
E5
E
F1
F2
F3
F
G
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CS-2B4_pt2
CS-3B1_pt1
Thin-3B4_pt1
Thin-3B4_pt3
Thin-3A3_pt2
Thin-1A3
Thin-3A3_pt3
Thin-1B3
Thin-2A1_pt1
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Thin-2A2_pt1
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Thin-3B4_pt2
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Thin-2A2_pt2
Thin-2A1_pt2
Thin-3B2_pt2
Thin-3A4
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HB-AJ517236_pt2
pSc119.2_pt1
SV-AJ517292
pSc119.2_pt3
SV-AJ517271
pSc119.2_pt2
Thin-1A1_pt2
CS-3B4_pt1
CS-2B4_pt1
CS-1B4
CS-3A3_pt2
CS-3B1_pt2
CS-3B3_pt1
Thin-2B3_pt1
Thin-2B4_pt1
Thin-3A2_pt2
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Thin-3A3_pt1
HB-AJ517288_pt2
Thin-3A2_pt3
HB-AJ517288_pt3
HB-AJ517288_pt1
CS-3B2_pt3
HC-AJ517289_pt2
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TA-EF455883
TA-EF455894
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TA-EF455887
TA-EF455886
TA-EF614977
TA-EF455888
TA-EF455898
CS-3B2_pt2
CS-3A1_pt1
Thin-1A4
Thin-3B3_pt1
Thin-2A3
Thin-2A4_pt1
Thin-2B2_pt2
Thin-3B3_pt3
TA-AJ517253
AS-AJ517249
CS-1A3
CS-3B4_pt3
CS-2B2_pt2
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CS-2B3_pt1
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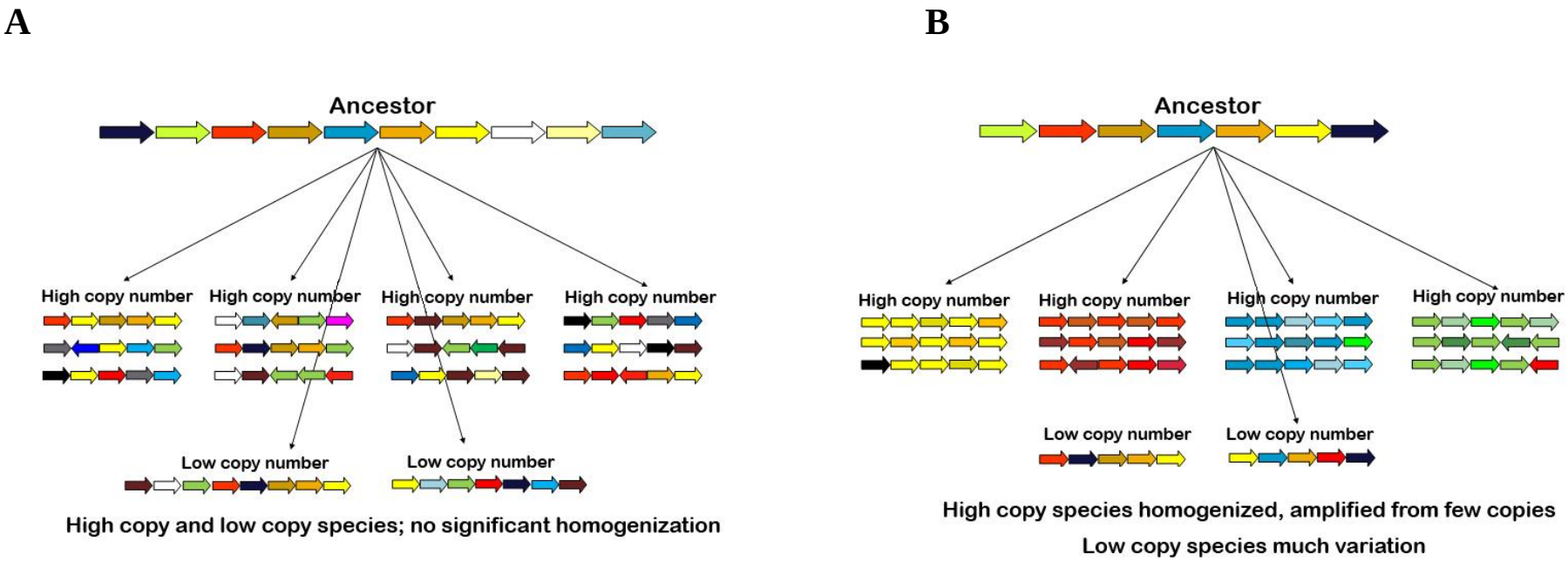


Figure 5.7: Phylogenetic relationships and homogenization models of pSc119.2 **(A)** and Afa-family sequences **(B)**. Both sequences are widely distributed among *Triticeae* members and show different sequence diversity. In pSc119.2 sequences no clusters from the same species nor deep branches are seen in the DNA sequence trees (Figure 5.6) indicating that the common ancestor probably had multiple sequences with a range of variation most of which is maintained within the species of today. Thus there are no strong homogenization events. Afa-family sequences show species and chromosome specific clusters in the DNA sequence tree (Figure 5.5) and branching is evident of strong homogenization events. Copies in the species with high copy number of Afa sequences have amplified from a few selected units from the ancestor.

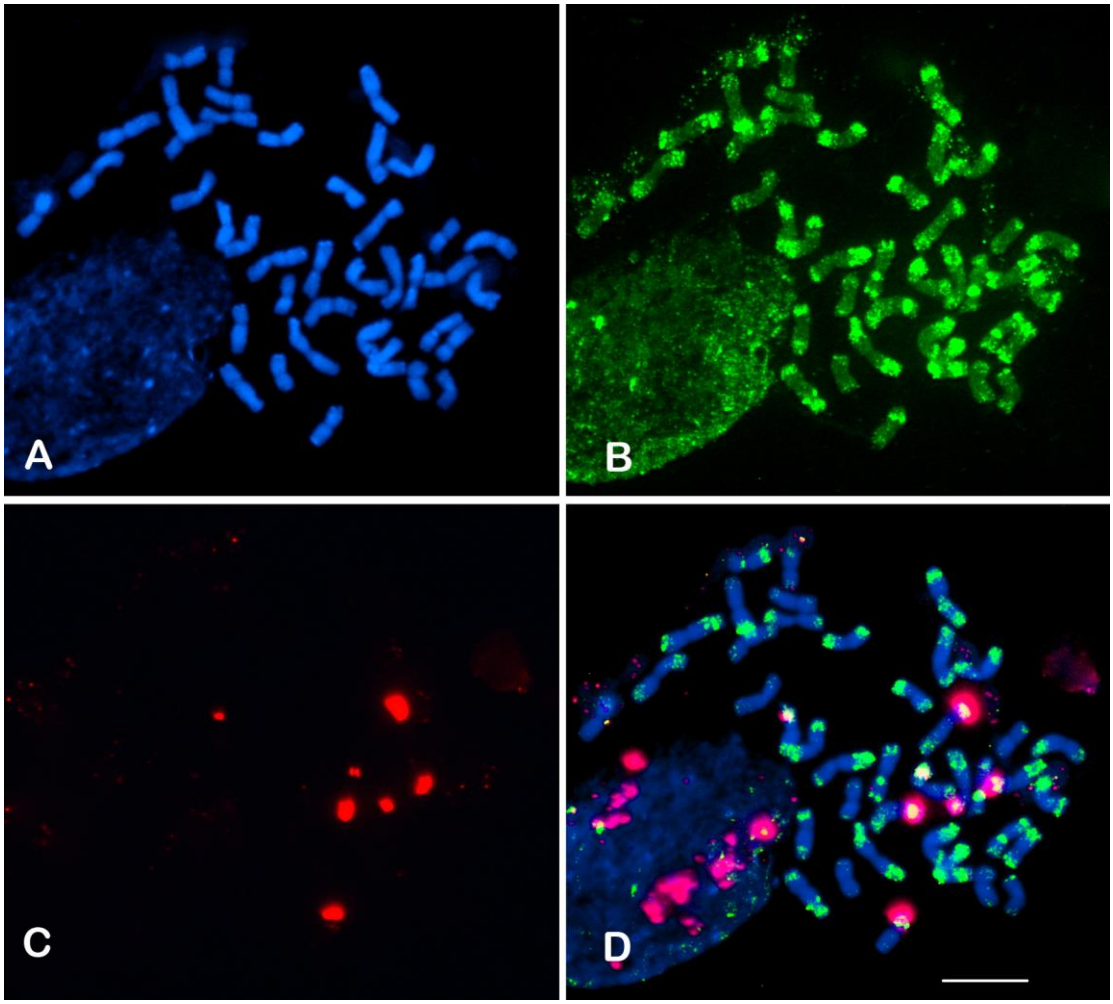


Figure 5.8: Root-tip metaphase chromosomes of *Th. intermedium* (2n=42) after fluorescent *in situ* hybridization (FISH). **(A)** *Th. intermedium* chromosomes are appearing blue with DAPI fluorescence. **(B)** Hybridization pattern of the dpTa1 DNA sequence labelled with digoxygenin 11-dUTP (detected in green) that hybridized preferentially to the D-genome chromosomes of wheat. **(C)** Hybridization pattern of the pTa71 clone labelled with biotin 16-dUTP (detected in red) showing the physical location of major 45S rDNA sites. **(D)** Overlay of A, B and C images. Bar represents 10µm.

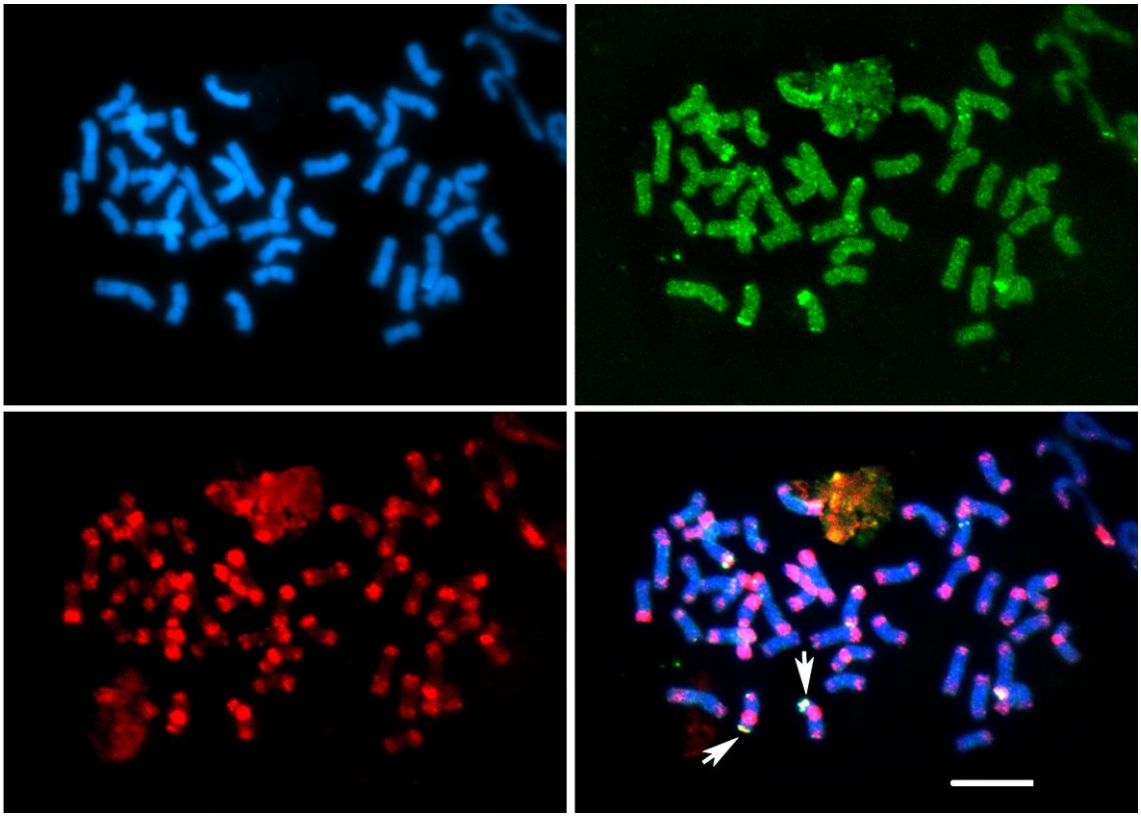


Figure 5.9: Root-tip metaphase chromosomes of *Th. intermedium* (2n=42) after fluorescent *in situ* hybridization (FISH). **(A)** *Th. intermedium* chromosomes are appearing blue with DAPI fluorescence. **(B)** Hybridization pattern of the pSc119.2 DNA sequence labelled with digoxygenin 11-dUTP (detected in green). **(C)** Hybridization pattern of the Afa DNA sequence labelled with biotin 16-dUTP (detected in red). **(D)** Overlay of A, B and C images, the chromosomal arms harbouring WSMV-resistant gene are indicated by arrows. Bar represents 10µm.

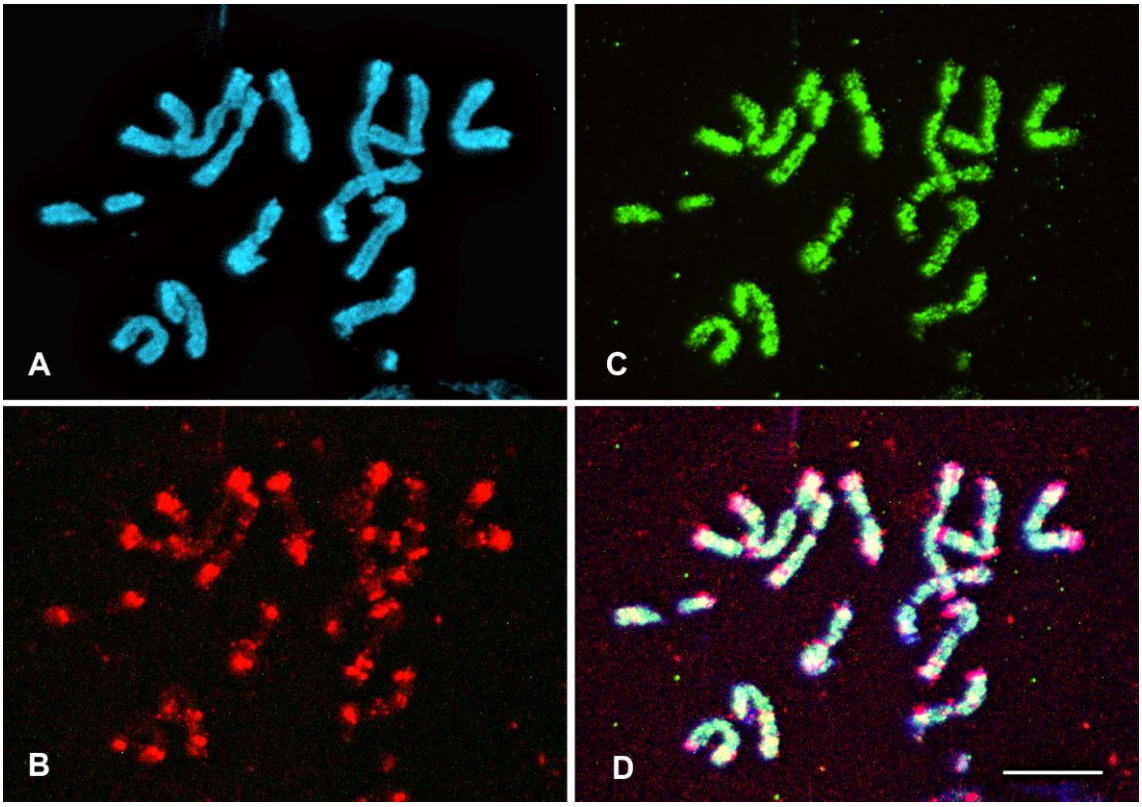


Figure 5.10: Root-tip metaphase chromosomes of *Ae. tauschii* (D-genome, 2n=14) after immunostaining with anti-5-mC antibody and fluorescent *in situ* hybridization (FISH). **(A)** *Ae. tauschii* chromosomes are appearing blue with DAPI fluorescence. **(B)** Methylated cytosine sites were detected with anti-5-mC antibody (detected in green). **(C)** Hybridization pattern of the dpTa1 DNA sequence labelled with biotin 16-dUTP (detected in red). **(D)** Overlay of A, B and C images. Bar represents 10μm.

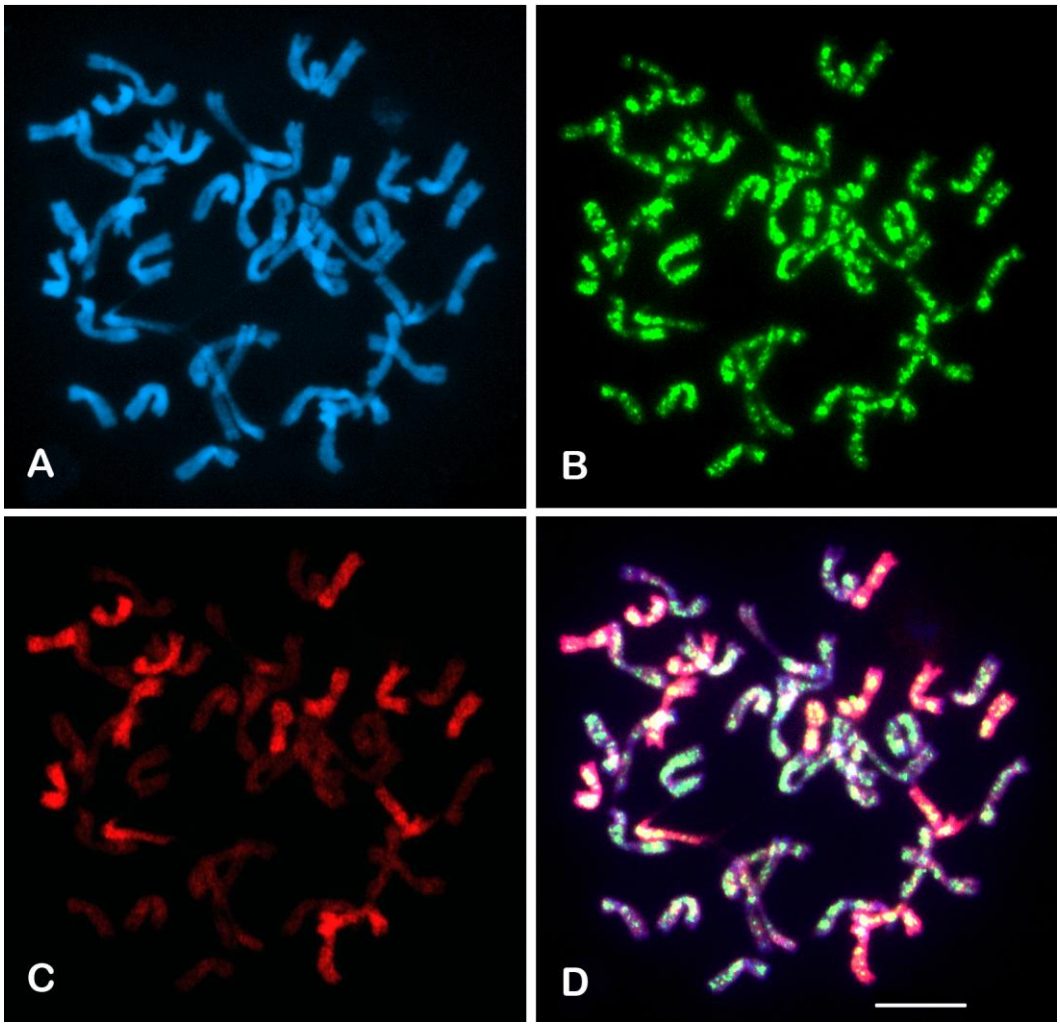


Figure 5.11: Root-tip metaphase chromosomes of *T. aestivum* 'Millennium' (ABD-genome, 2n=42) after immunostaining with anti-5-mC antibody and fluorescent *in situ* hybridization (FISH). **(A)** 'Millennium' chromosomes are appearing blue with DAPI fluorescence. **(B)** Methylated cytosine sites were detected with anti-5-mC antibody (detected in green). **(C)** *In situ* hybridization of the total genomic DNA from *Ae. tauschii* labelled with biotin 16-dUTP (detected in red). The genomic *in situ* hybridization allows the detection of D-genome chromosomes. **(D)** Overlay of A, B and C images. Bar represents 10μm.

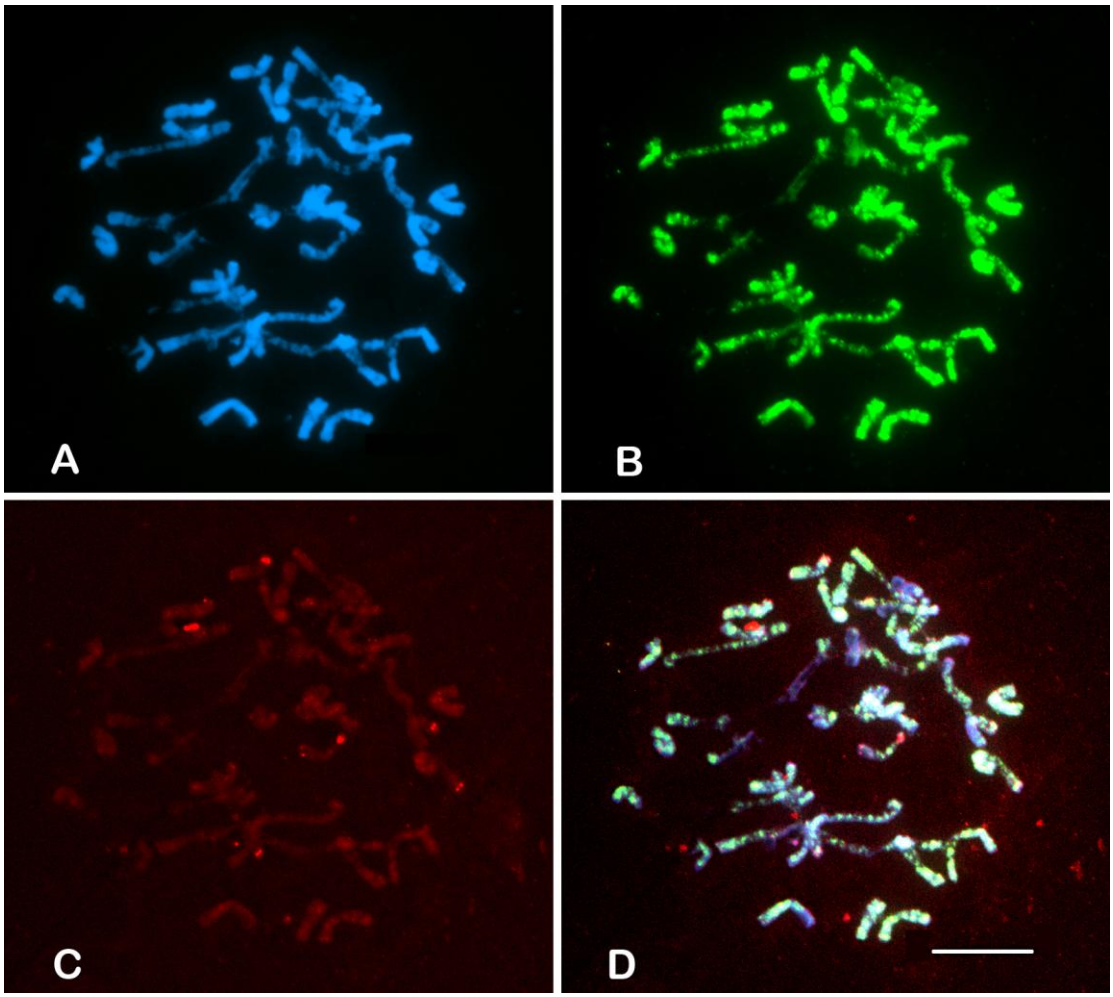


Figure 5.12: Root-tip metaphase chromosomes of *Th. intermedium* ($JJ^{S}S$-genome, 2n=42) after immunostaining with anti-5-mC antibody and fluorescent *in situ* hybridization (FISH). **(A)** *Th. intermedium* chromosomes are appearing blue with DAPI fluorescence. **(B)** Methylated cytosine sites were detected with anti-5-mC antibody (detected in green). **(C)** Hybridization pattern of the pSc119.2 DNA sequence labelled with biotin 16-dUTP (detected in red). **(D)** Overlay of A, B and C images. Bar represents 10μm.

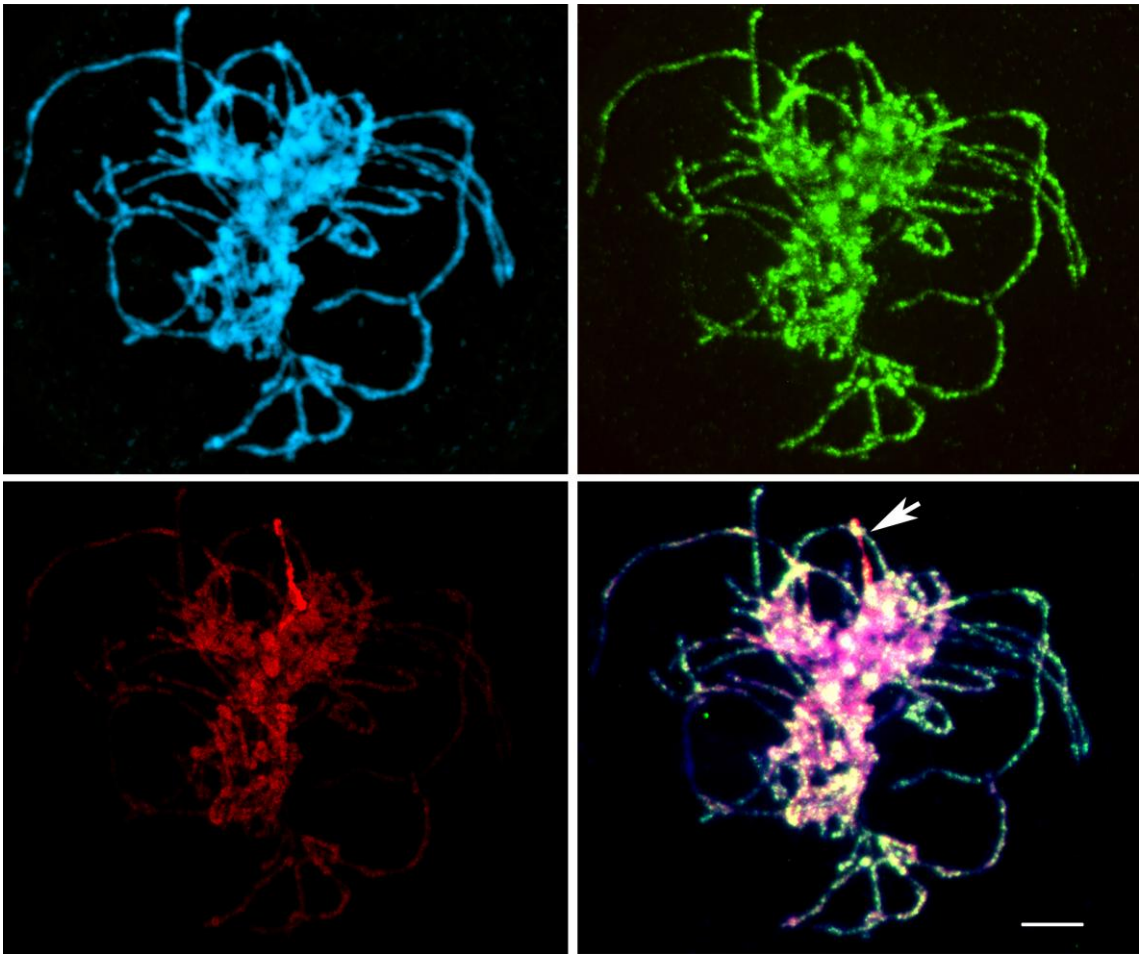


Figure 5.13: Pachytene chromosomes of the WSMV resistant-line N02Y5075 (2n=42) after immunostaining with anti-5-mC antibody and fluorescent *in situ* hybridization (FISH). **(A)** Wheat chromosomes are appearing blue with DAPI fluorescence. **(B)** Methylated cytosine sites were detected with anti-5-mC antibody (detected in green). **(C)** *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with biotin 16-dUTP (detected in red). The genomic *in situ* hybridization allows the detection of *Th. intermedium*-origin chromosome segments. **(D)** Overlay of A, B and C images, alien chromosomal arms are indicated by arrows. Bar represents 10µm.

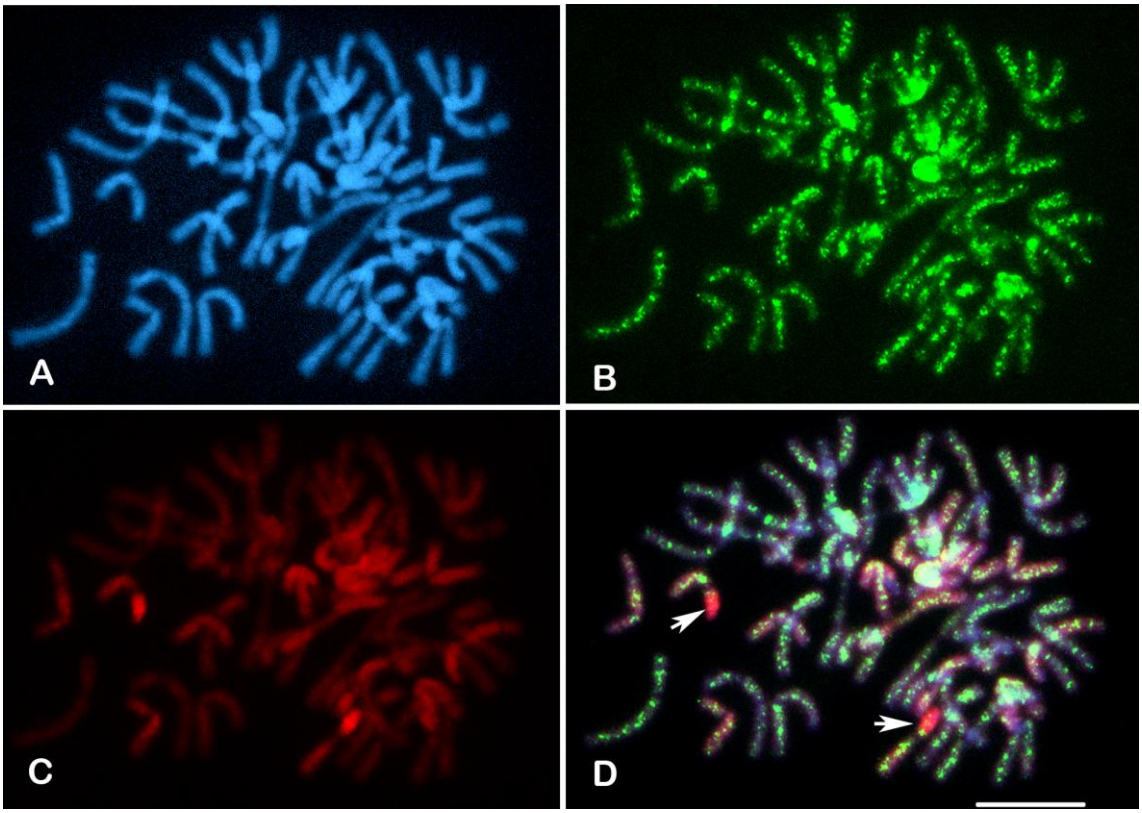
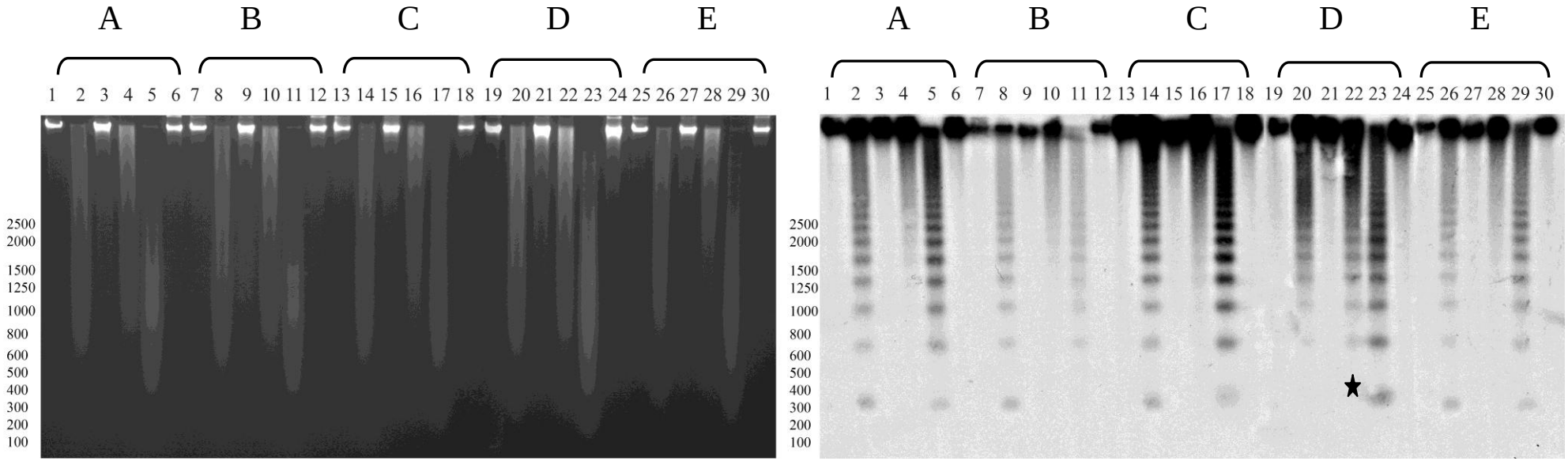
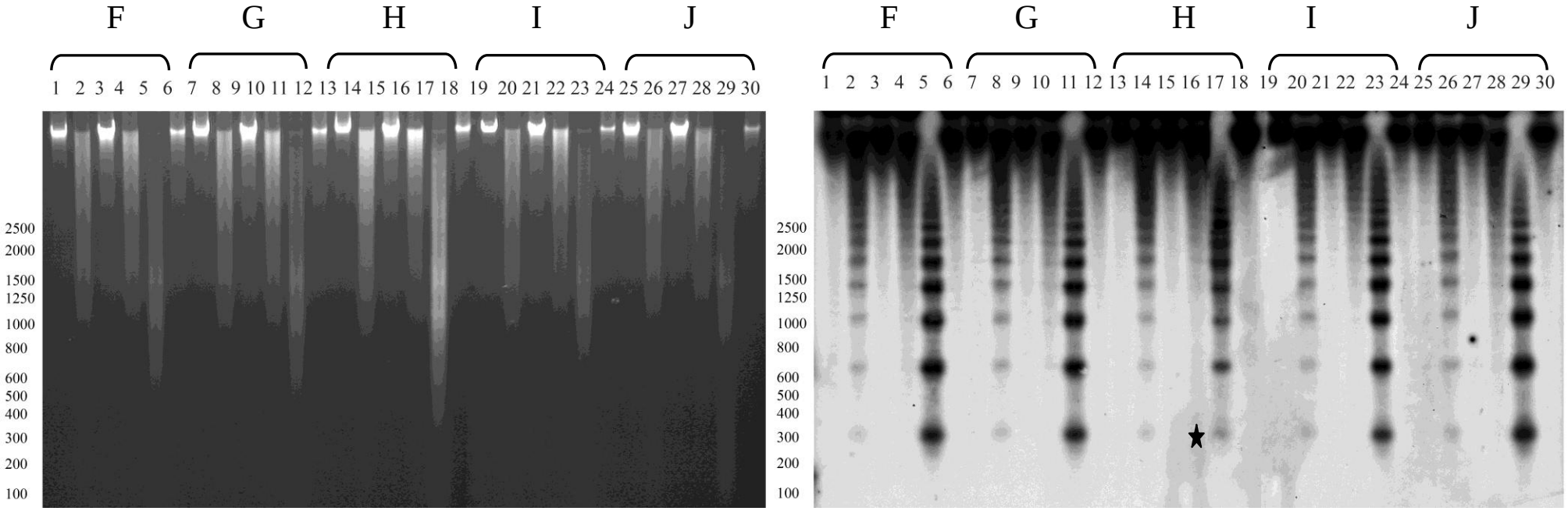


Figure 5.14: Root-tip metaphase chromosomes of the WSMV resistant-line N02Y2016 (2n=42) after immunostaining with anti-5-mC antibody and fluorescent *in situ* hybridization (FISH). **(A)** Wheat chromosomes are appearing blue with DAPI fluorescence. **(B)** Methylated cytosine sites were detected with anti-5-mC antibody (detected in green). **(C)** *In situ* hybridization of the total genomic DNA from *Th. intermedium* labelled with biotin 16-dUTP (detected in red). The genomic *in situ* hybridization allows the detection of *Th. intermedium*-origin chromosome segments. **(D)** Overlay of A, B and C images, alien chromosomal arms show reduced methylation and are indicated by arrows. Bar represents 10μm.



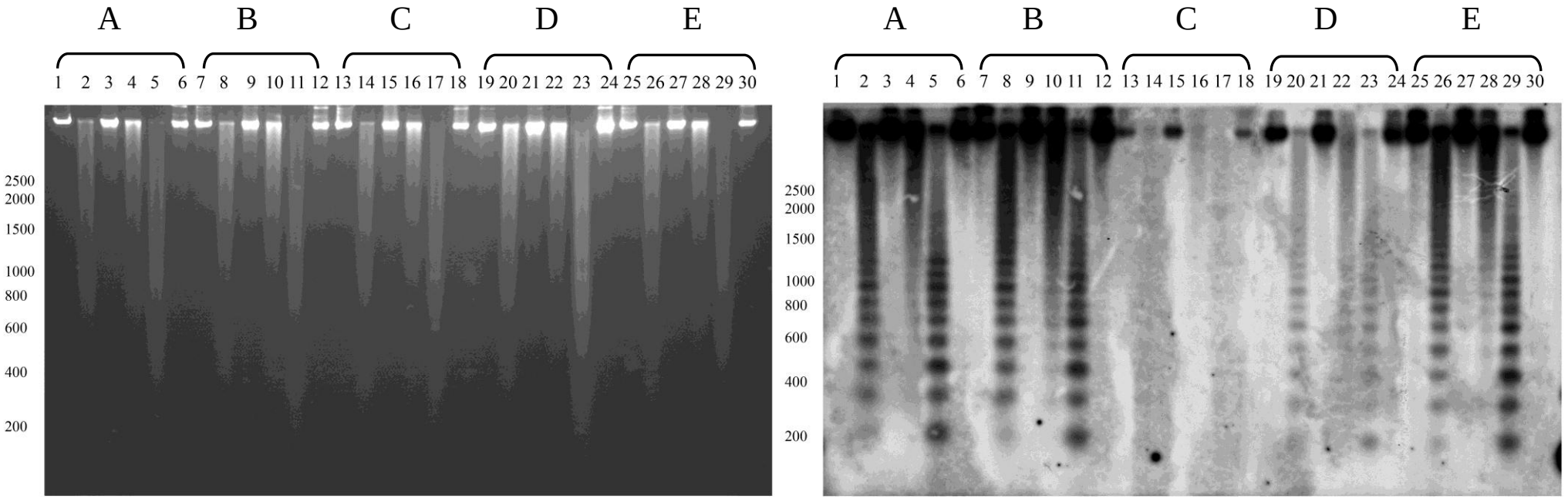
Lanes 1, 7, 13, 19, 25 undigested
Lanes 2, 8, 14, 20, 26 digested with *Msp*I
Lanes 3, 9, 15, 21, 27 digested with *Hpa*II
Lanes 4, 10, 16, 22, 28 digested with *Scr*FI
Lanes 5, 11, 17, 23, 29 digested with *Bst*NI
Lanes 6, 12, 18, 24, 30 digested with *Mcr*BC

Figure 5.15: Gel picture of the genomic restriction (left) and Southern hybridization (right) with radioactively labelled Afa-family sequence of DNA from (A) *T. aestivum* 'Chinese Spring' ABD-genome, (B) *T. turgidum* AB-genome, (C) *Ae. tauschii* D-genome, (D) *Th. intermedium* $JJ^{S}S$-genome, (E) *T. aestivum* 'Mace' ABD-genome.



Lanes 1, 7, 13, 19, 25 undigested
Lanes 2, 8, 14, 20, 26 digested with *Msp*I
Lanes 3, 9, 15, 21, 27 digested with *Hpa*II
Lanes 4, 10, 16, 22, 28 digested with *Scr*FI
Lanes 5, 11, 17, 23, 29 digested with *Bst*NI
Lanes 6, 12, 18, 24, 30 digested with *Mcr*BC

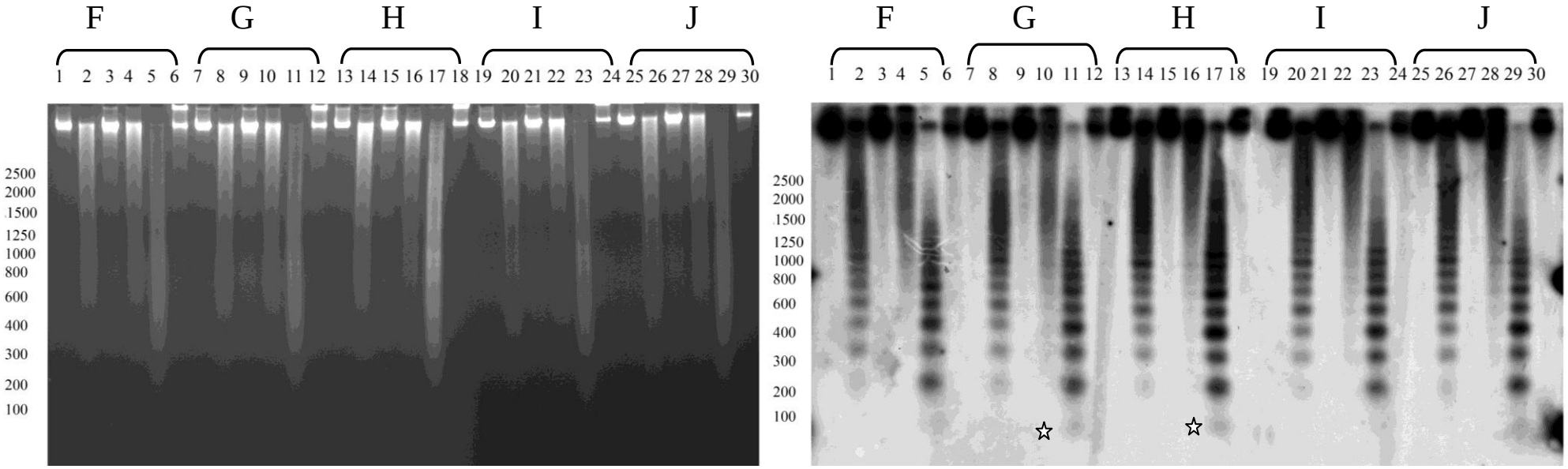
Figure 5.16: Gel picture of the genomic restriction (left) and Southern hybridization (right) with radioactively labelled Afa-family sequence of DNA from (F) *T. aestivum* 'Millennium' ABD-genome, (G) KS96HW10-1 ABD-genome, (H) N02Y5003 ABD-genome, (I) N02Y5109 ABD-genome, (J) *T.* N02Y5163 ABD-genome.



Lanes 1, 7, 13, 19, 25 undigested
Lanes 3, 9, 15, 21, 27 digested with *Hpa*II
Lanes 5, 11, 17, 23, 29 digested with *Bst*NI

Lanes 2, 8, 14, 20, 26 digested with *Msp*I
Lanes 4, 10, 16, 22, 28 digested with *Scr*FI
Lanes 6, 12, 18, 24, 30 digested with *Mcr*BC

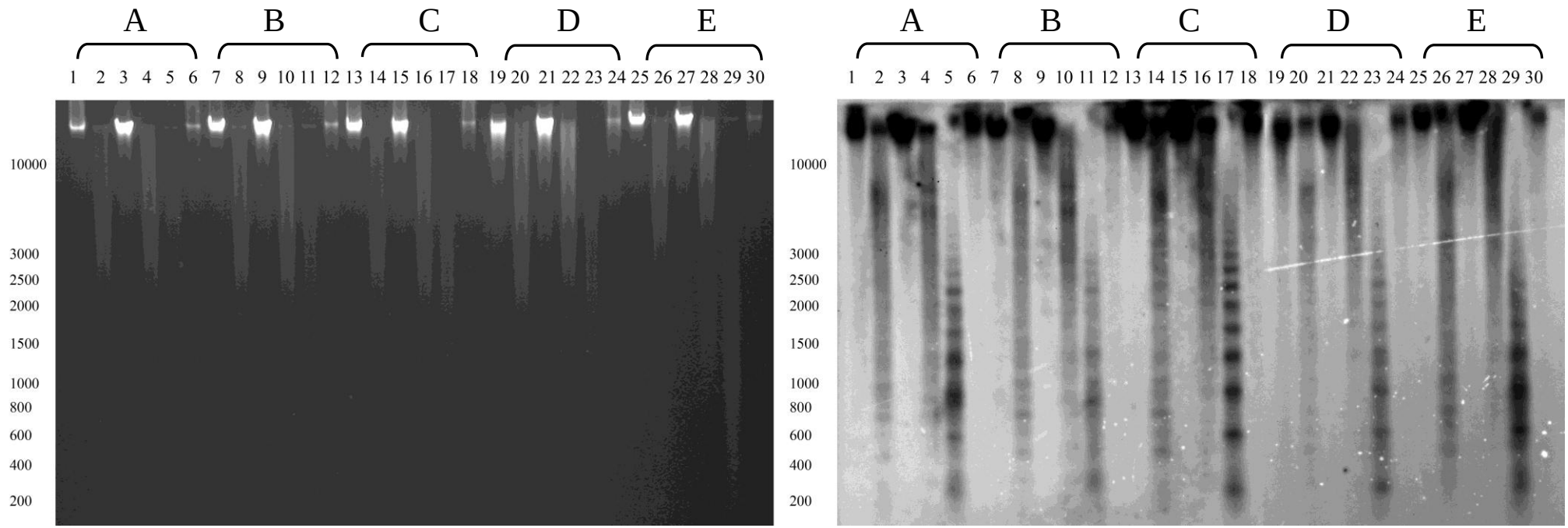
Figure 5.17: Gel picture of the genomic restriction (left) and Southern hybridization (right) with radioactively labelled pSc119.2 sequence of DNA from (A) *T. aestivum* 'Chinese Spring' ABD-genome, (B) *T. turgidum* AB-genome, (C) *Ae. tauschii* D-genome, (D) *Th. intermedium* $JJ^{S}S$-genome, (E) *T. aestivum* 'Mace' ABD-genome.



Lanes 1, 7, 13, 19, 25 undigested
Lanes 3, 9, 15, 21, 27 digested with *Hpa*II
Lanes 5, 11, 17, 23, 29 digested with *Bst*NI

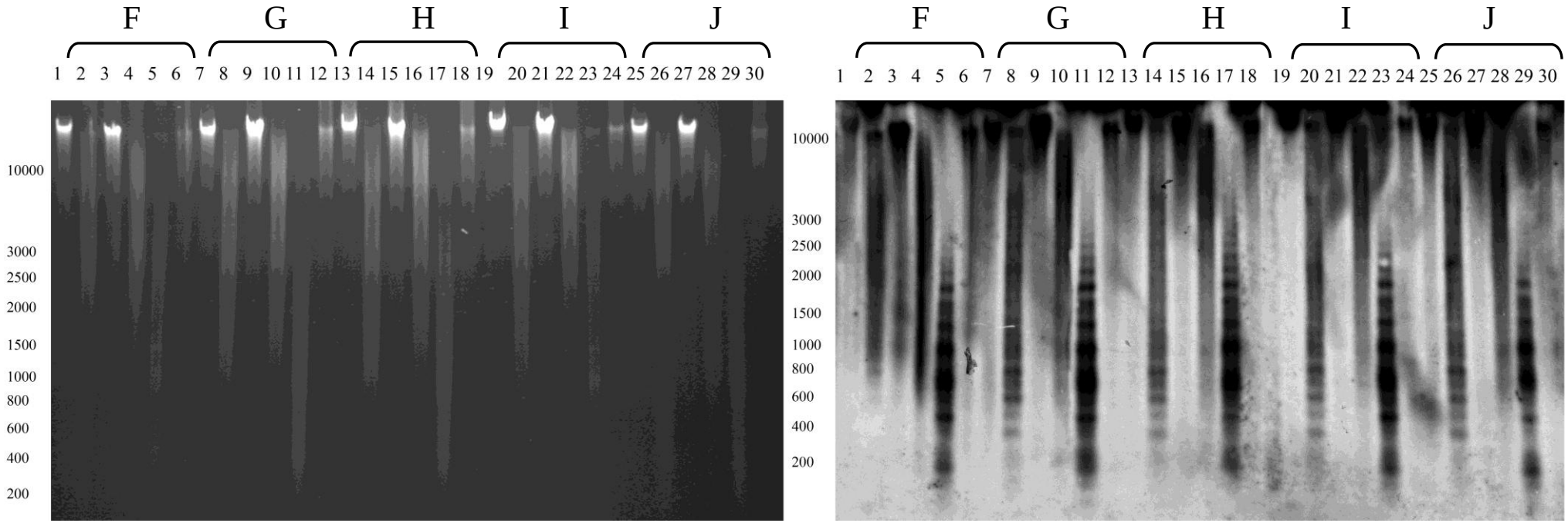
Lanes 2, 8, 14, 20, 26 digested with *Msp*I
Lanes 4, 10, 16, 22, 28 digested with *Scr*FI
Lanes 6, 12, 18, 24, 30 digested with *Mcr*BC

Figure 5.18: Gel picture of the genomic restriction (left) and Southern hybridization (right) with radioactively labelled pSc119.2 sequence of DNA from (F) *T. aestivum* 'Millennium' ABD-genome, (G) KS96HW10-1 ABD-genome, (H) N02Y5003 ABD-genome, (I) N02Y5109 ABD-genome, (J) *T.* N02Y5163 ABD-genome.



Lanes 1, 7, 13, 19, 25 undigested
Lanes 2, 8, 14, 20, 26 digested with *Msp*I
Lanes 3, 9, 15, 21, 27 digested with *Hpa*II
Lanes 4, 10, 16, 22, 28 digested with *Scr*FI
Lanes 5, 11, 17, 23, 29 digested with *Bst*NI
Lanes 6, 12, 18, 24, 30 digested with *Mcr*BC

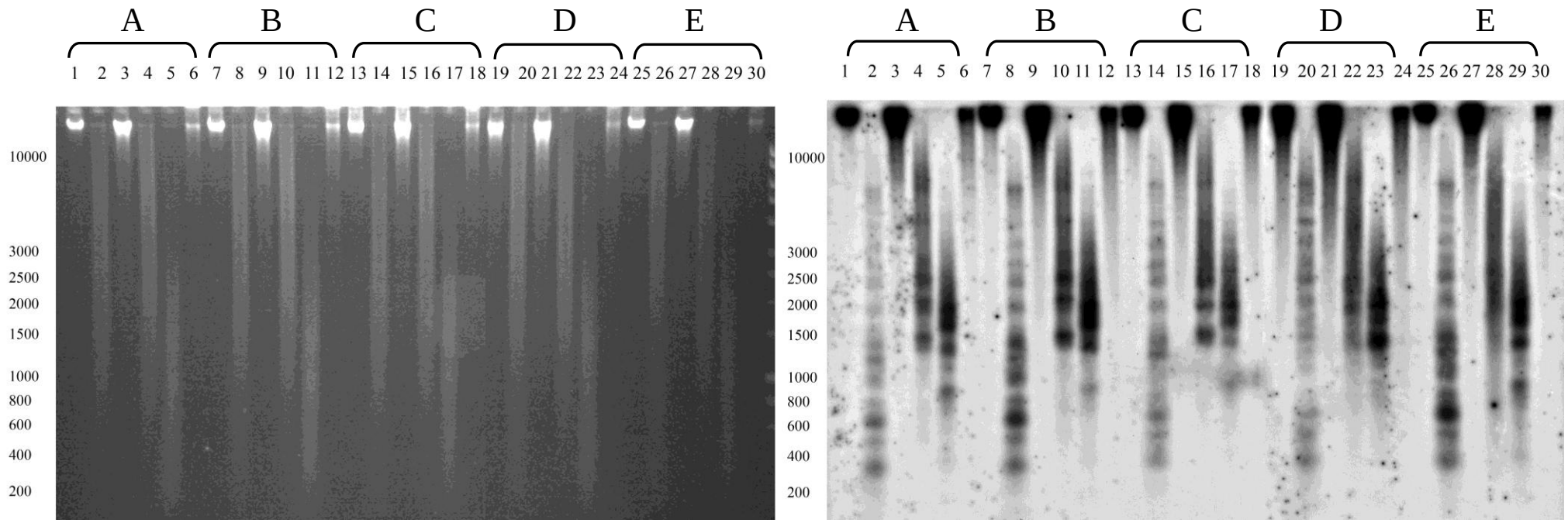
Figure 5.19: Gel picture of the genomic restriction (left) and Southern hybridization (right) with radioactively labelled LTR-probe of DNA from (A) *T. aestivum* 'Chinese Spring' ABD-genome, (B) *T. turgidum* AB-genome, (C) *Ae. tauschii* D-genome, (D) *Th. intermedium* $JJ^{S}S$-genome, (E) *T. aestivum* 'Mace' ABD-genome.



Lanes 1, 7, 13, 19, 25 undigested
Lanes 3, 9, 15, 21, 27 digested with *Hpa*II
Lanes 5, 11, 17, 23, 29 digested with *Bst*NI

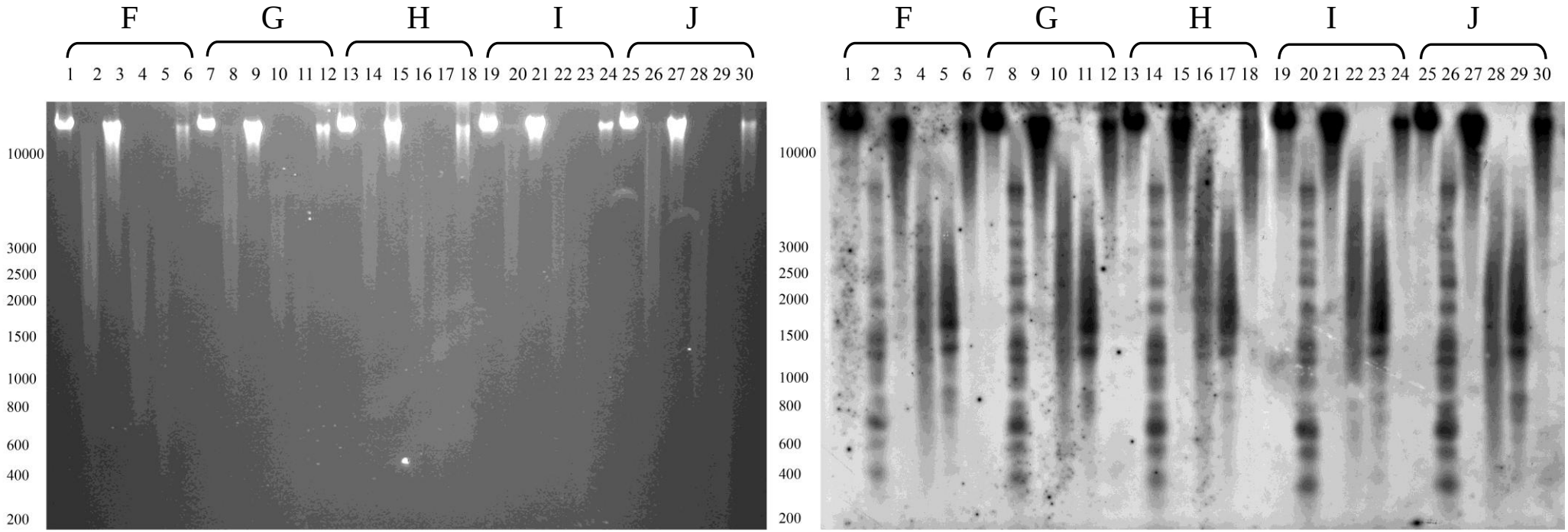
Lanes 2, 8, 14, 20, 26 digested with *Msp*I
Lanes 4, 10, 16, 22, 28 digested with *Scr*FI
Lanes 6, 12, 18, 24, 30 digested with *Mcr*BC

Figure 5.20: Gel picture of the genomic restriction (left) and Southern hybridization (right) with radioactively labelled LTR-probe of DNA from (F) *T. aestivum* 'Millennium' ABD-genome, (G) KS96HW10-1 ABD-genome, (H) N02Y5003 ABD-genome, (I) N02Y5109 ABD-genome, (J) *T.* N02Y5163 ABD-genome.



Lanes 1, 7, 13, 19, 25 undigested
Lanes 2, 8, 14, 20, 26 digested with *Msp*I
Lanes 3, 9, 15, 21, 27 digested with *Hpa*II
Lanes 4, 10, 16, 22, 28 digested with *Scr*FI
Lanes 5, 11, 17, 23, 29 digested with *Bst*NI
Lanes 6, 12, 18, 24, 30 digested with *Mcr*BC

Figure 5.21: Gel picture of the genomic restriction (left) and Southern hybridization (right) with radioactively labelled Cas2-probe of DNA from (A) *T. aestivum* 'Chinese Spring' ABD-genome, (B) *T. turgidum* AB-genome, (C) *Ae. tauschii* D-genome, (D) *Th. intermedium* $JJ^{S}S$-genome, (E) *T. aestivum* 'Mace' ABD-genome.



Lanes 1, 7, 13, 19, 25 undigested
Lanes 3, 9, 15, 21, 27 digested with *Hpa*II
Lanes 5, 11, 17, 23, 29 digested with *Bst*NI

Lanes 2, 8, 14, 20, 26 digested with *Msp*I
Lanes 4, 10, 16, 22, 28 digested with *Scr*FI
Lanes 6, 12, 18, 24, 30 digested with *Mcr*BC

Figure 5.22: Gel picture of the genomic restriction (left) and Southern hybridization (right) with radioactively labelled Cas2-probe of DNA from (F) *T. aestivum* 'Millennium' ABD-genome, (G) KS96HW10-1 ABD-genome, (H) N02Y5003 ABD-genome, (I) N02Y5109 ABD-genome, (J) *T.* N02Y5163 ABD-genome.

5.4 Discussion

5.4.1 Repetitive DNA sequences

The results indicated pSc119.2 sequences are as ancient as the *Triticeae* itself as they are present in most *Triticeae* lineages (Figure 5.6, see also McIntyre *et al.*, 1990, Contento *et al.*, 2005). Significant variation of pSc119.2 sequences exists throughout the *Triticeae*. Dispersion of repeat units form the same species is evident in the tree and there are not clear or well-supported clusters except in a few instances (e.g. see sequences in Clade E, Figure 5.6). There seem to be no genus or species specific120bp repeats, and it was speculated that the sequence variation was already present in the ancestor as well as in the *Triticeae* species of today confirming conclusions derived by Contento *et al.*, (2005). Chromosomal origin was not assigned to any of the pSc119.2 sequences, still it could be argued, and that if the chromosomal origin was known the sequences would still be as dispersed as they are now (Figure 5.6). However, to prove this hypothesis, BACs and sequences with chromosomal annotations are mandatory that are becoming increasingly available in the public databases and from the wheat sequencing project (http://www.wheatgenome.org).

It was presumed the ancestral *Triticeae* had multiple master copies and a range of variants of the 120bp repeats (Figure 5.7A). Since *Triticeae* split into sub families about 50-70MYA (Murat *et al.*, 2010), different species inherited multiple copies of pSc119.2 sequences and that diversity is maintained at large even today (see *T. aestivum* sequences in Figure 5.6). One possible explanation of maintaining this diversity could be that the sequences are present in the major heterochromatic blocks of *Triticeae* chromosomes (Mukai *et al.*, 1993, Taketa *et al.*, 2000, Badaeva *et al.*, 1998, Cuadrado and Jouve, 1995) and show heavy cytosine methylation in diploid and polyploid species at the chromosomal level except for the very large blocks in rye which have amplified after the divergence of rye (Contento and Schwarzacher unpublished data and below). Therefore, chromosomal regions with multiple copies of the variants do not undergo recombination and are transferred as blocks to maintain the original diversity. Comparative analysis of pSc119.2 sequences revealed a diverging pattern of evolution. Single nucleotide polymorphism is present along the entire sequence at intra and interspecific levels (see multiple sequence alignment Appendix 5.1). There is no strong homogenization of pSc119.2 sequences within or among

191closely related species (Figures 5.5&5.6A). Thus molecular drive leading to concerted-evolution of repeats is time independent for pSc119.2 sequences (Pérez-Gutiérrez *et al.,* 2012, Contento *et al.,* 2005).

Extensive distribution of Afa-family sequences in *Triticeae* genomes is evident from their chromosomal positions (Figures 5.7&5.8). They exist not only in the telomeric/sub-telomeric but also in the interstitial regions of chromosomes (Mukai *et al.,* 1993, Castilho and Heslop-Harrison, 1995, Pedersen and Langridge, 1997, Biagetti *et al.,* 1999, Carvalho *et al.,* 2009, Nagaki *et al.,* 1998b). Afa-family sequences of related genomes clustered in the tree (Figure 5.5). Copy number as well as chromosomal origin has a huge impact on sequence groupings (see clade A Figure 5.5). These results are consistent to those of previously obtained for other Afa homologous sequences (Tsujimoto *et al.,* 1997, Nagaki *et al.,* 1998b). Afa-family sequences have undergone several episodes of amplifications and deletions in their evolutionary history (Nagaki *et al.,* 1995, Tsujimoto *et al.,* 1997). Thus the enormous variation in copy number among *Triticeae* members could be assigned to such events (Nagaki *et al.,* 1999, Vershinin *et al.,* 1994). However, other mechanisms such as their incorporation in autonomous TEs may also result in their rapid turnover. Indeed, several BACs were found, where Afa-sequences were flanked or inserted into TEs (Figure 5.4C) and were amplified with PCR using Afa and LTR primers (Appendix 5.1 and above). However, some chromosomal regions are reported to be repeat-rich as they can efficiently accumulate repeats or they are inefficient in removing them (Ma *et al.,* 2007, Ma *et al.,* 2004, Vogel *et al.,* 2010).

The low sequence diversity of Afa-family sequences in different *Triticeae* genomes may be the result of recent amplifications (Nagaki *et al.,* 1998a, 1999). However, PCR reaction may also be influenced towards the amplification of certain subsets of the repetitive DNA sequences as a small amount of DNA is used as template for amplification, which could potentially affect the estimation of diversity (Tang *et al.,* 2011, Nagaki *et al.,* 1998a). This limitation was overcome, by using two rounds of PCR and the eluted fragments from both reactions were sequenced (see above). However, no significant variation in the sequencing result was evident (see multiple sequence alignment of Afa-family sequences Appendix 5.1). Furthermore, the results here also revealed, Afa sequences are not only genome or species specific but also show chromosomal specificity in their distribution (compare sub-clades A1&A2 Figure 5.5). It is very likely that the Afa-family sequences present on the same chromosome

have multiplied from the same or very few and closely related master copies (Figure 5.7).

It was difficult to predict when the first relic of Afa-sequences amplification has occurred in *Triticeae.* However, Nagaki *et al.,* (1998a) believed, the S-genome of *Ae. speltoides* as the possible ancestor, as it contains few and highly diverged copies. But in this analysis Afa-family were predicted as much older component of the *Triticeae* genomes. Intact tandem array of Afa-family sequences were identified in several whole genome shotgun sequences of rice (for example see GenBank accession number AACV01030552). Indicates the ancestor of the grass family contained Afa-sequences before its split into subfamilies some 65MYA (see Figure 1.2, Chapter I).

Rapid expansion and homogenization of variant repeats of satDNA is a major event in species diversification (Kuhn *et al.,* 2008). Sequence of the Afa-family exhibit species as well as chromosome specific clusters (compare sub-clade A1 and A2 Figure 5.5). Sequences from the genomes with high copy number clusters significantly while those with few copies do not cluster (see *T. turgidum* sequences in sub-clade B2 and C1 Figure 5.5). Presumably, the Afa-family sequence of today have been amplified several times using a limited number of master copies (Figure 5.7) and therefore, display species and chromosome specific repeats grouping (sub-clade A1 and A2 Figure 5.5). It is evident, Afa-family sequences both at the intra and interspecific levels have undergone strong homogenization events and the molecular forces leading to the concerted-evolution of sequence family is time dependent.

5.4.2 *In situ* hybridization

At present, the Afa-family (Rayburn and Gill, 1986) and pSc119.2 sequences (McIntyre *et al.,* 1990) are extensively applied as cytological markers in *Triticeae* research for detecting alien chromatin in hybrid wheat backgrounds (Mukai *et al.,* 1993, Castilho and Heslop-Harrison, 1995, Carvalho *et al.,* 2009). Here, both repeat families were applied not only to detect the recombinant wheat chromosomes (see Chapter III) but also in mapping of WSMV-resistance gene in the wild *Th. intermedium* genome (Figure 5.9).

5.4.3 DNA methylation

5.4.3.1 Immunostaining with anti-5-mC

Cytosine methylation is a stable epigenetic mark and has a prominent role in plants regulatory machinery, including silencing TEs and other repetitive DNA sequences as well as in the expression of endogenous genes (Finnegan *et al.*, 1998, Finnegan *et al.*, 2000, Kubis *et al.*, 2003a). Stress tolerance is largely associated with changes in the levels of cytosine methylation (Chinnusamy and Zhu, 2009, Finnegan and Kovac, 2000b, Bender, 2004). Previous studies have reported rapid and reproducible alterations in the DNA methylation in response to allopolyploidization events (Yaakov and Kashkush, 2011). Therefore, the focus of this study was to correlate possible effects of interspecific hybridization (see Chapter III) in relation to changes in levels of DNA methylation.

Methylation levels of diploid and polyploid *Triticeae* (Table 5.1) was assessed with both anti-5-mC antibody and methylation sensitive restriction enzymes in Southern hybridization (see below). Since, the D-genome of most lines included in this study was the recipient of *Th. intermedium* chromatin (see Chapter III&IV). Therefore methylation pattern of the D-genome was studied both at diploid and polyploid level. The B-genome component of wheat is the largest among the three wheat gnomes (Gustafson *et al.*, 2009) and it was also the recipient of small *Th. intermedium* fragments (Chapter III and Table 5.1). Therefore, *T. turgidum* (AB-genome) was included in the study to compare changes originating from the AB-genome alone in response to alien introgression.

The immunostaining with anti-5-mC antibody showed uniform methylation pattern along most of the *Ae. tauschii* chromosomes (Figure 5.10). However, as reported polyploidization induce alteration of cytosine methylation (Salmon *et al.*, 2005, Xu *et al.*, 2009). The immunostaining results also consistently showed all polyploids species irrespective of the genomes involved exhibited uneven and patchy distribution of the methylated cytosines along the chromosomes (Figures 5.11-5.14). Effects of the allopolyploidization events were obvious, the D-genome that show uniform 5-mC in the diploid state showed unevenly distributed 5-mC signals at the polyploid level (compare Figure 5.10 and Figure 5.11). In flowering plants, interspecific hybridization acts as a stimulus and causes heritable changes of cytosine methylation (Slotkin *et al.*, 2009, Feldman and Levy, 2005b, Matzke *et al.*, 2009).

Therefore, the recipient D-genome and the introgressed alien arm were targeted (arrows in Figure 5.13&5.14) by combing ani-5-mC antibody with total genomic DNA from *Ae. tauschii* and *Th. intermedium* (see Figures 5.11&5.14).

Methylation patterns of the D-genome was assessed in 'Millennium' which lacks alien chromatin (Table 5.1) and most of its D-genome chromosomes exhibited DNA methylation levels comparable to the other wheat or *Th. intermedium* chromosomes (compare Figure 5.11&5.14). Possible alterations of the 5-mC levels were studied in the complete alien arm using both mitotic and meiotic spreads (Figures 5.13&5.14). This introgressed alien arm is present in the form of 4Ai#2S.4DL chromosomal translocation in the lines analysed here and is the vastly deployed source of natural resistance against WSMV (see Chapter III&IV). So far, genes conferring resistance to WSMV, WCM and the fungus *Tapesia yallundae* have been mapped to the same chromosomal arm (Chen *et al.,* 1998a, Friebe *et al.,* 2009, Qi *et al.,* 2010, Schwarzacher *et al.,* 2011). However, still other genes of agronomic and bread making quality may be present on the same arm (Divis *et al.,* 2006 and Chapter III).

In both plants and animals, most of the methylated cytosine is found in heterochromatic regions (see Chapter I and above), where repetitive DNA and TEs are abundant (Heslop-Harrison, 2000b, Turner, 2009, Grafi *et al.,* 2007). Conversely, reduced methylation levels are characteristic features of the actively transcribing chromatin (Kubis *et al.,* 2003, Josefsson *et al.,* 2006, Argen and Wright 2011, Yaakov and Kashkush 2011). Hypomethylation is very well documented and associated with actively transcribing genes (Heslop-Harrison 2000a, Josefsson *et al.,* 2006, Argen and Wright, 2011, Yaakov and Kashkush, 2011). DNA methylation levels of the *Th. intermedium* arm were found more or less similar to the average wheat chromatin at meiotic pachytene (Figure 5.13). Notably, the alien arm show significantly reduced levels of 5-mC in mitotic metaphase (arrows in Figure 5.14). Due to time constraint, the same experiment with meiotic spreads was not repeated (as flowering season is once in a year). However, reproducible results of reduced DNA methylation were obtained using mitotic spreads from different lines at different times (results not shown). Thus, the prospect for expression of transferred alien genes is given at the global chromatin level (arrows in Figure 5.14). It needs to be seen now that DNA methylation is low at the gene level and can be maintained in the hybrid backgrounds.

Except the green alga *Chlamydomonas*, that exhibits the most unusual pattern of DNA methylation, abundant in the exons of genes rather than in repetitive DNA and TEs (Feng *et al.,* 2010), all other plants DNA employ methylation as a conserved role in silencing genes, endogenous selfish elements and other non-coding regions (Chinnusamy and Zhu, 2009, Finnegan and Kovac, 2000a, Suzuki and Bird, 2008). However, the levels and patterns of DNA methylation vary considerably among different species (Bender, 2004, Kato *et al.,* 2003, Law and Jacobsen, 2010). Plant genomes are incredibly large (see Bennett and Leitch, 2011) with a vast majority of their DNA being potentially deleterious and selfish (Feldman and Levy, 2005, Matzke *et al.,* 2009, Slotkin *et al.,* 2009, Yaakov and Kashkush, 2011). However, most if not all of these elements are epigenetically silenced (see Chapter I and above).

The lack of activity in repetitive DNA and TEs is due to highly evolved mechanisms that plants have, to recognize and silence the repetitive DNA that act as a genomic immune system (Huda and Jordan, 2009, Slotkin and Martienssen, 2007). Not only are these elements silenced, but they are also remembered and maintained over generations (Lisch, 2009). Allopolyploids formation is accompanied by a variety of evolutionary and revolutionary genomic changes (Feldman and Levy, 2009). Previous studies have reported rapid and reproducible alterations in the DNA methylation patterns in the early stages of the life of nascent allopolyploid species (Yaakov and Kashkush, 2011). In the genome of model plant *Arabidopsis*, reawakening of silenced transposable elements and burst of retrotransposon was associated with reduced DNA methylation (Tsukahara *et al.,* 2009). Hybridization introduces novel TEs into a host, lacking effective silencing mechanisms and thus results in increased TEs activity (Argen and Wright, 2011).

Therefore, genomic methylation pattern of the repetitive DNA and TEs was investigated using methylation sensitive restriction enzymes, which was presumed, would be the first component to be triggered to such genomic changes (alien introgression). However, by and large no massive alterations of the genomic methylation patterns were revealed. Some changes may be associated with the alien chromatin, such as the appearance of extra bands (see stars in Figures 5.16&5.18 right, and above) or increase-decrease in the smears resulting from restriction of overlapping sites (compare Figures 5.21&5.22 right) or the existence of low CCNGG methylation in the *Th. intermedium*, evident in the Afa-family blot restricted with *Scr*FI (compare 4th lane in D with A, B, C, E and F-J Figures 5.15&5.16 right). These all are indicative

of the demethylation around some unknown regions in the wheat genome and may have many evolutionary implications in terms of regulating gene expression, especially the resistant genes in case of *Th. intermedium* genome (see also chapter VI).

CHAPTER VI: GENERAL DISCUSSION

6.1 Novel sources of WMSV-resistance in *Th. intermedium* chromatin transferred to wheat

In this study, wheat-*Th. intermedium* derivatives that conferred effective WSMV-resistance in both green-house and field trials (Divis *et al.*, 2006) were screened with fluorescent *in situ* hybridization (Chapter III) and molecular markers (Chapter IV), that allowed determination of the nature and size of *Th. intermedium* chromatin in these lines. Molecular cytogenetics with repetitive DNA probes that have characteristic banding patterns for most wheat chromosomes (see Chapter I, Fig 1.5) was very effective in identifying the recipient wheat chromosomes (Chapter III, Figures 3.24 and 3.25). Subsequent selection of molecular markers for breakpoint mapping could concentrate on the identified chromosome arm (Chapter IV, Table 4.5) rather than needing a genome wide approach of testing for the 42 different wheat chromosome arms. In most of the resistant breeding lines tested here, the previously known *Th. intermedium* group-4 derived resistance translocated to wheat chromosome 4D, was identified as a whole short arm translocation (Chapter III, Table 3.1), but also potential novel resistances were associated with the group-1 and group-3 of *Th. intermedium* translocated as small fragments to wheat chromosomes 1BS and 3DL respectively (see IV). The transfer of desirable genes and development of crops with durable and non race-specific resistance constitute the core objectives of plant breeding (Ayala-Navarrete *et al.*, 2007, King *et al.*, 1997a, Krattinger *et al.*, 2011, Mujeeb-Kazi and Hettel, 1995, Schwarzacher *et al.*, 1992). Often, translocations involving small alien fragments are preferred due to the less likelihood of linkage drag, compared to addition or substitution lines (Carvalho *et al.*, 2009, Friebe *et al.*, 2009, Lukaszewski, 2000, Qi *et al.*, 2007). However, not all larger alien fragments are disadvantageous, they may potentially introduce more variation. The short arm of *Th. intermedium* chromosome 4Ai#2 has no, or unrevealed negative effects and under field conditions is stable and provides complete protection against WSMV and its vector the WCM (Chen *et al.*, 1999a, Divis *et al.*, 2006, Graybosch *et al.*, 2009, Schwarzacher *et al.*, 2011). The same alien arm (4Ai#2S) also carries the resistance gene(s) for the notorious fungal pathogen *Tapesia yallundae* along with those of WCM and WSMV-resistance (Chen *et al.*,

2003a, Li *et al.,* 2005b, Mutti *et al.,* 2011). Recently, other resistance genes, such as the *Lr19/Lr25* and *Lr24/Lr26* complexes have also been mapped to the long arms of *Th. ponticum* chromosomes 7 and 3, that are transferred as blocks (Li and Wang, 2009). In contrast, the smaller introgressed fragments may not supply the additional resistances and might not retain the desirable traits for which they are selected (see recombinant S-lines Chapter III).

Currently *Wsm1* and *Wsm2* are the two genes used in cultivar improvement and both genes show temperature dependency. However, *Wsm1* provides superior resistance and can provide protection above 18°C, while the resistance offered by *Wsm2* fails at 18°C (Graybosch *et al.,* 2009, Mutti *et al.,* 2011, Seifers *et al.,* 1995, Seifers *et al.,* 2007, Seifers *et al.,* 2006). Often, WSMV interacts with *High Plains virus* (HPV) and *Triticum mosaic Virus* (TriMV) to co-infect a single host, that causes severe damages due to synergistic interaction (Mette *et al.,* 2002, Seifers *et al.,* 2009b, Stenger *et al.,* 2007a). However, it has been shown that the released cultivar 'Mace' (from the same populations as studied in the current work) that carries the *Wsm1* gene, resists the co-infection of WSMV and TriMV up to 19°C and prevents disease synergism (Tatineni *et al.,* 2010).

All known sources of WSMV-resistance that only carry the short arm of *Th. intermedium* chromosome only, exhibit characteristic symptoms of WSMV at 27°C (Fahim *et al.,* 2010b, Seifers *et al.,* 1995) and the resistance offered by *Wsm2* can only be exploited by planting wheat in months with cool autumn temperatures (Lu *et al.,* 2011, Martin *et al.,* 2007). However, wheat substitution lines that carry the entire *Th. intermedium* chromosomes exhibit stable WSMV-resistance even at 27°C (Seifers *et al.,* 1995, Fahim *et al.,* 2011), suggesting the presence of further resistance genes in *Th. intermedium*. This urges the need for more effort to exploit the potential in *Th. intermedium,* and the results shown here provide the first concrete evidence of new WSMV-resistance genes, designated as *Wsm4* and *Wsm5* present on the homoeologous group-1 and group-3 of *Th. intermedium* chromosomes respectively (Chapter III for detail).

Presence of the 4D recombinant chromosome has always been correlated with WSMV-resistance in the field (Divis *et al.,* 2006, Qi *et al.,* 2007, Wells *et al.,* 1982, Wells *et al.,* 1973). However, lines N02Y5003 and N02Y5109 without a 4D translocation, but with the largest distal alien chromatin insertion corresponding to the 28.3±4.9% and 42.9±2.5% of the recombinant 1BS and 3DL arms also showed

WSMV-resistance (see Chapter III). In contrast, experimental lines without alien chromatin (N02Y5021, N02Y5082, N02Y5096, N02Y5105 and N02Y5121) and lines with small 1BS fragments (N02Y5019, N02Y5156, N02Y5163) do not show resistance. However, the WSMV-susceptible lines with small 1BS fragments identified in this study, are still worthy for further screening to other biotic and abiotic stresses, as recent studies have revealed the presence of quality and resistance genes on the group-1 of *Th. intermedium* (Hu *et al.,* 2011). Additionally, they provide useful tools for mapping the *Wsm4* gene (see below).

6.2 Molecular markers detecting *Th. intermedium* chromatin and confirmation of novel WSMV-resistance genes

To ensure maximum exploitation of the known and novel WSMV-resistance and their earliest availability to wheat growers, a number of previously known PCR markers linked to the resistance were employed to facilitate MAS-breeding approaches (see Chapter IV). PCR-based marker analysis is reliable, time and cost-effective as well as convenient in terms of manipulation and application (Collard and Mackill, 2008, Korzun, 2002, Todorovska *et al.,* 2005). ESTs-derived markers are very useful in determining the homoeologous relationships of chromosomes from different grass species by comparative mapping (Heslop-Harrison, 2000b). Therefore, EST-markers are extensively used as an effective tool for genetic analysis in *Triticeae* (Liu *et al.,* 2011, Peng *et al.,* 2004, Qi *et al.,* 2007, Wang *et al.,* 2010). Recently, ESTs from the interspecific conserved exonic regions designed for wheat group-4 chromosomes were reported to be useful in amplifying the group-2 chromatin of *Th. intermedium* and vice versa (Fahim *et al.,* 2011). Furthermore, it is also known that PCR often amplifies products from orthologous genes simultaneously, however the amplicons show length polymorphisms (Hu *et al.,* 2011). Therefore, to overcome any potential shortcoming that may appear from the low levels of polymorphism and the conserved nature of ESTs present on different homoeologous groups, the study concentrated on 26-EST markers previously tested and reported polymorphic for *Th. intermedium* and 'Chinese Spring' in the literature (Fahim *et al.,* 2011, Gao *et al.,* 2009, Kong *et al.,* 2009, Qi *et al.,* 2007, Wang *et al.,* 2010, Zhang *et al.,* 2002). These included group-2, group-4 and group-7 derived dominant ESTs-markers (see Chapter IV). Polymorphism for some of these markers was successfully established in the material used and 6 new polymorphic

markers were identified. Thus a good selection of markers is now available for large scale screening of WSMV-resistance (see Chapter IV) in marker-assisted WSMV-resistance breeding and gene pyramiding

Another aim of the marker analysis was to ascertain the novelty of 1B and 3D resistances, and that it is not the known *Wsm1* gene derived from 4AiS#2S that could transfer through translocation. Two approaches were followed, first extensive cytogenetics using GISH and repetitive DNA sequences were applied and the 4Ai#2S and 4D chromosomes in lines carrying the recombinant 1B and 3D chromosomes, were found intact (Chapter III for detail). Secondly, known markers linked to 4D derived resistances were used (see above), in order to identify the resistance genes on 1BS and 3DL if they have a common chromosomal origin. The PCR markers screen also supported the different origin of these resistances. Apart from the group-4 derived markers none could reveal useful polymorphisms in the lines applied here (Chapter IV, Table 4.1 and Appendix 4.1). Group-4 markers amplified DNA from the group-4 recombinants only, except the WSR9, but could not identify useful polymorphism for alien-derived resistance associated with 1BS or 3DL (see Figure 4.1, Chapter IV).

Consequently, both the cytogenetics and MM results confirm the newly identified recombinant chromosomes in line N02Y5003 and N02Y5109 as recipients of novel WSMV-resistance genes, designated as *Wsm4* and *Wsm5*. However, none of the known markers showed linkage to 1BS or 3DL resistance. Therefore, attempts were made to find linked markers to the newly identified genes, but only one potential marker for the 1B resistance was identified (Figure 4.11).

6.3 Molecular breakpoints detection in the recombinant 1BS

The recent advances in DNA sequencing projects and analytical approaches have greatly increased our understanding of the grass genome (Devos, 2010). Accumulation of the genetic markers, combined with the availability of large sets of DNA sequence data have made it possible to carry out comparative genomic studies in the grass family (Feuillet and Keller, 2002b, Heslop-Harrison, 2000b, Hu *et al.*, 2011, Peng *et al.*, 2004). Among the various MMs, microsatellites offer an attractive and reliable approach for wheat mapping studies because of their high degree of polymorphism (Röder *et al.*, 1998b, Röder *et al.*, 1998a, Somers *et al.*, 2004, Song *et al.*, 2005, Sourdille *et al.*, 2001, Mangini *et al.*, 2010). In the current study 32-MMs were applied

thoroughly, that were distributed across the three distal deletion bins of wheat above the NOR of 1BS (see Chapter IV, Table 4.3).

The sizes of the seven *Th. intermedium* group-1 derived translocations were compared (Table 4.5). Firstly, the sizes of these translocations cytogenetically were estimated using GISH measuring the *Th. intermedium* chromatin along the chromosome in percentage and ranked them in order of size (Chapter III, Table 3.2). As deletion stocks with known fraction length (FL) values (Endo and Gill, 1996) were not applied in this study, therefore physical length measurements of the recombinant chromosomal arm to the lost wheat arm cannot be correlated directly with MM. However, the genetic map position of some MMs is published (Reddy *et al.*, 2008, Somers *et al.,* 2004, Song *et al.*, 2005, Sourdille *et al.*, 2004b, Sourdille *et al.*, 2004a, also see Chapter IV section 4.4.5). Hence, an attempt was made to order the MMs on a combined map and estimate the genetic position of each breakpoint (compare Figure 4.11, and 4.9 Chapter IV). The two results however differ slightly in the ranking of the breakpoints (compare Figure 4.11 and 4.10 Chapter IV) and while line N02Y5003 contains the largest alien fragment with both estimates, the other fragment sizes are ranked differently. The differences between estimated size based on cytogenetic methods and MMs are common (Ayala *et al.* 2009, Friebe *et al.* 2009). The fluorescent *in situ* hybridization signal is often very large and extends beyond the physical boundary of the chromosome giving overestimation of signal width, particularly with small fragments (Lukaszewski *et al.,* 2005). Because chromosomal condensation during metaphase is uneven along chromosomes and can vary between early and late metaphases and is influenced by the duration of the metaphase arresting pre-treatment before fixation, fragment size depends on the stage and overall lengths of the chromosomes measured (Schwarzacher and Heslop-Harrison, 2000).

To identify BPs and map-based position of the newly identified WSMV-resistant gene in line N02Y5003, two approaches were used. Firstly, the polymorphic were applied markers to develop a consensus map corresponding best with the physical data and showing the least postulated rearrangements of markers between lines, and then the map was used to identify the breakpoints (see Figure 4.11 and Table 4.6 Chapter IV). Secondly, to reconfirm the order and interval of MMs, the markers order obtained here was compared to published 1B maps (Figure 4.9). Absence of a marker from a recombinant line and its presence in another provided the basis of identifying a break point interval (Table 4.6, Figure 4.11). Further the authenticity of markers was

confirmed by their presence-absence in the nulli-1B line and 'Chinese Spring'. To double check and refine the BP intervals, comparative map analysis of the 1B published maps was carried out (see Figure 4.9, 4.10).

The present results indicated that all recombination (Table 4.6, Figure 4.11) involved the distal 28.3% region of the physical 1BS and no recombination in the proximal regions toward the centromere (see also Chapter III). Thus, suggesting the presence of recombination hot-spots along the distal 1BS. Sharma *et al.,* (2009) studied the physical distribution of 68 recombinant breakpoints on the 1R and 1S, and mapped all recombination within the distal 40% of the physical arm. Similar results of low recombination frequency close to the centromeres compared to the telomeres have been reported for the group-1 of wheat (Sourdille *et al.,* 2004a). Endo and Gill (1996), recognized that most of the hot spots along wheat chromosome exist at the junction of heterochromatic and euchromatic regions. They also identified localized hot spots with relatively inert adjacent regions in a few wheat chromosomes including the NOR region of IBS.

6.3.1 Physical and map-based position of the novel WSMV-resistant gene on 1BS arm of wheat

The micro-collinearity of genetic markers (Bennetzen, 2005, Luo *et al.,* 2009, Paterson *et al.,* 2009, Qi *et al.,* 2009) was evident in the MMs analysis of the homoeologous group-1 of wheat, Beaver and *Th. intermedium* (compare Figure 4.3-4.7). Initially Beaver (1RS.1BL) was used as a control 1BS line. However, the low polymorphism between wheat 1BS and rye 1RS was well pronounced (Table 4.5). That could be attributed to the high gene density along the small arm of group-1 chromosomes (Peng *et al.,* 2004, Sharma *et al.,* 2009).

The precise physical map location of the newly identified WSMV-resistance gene was not detected. However, with enough confidence, it is not located within the distal 20% of the recombinant 1BS arm (see Chapter III). These results are consistent to those obtained by Wells *et al.,* (1983) by analysing a less desirable disomic substitution line. They reported the location of WSMV-resistance gene towards the centromere rather than toward the distal end in the *Th. intermedium* chromosome. Friebe *et al.,* (2009) mapped the *Wsm1* gene to the distal 20% of the recombinant 4DS.

However, as *Wsm1* is a group-4 derived gene, therefore these results do not contradict to those of Friebe *et al.,* (2009).

Finally, the resistance gene was pinpointed to a ~6% region of the recombinant 1BS arm (comparing translocation sizes of alien fragments in R and S lines, Table 3.2 Chapter III). The Ganal and Röder (2007) markers *Xgwm1100, Xgwm1028* and *Xgwm4435* delimited the resistance gene in line N02Y5003 (see PB-V, Table 4.6 Chapter IV). Thus taking the order of markers in these results this resistance gene is flanked between *Xgwm4144* and *Xgwm1100* markers (Table 4.6, Figure 4.11). However, if the order of the Ganal and Röder (2007) map is followed (in the absence of recombination) then the resistance gene would lie between Xgwm0911 and *Xgwm1100* markers (Figure 4.10A).

6.4 Significance and potential of the novel resistance genes derived from *Th. intermedium*

Since the 1960s, at least 15-genes for fungal or viral resistance have originated from *Th. intermedium* chromosomal segments (Li and Wang, 2009). Though, *Th. intermedium* is a member of the tertiary gene pool of wheat, the results with GISH (Chapter III), PCR markers (Chapter IV) and repetitive DNA studies (Chapter V) show the shared homology of *Th. intermedium* and wheat, especially to the D-genome. The present results indicated *Th. intermedium* has more potential to be exploited, it does not cause meiotic instability (see Chapter III) and once the *Th. intermedium* fragments are transferred, they show reduced cytosine methylation (Chapter V, and below), a characteristic of the actively transcribing chromatin (Bender, 2004, Law and Jacobsen, 2010, Schmidt and Heslop-Harrison, 1998a). Furthermore, no significant epigenetic changes were revealed in the current study that are often associated with intergeneric crosses and alien chromatin transfer (see Chapter V and below).

Plant breeders have been remarkably successful in manipulating novel variations required for resistance and productivity (Borlaug, 1983). Though, some of the high yielding cultivars suffer from low resistance, while many of the highly resistant cultivars show poor agronomic performance (Ayala-Navarrete *et al.*, 2007, Divis *et al.,* 2006, Schwarzacher *et al.,* 1992).

This natural WSMV-resistance derived from the group-4 is currently the most important source of resistance available to wheat breeders (Friebe *et al.,* 2009, Graybosch *et al.,* 2009). The basis for such wide utilization is effective resistance, and the lack of linkage drag which could potentially depress the essential agronomic and end-use quality traits (Divis *et al.,* 2006, Mutti *et al.,* 2011, Schwarzacher *et al.,* 2011). Alien material introduces new diversity at the expense of wheat genes (Chen *et al.,* 1999a, King *et al.,* 1997a, King *et al.,* 1997b, Mujeeb-Kazi and Hettel, 1995, Wang *et al.,* 2010), but sometime the inserted fragments replace important wheat quality genes (Ayala-Navarrete *et al.,* 2007, Friebe *et al.,* 2009, Liu *et al.,* 2011, Qi *et al.,* 2007).

Two novel WSMV-resistance genes were identified (Chapter III and above), time constraint allowed us neither to develop MMs linked to 3DL resistance nor to exploit the available markers to determine the BP (Chapter III and above). Of the seven homoeologous groups, the group-1 of wheat is well studied and most understood. Primarily, clusters of important agronomic genes (Endo and Gill, 1996, McIntosh *et al.,* 2010, Reddy *et al.,* 2008) including at least 22 genes and QTLs have been found on chromosome 1B that confer disease resistance (Peng *et al.,* 2004). The value of 1B in relation to WSMV-resistance has never been documented before (Chapter III and above) so it is important to check for possible linkage drag. Indeed, some PCR markers that were previously reported as flanking important agronomic genes were lost from the recombinant 1BS arms. For example *Xpsp3000*, a dominant marker, applied in MAS-breeding for three genes including *Gli-1* gene (Bryan *et al.,* 1997) *Yr10* (Wang *et al.,* 2002) and *Snn1* (Reddy *et al.,* 2008) was lost and replaced by *Th. intermedium* chromatin in a few susceptible lines as well as from the resistant line N02Y5003 (Table 4.6). Similarly, on the genetic map the *Glu-3, Pm8, Lr26, Sr31* and *Yr9* loci are positioned above the dominant EST-marker *Xucr*_6 (Sharma *et al.,* 2009). The current results revealed that the *Xucr_6* marker is lost by the WSMV-resistance line N02Y5003 (Table 4.6).

Nowadays, *Wsm1* confers resistance and disadvantages (Divis *et al.,* 2006, Graybosch *et al.,* 2009). However, original sources that carried *Wsm1* were frequently associated with undesirable traits such as yield penalties and poor bread-making qualities (Baley *et al.,* 2001, Seifers *et al.,* 1995, Sharp *et al.,* 2002). The subsequent backcrosses and hybridization eliminated the potential negative effects of the introgressed *Th. intermedium* chromatin (Divis *et al.,* 2006). The importance of similar

crossings is highlighted, to restore the desirable wheat genes while maintaining the WSMV-resistance of recombinant chromosome 1BS.

Reduction in the size of alien translocations through chromosome engineering has been met with great success (Ayala-Navarrete *et al.,* 2007, Friebe *et al.,* 2009, Lukaszewski, 2000, Qi *et al.,* 2007). Recently the group-1 substitution lines of wheat-*Th. intermedium* were exposed to stripe rust pressure and were reported as potential sources of *Sr*-resistance (Hu *et al.,* 2011). In the same study the authors also reported the presence of a novel high molecular weight glutenin (*Glu*) subunit of *Th. intermedium* origin. Earlier, when line N02Y5003 was analysed for the potential negative agronomic and bread making quality traits, none were discovered (Divis *et al.,* 2006). Sequence data also suggest, gene families are more or less conserved across different taxa of grasses (Heslop-Harrison and Schwarzacher 2011a) except some genes, like storage proteins and disease resistance which expand in a lineage-specific manner across grasses (Leister *et al.,* 2004, Xu *et al.,* 2008, Devos 2010). Therefore, the possible presence of further genes on the introgressed arm harbouring the WSMV-resistance as lineage specific genes is speculated.

The same *Th. intermedium* arm harbouring the *Wsm1* gene, and identified with terminal pSC119.2 sites (Friebe *et al.,* 1991, and Chapter III) has been used to enrich wheat cultivars of diverse backgrounds for almost 40 years now (Wells *et al.,* 1973). Studies indicate, pathogens mostly stay ahead of the hosts in their co-evolutionary race, probably due to their relatively short life and abundance (Zhan *et al.,* 2002). The current strains of fungi and viruses are evolving much faster to adapt to changing environmental conditions (Hovmøller *et al.,* 2011). The genetic information of most plant viruses including WSMV are encoded in their single stranded RNA genome (Fahim *et al.,* 2010b, Stenger *et al.,* 2007b, Tatineni *et al.,* 2011). The lack of proofreading activity in RNA viruses tends to be the main reason for their high mutation rate and enormous adaptability (Elena and Sanjuán, 2005). The increased virulence of fungi may be deduced from their ability to produce 2-3 times more spores than they would produce earlier, and infect cultivars that were previously resistant (Hovmøller *et al.,* 2011). The Ug99 races have been shown previously, to overcome the resistance of more than 23 catalogued wheat stem rust-resistant genes (Singh *et al.,* 2006, McIntosh *et al.,* 2008, Liu *et al.,* 2010). Similar mutations in the RNA-genome of WSMV or in the resistant gene *Wsm1* may potentially put all the deployed sources of WSMV-resistance with *Wsm1* at risk or even render it ineffective. Therefore, the

best means to improve resistance would be to stack the novel group-1 or group-3 derived resistances in a germplasm carrying the known 4DS resistance. Such deployment of the combinations of effective "stacked" genes should reduce the probability of simultaneous mutation events in the pathogen as well as in the resistant genes.

Therefore, the newly identified sources of WSMV-resistance in this study are extremely important, especially for gene pyramiding. It is reasoned with enough confidence that this study will be helpful, not only in MAS-breeding but may also provide an opportunity for targeted-gene cloning. Still, the development of new linked markers to these novel genes is importance and, so that the lack of markers will not hamper their effective utilization.

Production of high yielding and resistant cultivars remains the primary goal of wheat breeding (Wells *et al.,* 1973, Feldman and Sears 1981, Borlaug 1983). However, despite successful introgression, the actual value of hybrids remains obscure. However, integration of new genomic approaches with traditional breeding strategies may further multiply the value of identified desirable traits (Heslop-Harrison *et al.,* 1990, King *et al.,* 1993, 1997a, Graybosch *et al.,* 2009, Schwarzacher *et al.,* 2011). The better understanding of plant genomics has been possible due to the availability of large sets of genomic sequences (Varshney and Dubey 2009, Mochida and Shinozaki 2010). It has made it possible to develop a variety of functional molecular markers detecting desirable traits and is shaping our approaches of the plant breeding (Schwarzacher et al. 1989, Miflin 1999, Gutterson and Zhang 2004, Varshney and Dubey 2009, Heslop-Harrison and Schwarzacher 2011b).

Alien fragments continue to allow transfer of major traits into wheat varieties through crossing. Although timescales are long, the new characters are unique and benefits exceed any linkage drag in appropriate selection programmes. The importance of future research and directed efforts to combine different sources of known and novel resistances against WSMV is recommended (*Wsm1, Wsm2, Wsm3, Wsm4* and *Wsm5* the latter two are identified in this study) in a single genotype to safeguard wheat against the threats of WSMV.

6.5 Repetitive DNA sequences in wheat and *Th. intermedium* chromosomes

In this study, diversity and the contrasting evolutionary dynamics of two repetitive DNA sequences from *Th. intermedium* and 'Chinese Spring' were investigated. To date, many members of the repetitive DNA families have been isolated from the *Triticeae* (Bedbrook *et al.,* 1980, Rayburn and Gill 1986, McIntyre *et al.,* 1990, Anamthawat-Jonsson and Heslop-Harrison 1993, Vershinin *et al.,* 1994, 1995, Cuadrado *et al.,* 1995, Tsujimoto *et al.,* 1997, Nagaki *et al.,* 1998a, Contento *et al.,* 2005). Until recently, members of the repetitive families isolated, and their phylogeny or chromosomal distribution studied alone. However, fewer attempts have been made to understand the phylogeny of different repeat types (Anamthawat-Jonsson and Heslop-Harrison 1993, Tang *et al.,* 2011) or focus on mechanisms of concerted evolution (Perez-Gutierrez *et al.,* 2012) for different *Triticeae* repeat types. An attempt was made to bridge this gap by isolating two important repetitive DNA sequences (Afa and pSc119.2) from *Th. intermedium* and 'Chinese Spring', undergoing different selection and homogenization mechanisms (Figure 5.7). In addition, their chromosomal distribution was studied (Figure 5.9 and Chapter III) along their phylogeny (Figure 5.5 and Figure 5.6) and has given a comparative insight of both repeat types (Figure 5.7). Further, these repeats were used as finger prints to target alien introgression and their methylation pattern was studied (see Chapter III, V and below).

No function was ascribed for pSc119.2 sequences in the published data, however Afa-sequences were repeatedly found in BACs as integral part of resistant genes (GenBank accession EF567062, is Lr1 genomic region). The Afa repeats were found as component of some *Caspar* elements associated with gene and regulatory regions influencing gene expression (Wicker *et al.,* 2003). Afa-family sequences were also seen at the centromere of wheat-*Th. intermedium* hybrid lines and *Th. intermedium* itself (Figure 5.9). Thus Afa-sequences provide a hotspot for recombination between wheat and *Th. intermedium* (see Chapter III). Sequence analysis of Afa revealed two penta-nucleotide motifs 5'-CAAAA- 3', previously reported from rye heterochromatin and were considered to be involved in crossing over and transposition (Appels *et al.,* 1986, Grotewold*et al.,* 1991). More recently, similar

functions were proposed for the same motifs in Flying Dragon satellites sequences (Felice *et al.*, 2006).

6.6 DNA methylation of wheat-*Th. intermedium* hybrid lines

Organization of chromatin plays an important role in gene expression (Suzuki and Bird 2008, Slotkin *et al.*, 2009, Turner 2009). Heterochromatin is highly condensed compared to euchromatic regions and is not easily accessible for transcription. Therefore, the same nucleotide sequence may be either well-expressed or not at all depending on where it is located (Heslop-Harrison, 2000b, Bender, 2004, Matzke *et al.*, 2009). Most methylated cytosine is found in heterochromatic regions, where most of the repetitive sequences and transposable elements are found (Kubis *et al.*, 2003, Fuchs *et al.*, 2006, Grafi *et al.*, 2007, Lisch, 2009). DNA methylation is essential for normal development in higher eukaryotes. It reduces the transcriptional noise of mobile and other invading DNA and thus reduces the potential negative impacts of transposition (Finnegan 1998, Argen and Wright 2011). On the other hand, in plants cytosine methylation has a significant role in the regulatory machinery throughout the development. It has been recognized that stress or unusual environmental stimuli like interspecific hybridization or tissue culture may cause heritable changes to the cytosine methylation in plants and has evolutionary consequences (Feldman and Levy 2005, Matzke *et al.*, 2009, Slotkin *et al.*, 2009). Reduction in methylation level was reported to be associated with conspicuous effects on morphology, development and fertility (Finnegan *et al.*, 1998, Kashkush *et al.*, 2002, 2003, Jin *et al.*, 2008, Feldman and Levy 2009). In addition processes such as vernalization, flower and seed development and stress tolerance are largely associated with cytosine methylation (Chinnusamy and Zhu 2009). For example, in *Arabidopsis*, hypomethylation results in pleiotropic phenotypic and developmental disorders (Finnegan and Kovac 2000, Bender 2004). Thus, like other higher eukaryotes DNA methylation plays dual role in plants, providing defence against endogenous selfish elements and regulates gene expression (Finnegan 1998, Heslop-Harrison 2000a, Slotkin *et al.*, 2009). Similarly, aberrant DNA methylation has been associated with other conspicuous effects on morphology, development, fertility, aging, mental health abnormalities and diseases such as cancer (Finnegan *et al.*, 1998,

Kashkush *et al.,* 2002, 2003, Yang *et al.,* 2004, Jin *et al.,* 2008, Feldman and Levy 2009, Murgatroyd *et al.,* 2009).

Polyploidization plays a major role in the evolution of plants. Allopolyploid species can tolerate genomic changes that are either unattainable or unfavourable at the diploid level (Feldman and Levy 2009). Wheat is remarkably stable, and tolerates wide genomic changes, such as gain or loss of chromosomes (Sears 1966, Feldman and Sears 1981), that may be one of the contributing factors for the lack of radical modification in the DNA methylation patterns. In case of Afa-family or pSc119.2 sequences, it is known these elements comprise the oldest components of the *Triticeae* genomes (Vershinin *et al.,* 1994, Nagaki *et al.,* 1999, Contento et a. 2005). Therefore, irrespective of the alien introgression, not only the sequence itself, but the methylation pattern is also conserved (Figures 5.15-18).

Similarly, all polyploidization events are not necessarily accompanied with rapid genomic changes. For example in newly synthesized allotetraploid and allohexaploid cotton, no rapid genomic changes were recorded (see He *et al.,* 2003). The recent availability of DNA sequence data from the A and B genomes of wheat has made it possible to trace the footprints of TE insertion in the two genomes (Salina *et al.,* 2011). Studies have shown that the majority of the TEs actively proliferated in the A and B genomes some 0.5-0.6MYA, before the allopolyploidization events. They further concluded, the polyploidization events did not enhanced or repressed the transposition of mobile elements (Charles *et al.,* 2008). The presence of at least two independent methylation codes (CpG and CpNpG) might be related to the fact the plants are sessile and require a fine adaptation of their genomes to environmental conditions (Jeltsch, 2002).

Hybridization introduces novel TEs, for which the host genomes lack efficient silencing mechanisms (Argen and Wright, 2011). However, the FISH (see Chapter III) and preliminary sequence data of repetitive DNA (Chapter V) revealed the existence of largely unknown affinity between the *Th. intermedium* and wheat. It is reasoned to believe, if such significant homology in sequence context of repetitive DNA and other TEs is present on a higher scale (compare multiple sequence alignment files Appendix 5.1), between wheat and the *Th. intermedium*, less background effects will be evident in the hybrids as most of the TEs and repetitive DNA could be efficiently silenced. Such a possibility will increase the significance of *Th. intermedium* as an invaluable source of important genes for wheat improvement. The aim is, genes to be transcribed

as they show lineage-specific amplification (Leister *et al.*, 2004, Xu *et al.*, 2008, Devos 2010, Heslop-Harrison and Schwarzacher 2011, also see arrows in Figure 5.14), while the TEs and other repetitive DNA are more widely present and are effectively silenced.

Some 10,000YA, humans started a gigantic evolutionary experiment of adaptation and speciation (Darwin, 1905, Pringle, 1998, Eckardt, 2010). Humans consciously or unconsciously selected wheat, a crop of immense significance. The success of which, as a modern cultivated crop is evident from the fact that it has spread geographically more than any other crop in a very short period of time, and is able to thrive under extreme environmental conditions (see Peng *et al.*, 2011 and Chapter I). Wheat has achieved this enormous plasticity by compensating for genetic bottlenecks by conserving high variability from its ancestors and by rapidly generating new diversity (Dubcovsky and Dvorak, 2007).

APPENDIX 4.1

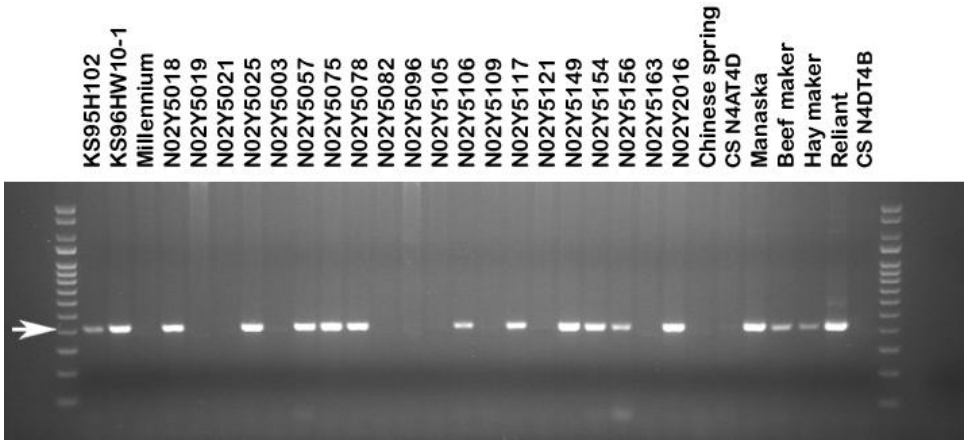


Figure 4.1: PCR amplification pattern of the STS-J15 marker from wheat-*Th. intermedium* hybrid lines (Table 4.4). Arrow indicates the ~420bp amplicons produced by *Th. intermedium* and the WSMV-resistant lines with 4Ai#2S chromosomal translocation. On either side of the agarose gel (2%) is a DNA length marker Q-Step 2.

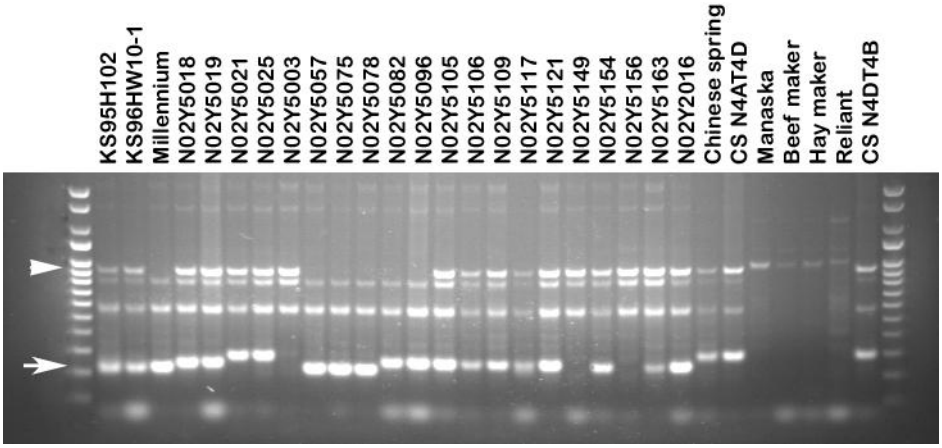


Figure 4.2: PCR amplification pattern of the *Xpsp3000* marker from wheat-*Th. intermedium* hybrid lines (Table 4.4). Arrow indicates the ~250-286bp amplicons produced by the WSMV-resistant lines with 4Ai#2S chromosomal translocation. Arrow head indicate another polymorphic band but it could not be related to the presence or absence of *Th. intermedium* chromatin. On either side of the agarose gel (2%) is a DNA length marker Q-Step 2.

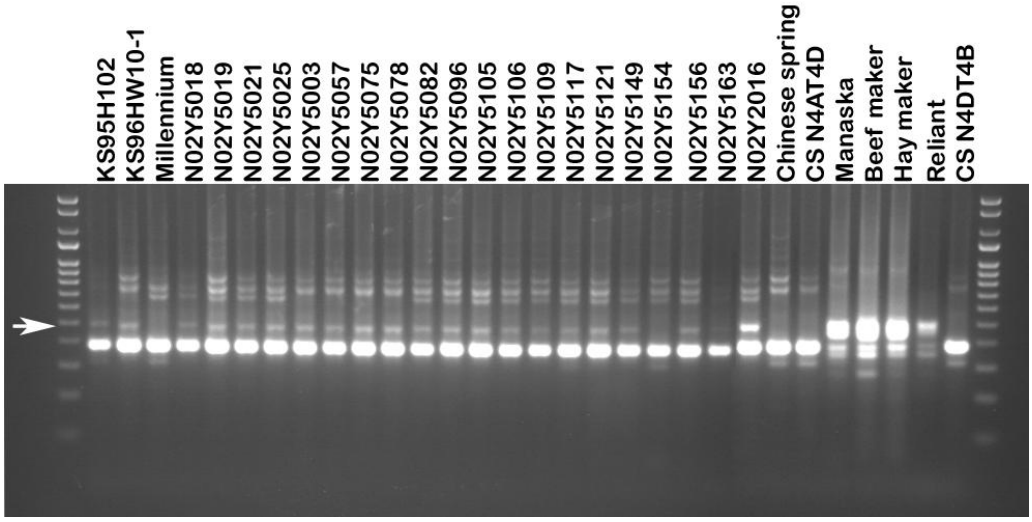


Figure 4.3: PCR amplification pattern of the P4 marker from wheat-*Th. intermedium* hybrid lines (Table 4.4). Arrow indicates a DNA band, present in only few WSMV-resistant lines. On either side of the agarose gel (2%) is a DNA length marker Q-Step 2.

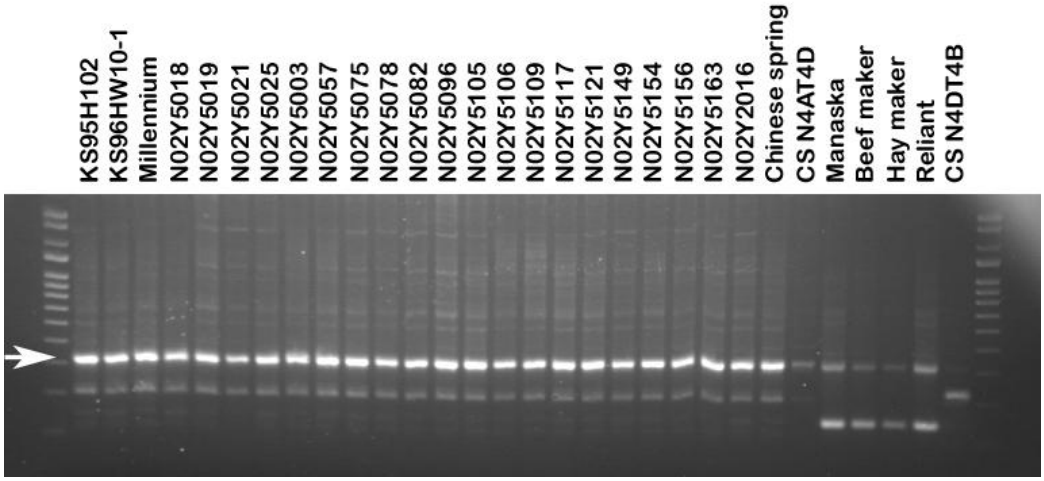


Figure 4.4: PCR amplification pattern of the WSR2 marker from wheat-*Th. intermedium* hybrid lines (Table 4.4). Arrow indicates a DNA band, present in all lines except nulli-4D line. On either side of the agarose gel (2%) is a DNA length marker Q-Step 2.

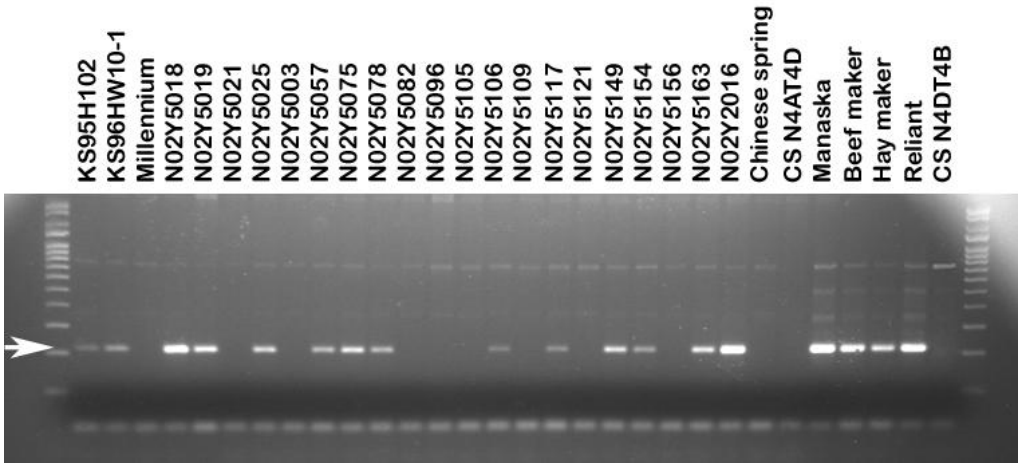


Figure 4.5: PCR amplification pattern of the WSR11 marker from wheat-*Th. intermedium* hybrid lines (Table 4.4). Arrow indicates the ~200bp amplicons produced by *Th. intermedium* and the WSMV-resistant lines with 4Ai#2S chromosomal translocation. On either side of the agarose gel (2%) is a DNA length marker Q-Step 2.

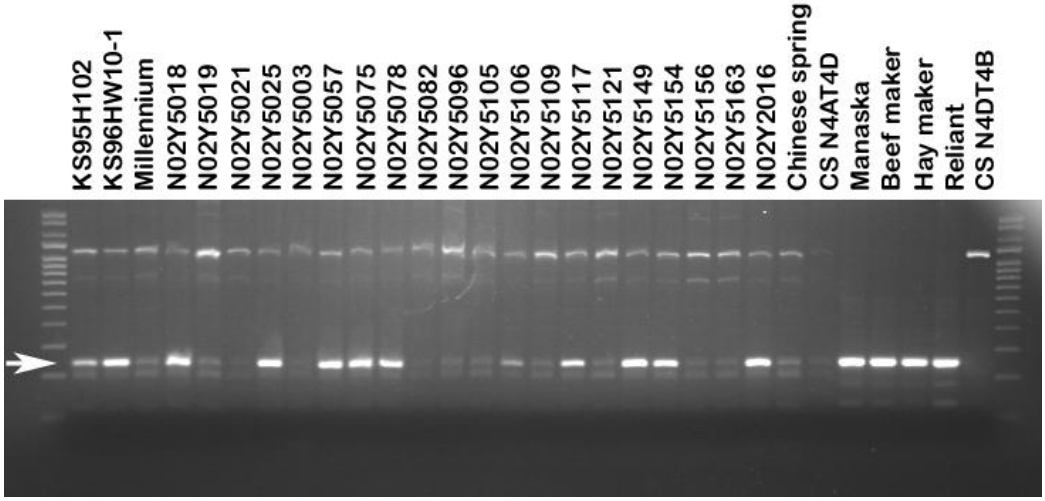


Figure 4.6: PCR amplification pattern of the WSR17 marker from wheat-*Th. intermedium* hybrid lines (Table 4.4). Arrow indicates the ~200bp amplicons produced by *Th. intermedium* and the WSMV-resistant lines with 4Ai#2S chromosomal translocation. On either side of the agarose gel (2%) is a DNA length marker Q-Step 2.

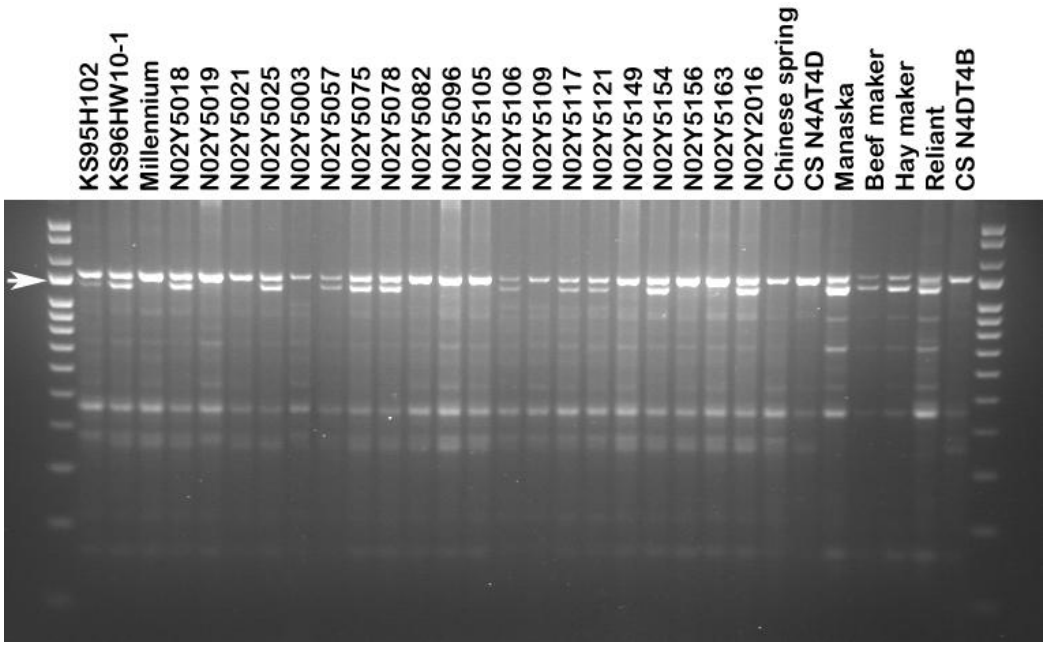


Figure 4.7: PCR amplification pattern of the WSR65 marker from wheat-*Th. intermedium* hybrid lines (Table 4.4). Arrow indicates the ~1300bp amplicons produced by *Th. intermedium* and the WSMV-resistant lines with 4Ai#2S chromosomal translocation. On either side of the agarose gel (2%) is a DNA length marker Q-Step 2.

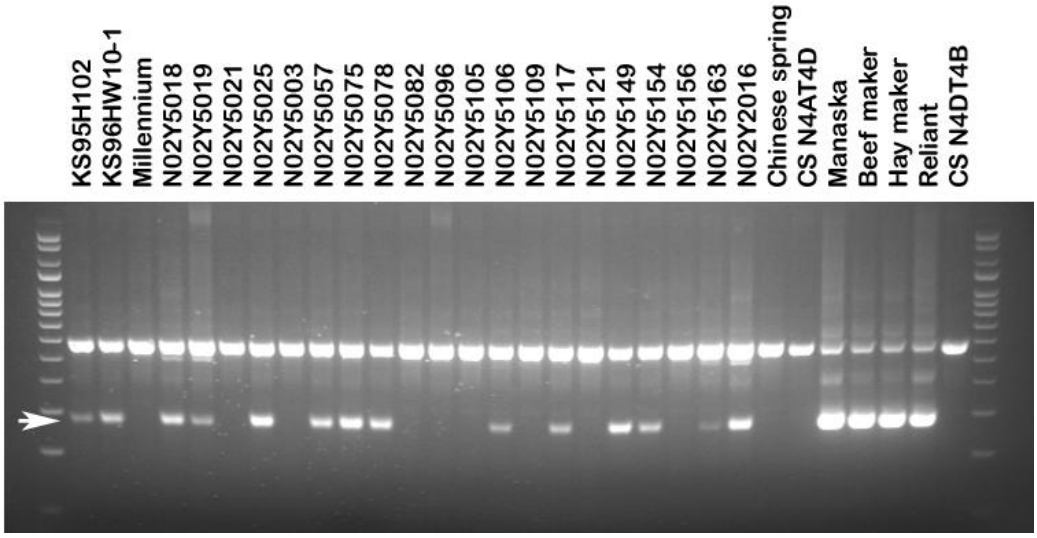


Figure 4.8: PCR amplification pattern of the UL-Thin-2 marker from wheat-*Th. intermedium* hybrid lines (Table 4.4). Arrow indicates the ~269bp amplicons produced by *Th. intermedium* and the WSMV-resistant lines with 4Ai#2S chromosomal translocation. On either side of the agarose gel (2%) is a DNA length marker Q-Step 2.

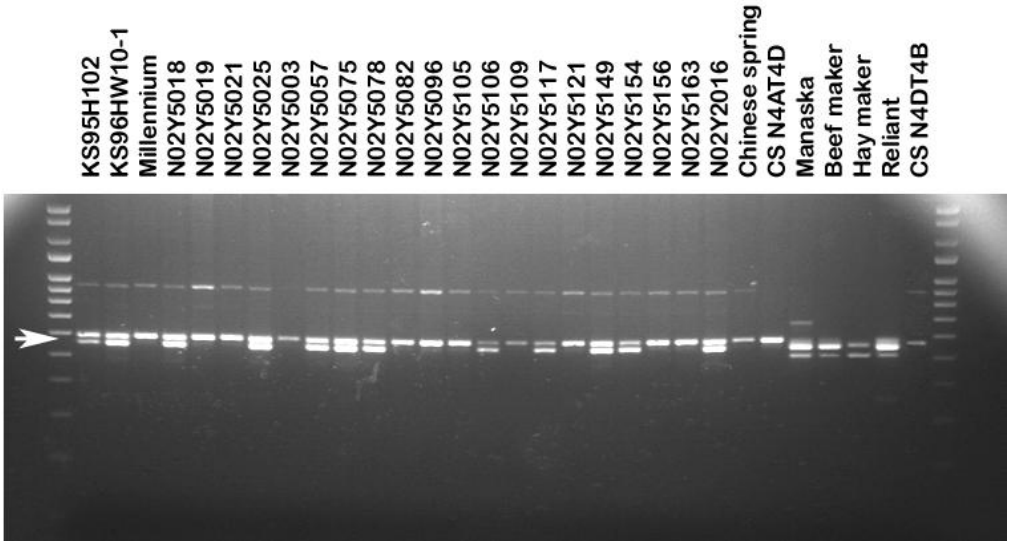


Figure 4.9: PCR amplification pattern of the UL-Thin-3 marker from wheat-*Th. intermedium* hybrid lines (Table 4.4). Arrow indicates the ~550bp amplicons produced by *Th. intermedium* and the WSMV-resistant lines with 4Ai#2S chromosomal translocation. On either side of the agarose gel (2%) is a DNA length marker Q-Step 2.

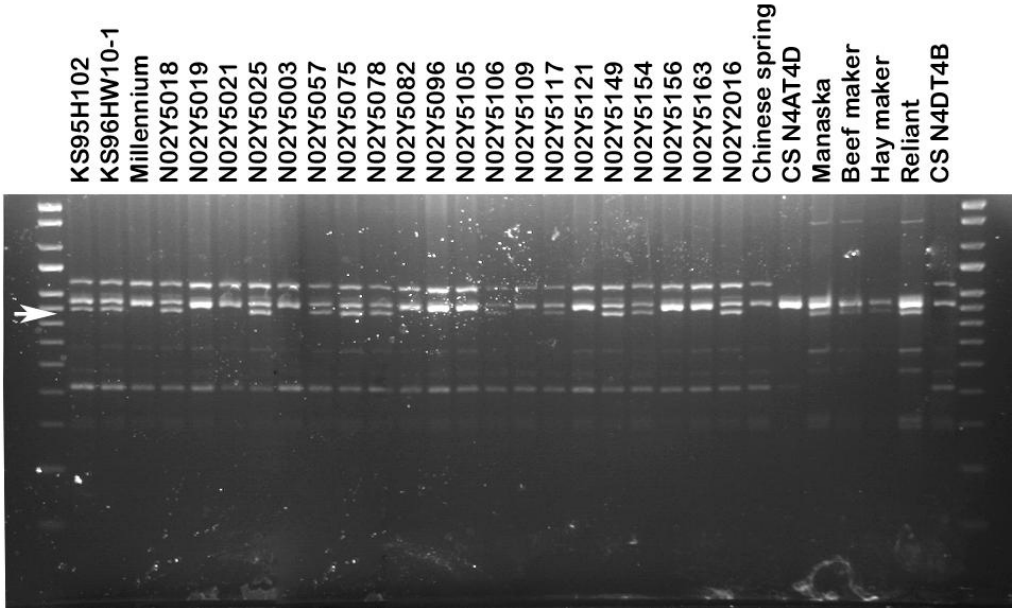


Figure 4.10: PCR amplification pattern of the UL-Thin-4 marker from wheat-*Th. intermedium* hybrid lines (Table 4.4). Arrow indicates the ~890bp amplicons produced by *Th. intermedium* and the WSMV-resistant lines with 4Ai#2S chromosomal translocation. On either side of the agarose gel (2%) is a DNA length marker Q-Step 2.

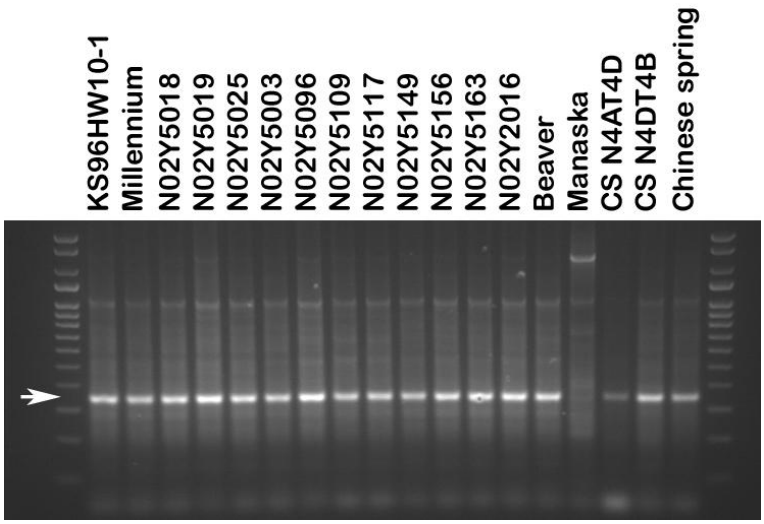


Figure 4.11: PCR amplification pattern of the *Xwmc500* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates ~350bp amplicons produced by all lines except Manaska (*Th. intermedium).* On either side of the agarose gel (1.5%) is a DNA length marker Q-Step 2.

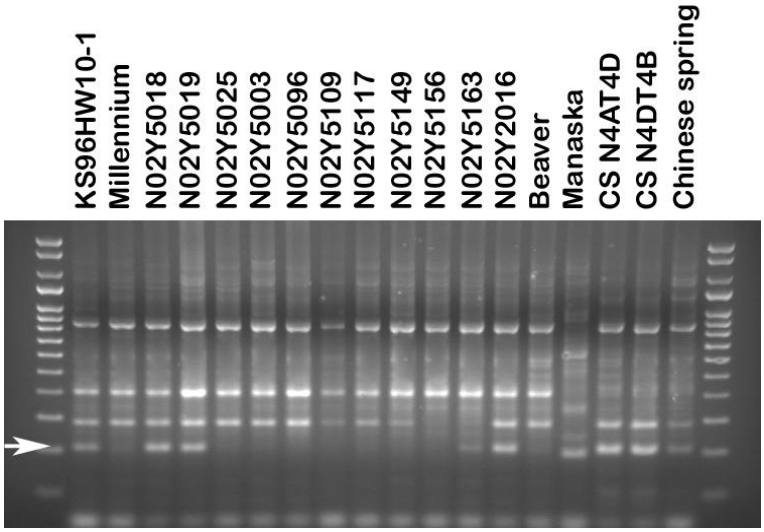


Figure 4.12: PCR amplification pattern of the *Xwmc49* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates 206bp amplicons produced by few lines. On either side of the agarose gel (2%) is a DNA length marker Q-Step 2.

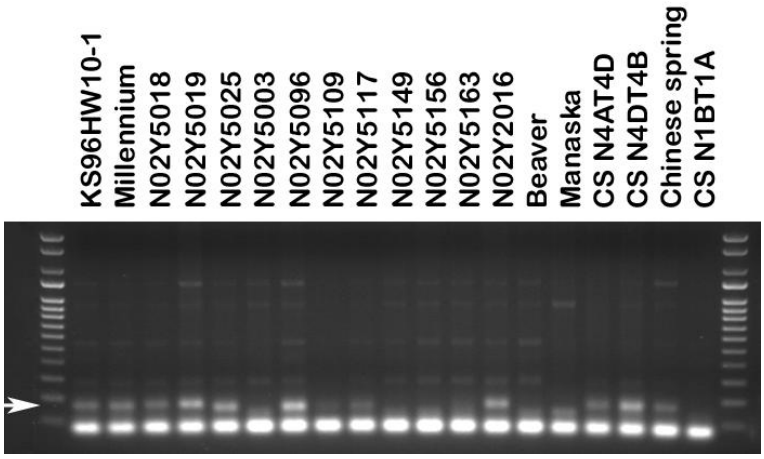


Figure 4.13: PCR amplification pattern of the *Xbarc194* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates *166bp* amplicons produced by lines with normal or small 1BS alien fragments. On either side of the agarose gel (1.5%) is a DNA length marker Q-Step 2.

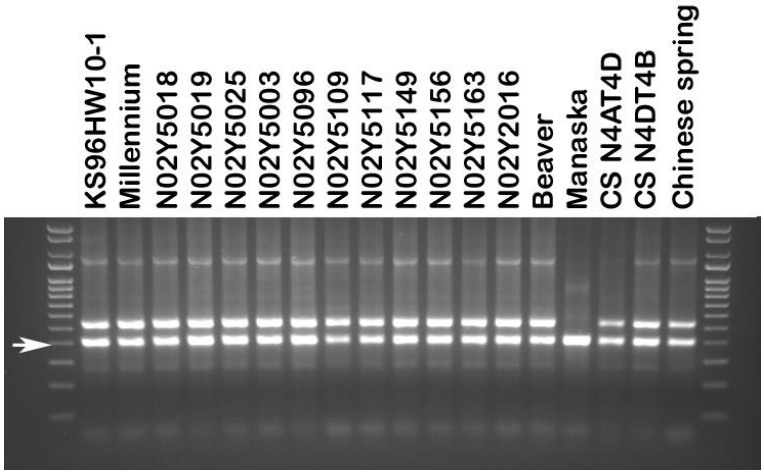


Figure 4.14: PCR amplification pattern of the XBF293222 marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates ~400bp amplicons produced by all lines. On either side of the agarose gel (1.5%) is a DNA length marker Q-Step 2.

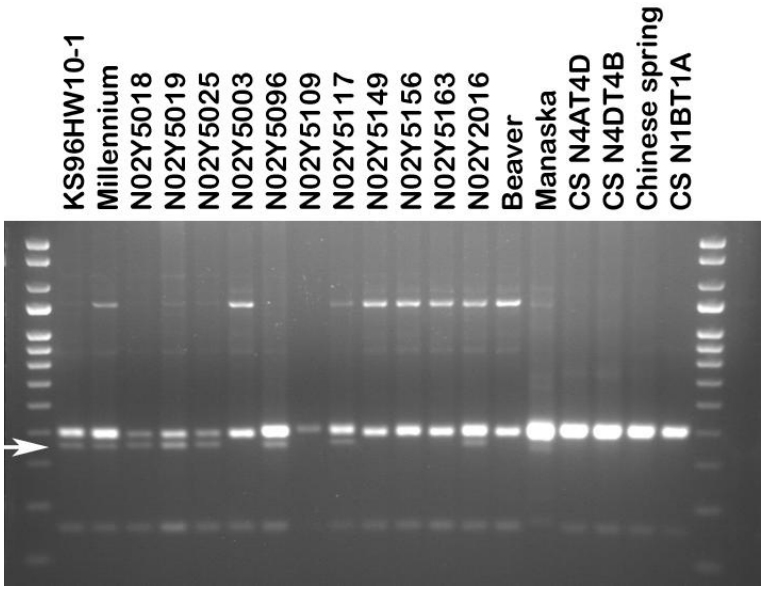


Figure 4.15: PCR amplification pattern of the XBF474204 marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates 480bp amplicons produced by lines with normal or small 1BS alien fragments. On either side of the agarose gel (3%) is a DNA length marker Q-Step 2.

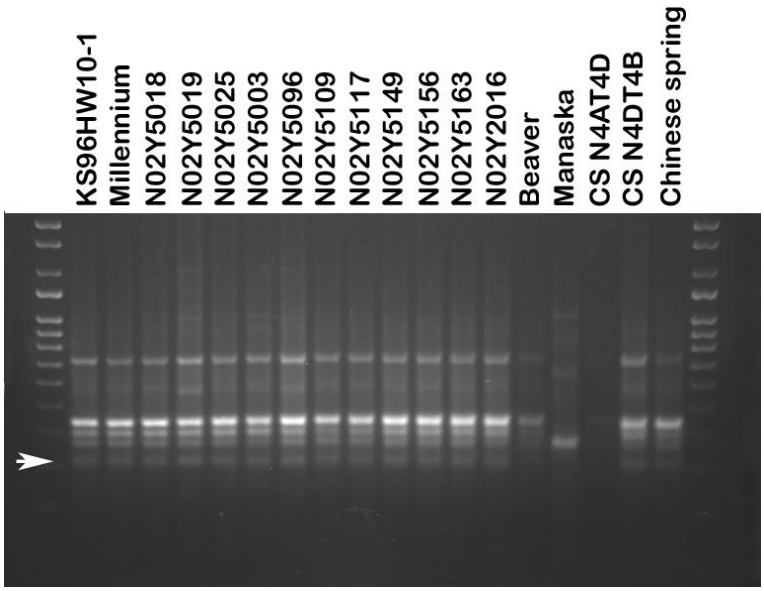


Figure 4.16: PCR amplification pattern of the *Xgwm0550* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates ~150bp amplicons produced by all lines except the nulli-4D line. On either side of the agarose gel (1.5%) is a DNA length marker Q-Step 2.

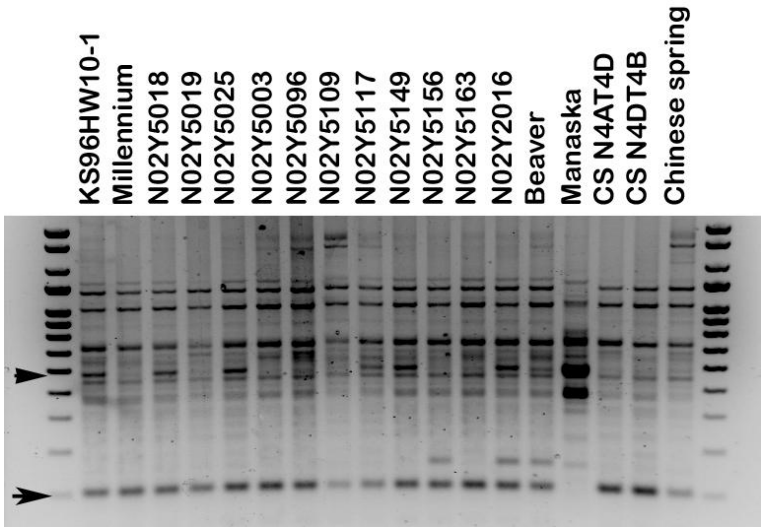


Figure 4.17: PCR amplification pattern of the Xpsp2530.1 marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates 100bp amplicons produced by lines except Manaska. Arrow head indicates the polymorphic band produced by lines with 4Ai#2S chromosomal arm. On either side of the agarose gel (3%) is a DNA length marker Q-Step 2.

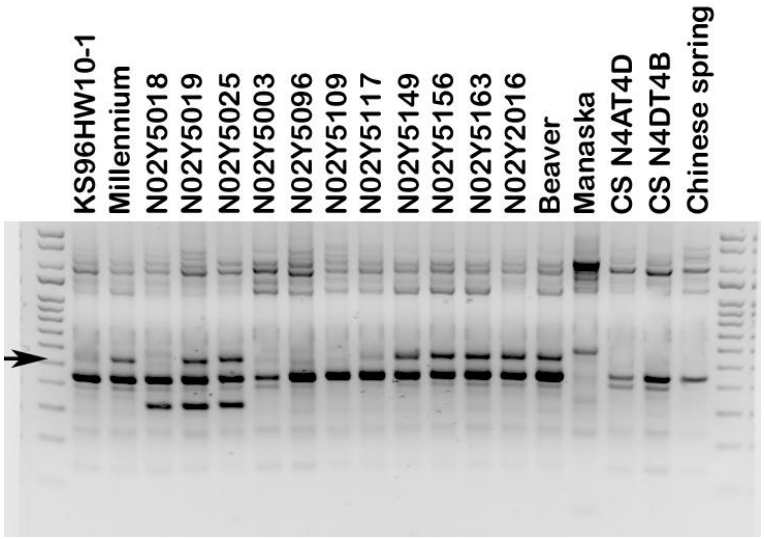


Figure 4.18: PCR amplification pattern of the *Ksud14a* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates ~550bp polymorphic band produced by most lines with small 1B alien fragments. On either side of the agarose gel (1.5%) is a DNA length marker Q-Step 2.

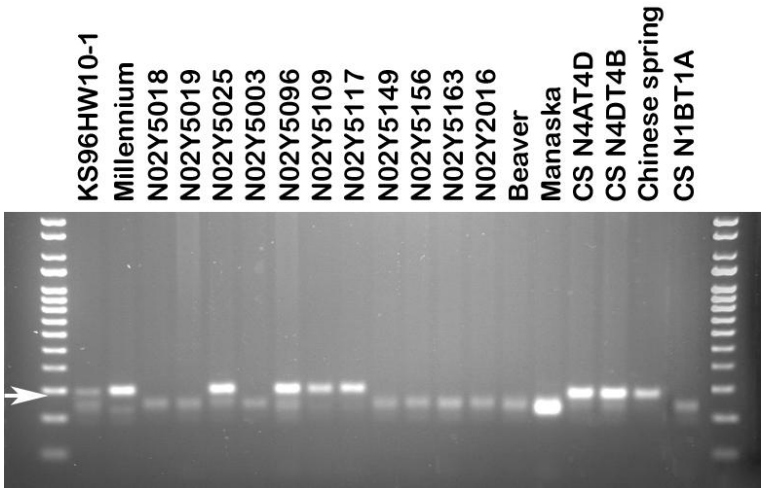


Figure 4.19: PCR amplification pattern of the *Xgwm0911* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates 272bp amplicons produced by lines with normal 1B chromosome. On either side of the agarose gel (1.5%) is a DNA length marker Q-Step 2.

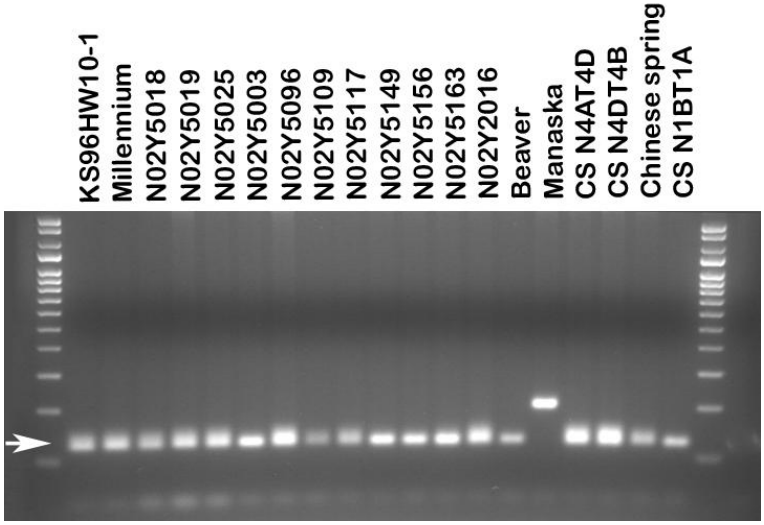


Figure 4.20: PCR amplification pattern of the *Xgpw1170* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates 166bp amplicons produced by most lines except Manaska. On either side of the agarose gel (1.5%) is a DNA length marker Q-Step 2.

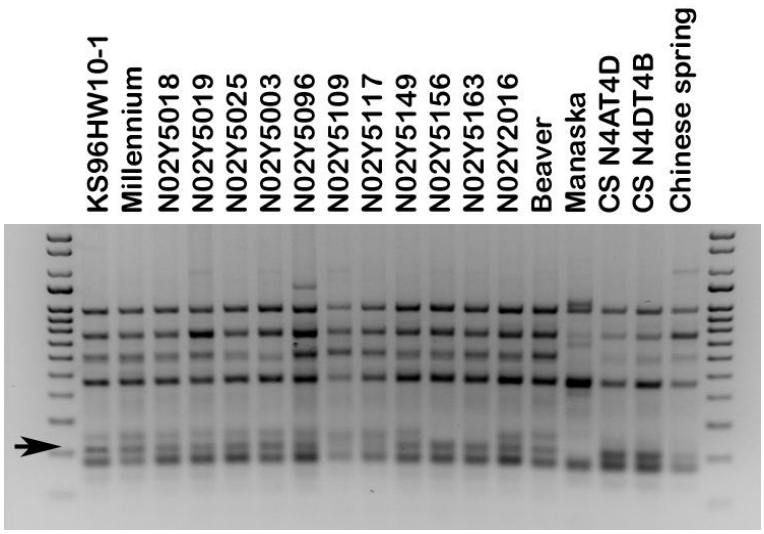


Figure 4.21: PCR amplification pattern of the *Xwmc85* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates 228bp amplicons seen in most of the lines except Manaska. On either side of the agarose gel (3%) is a DNA length marker Q-Step 2.

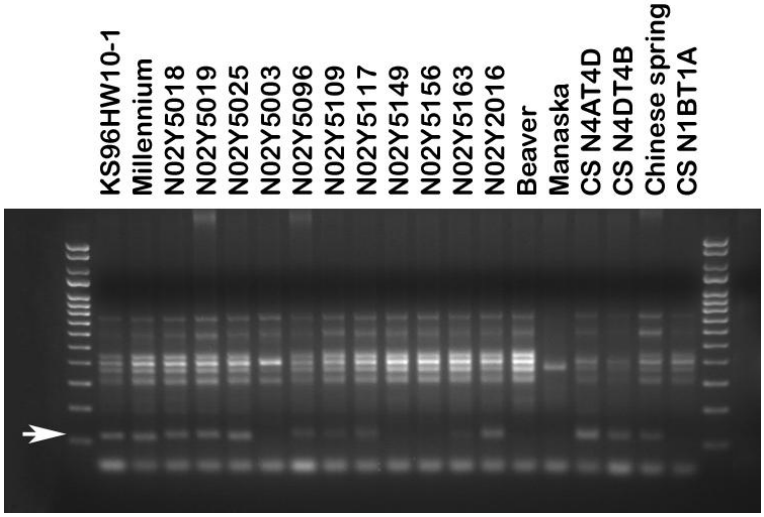


Figure 4.22: PCR amplification pattern of the *Xgwm1130* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates 116bp amplicons produced by lines with normal or small 1B alien fragments. On either side of the agarose gel (1.5%) is a DNA length marker Q-Step 2.

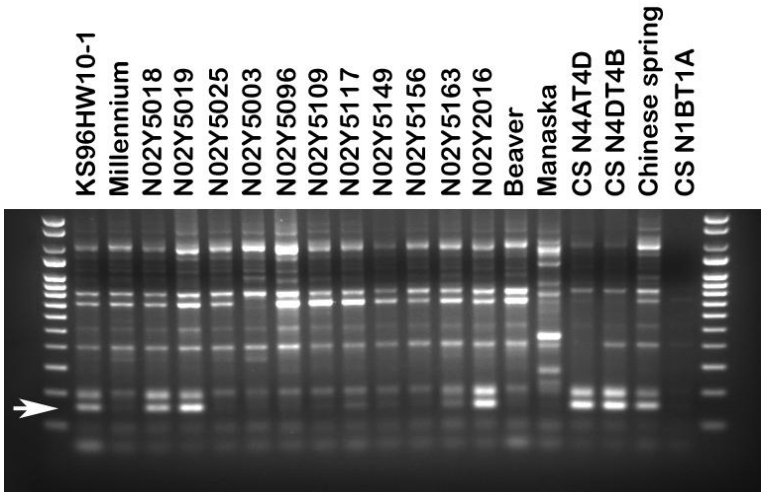


Figure 4.23: PCR amplification pattern of the *Xgwm4144* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates 191bp amplicons produced by lines with normal or small 1B alien fragments. On either side of the agarose gel (1.5%) is a DNA length marker Q-Step 2.

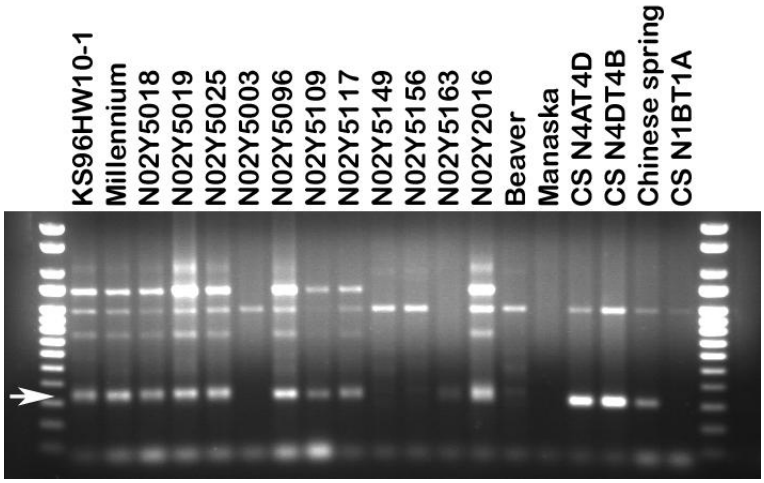


Figure 4.24: PCR amplification pattern of the *Xwmc230* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates ~230bp amplicons produced by lines with normal or small 1B alien fragments. On either side of the agarose gel (1.5%) is a DNA length marker Q-Step 2.

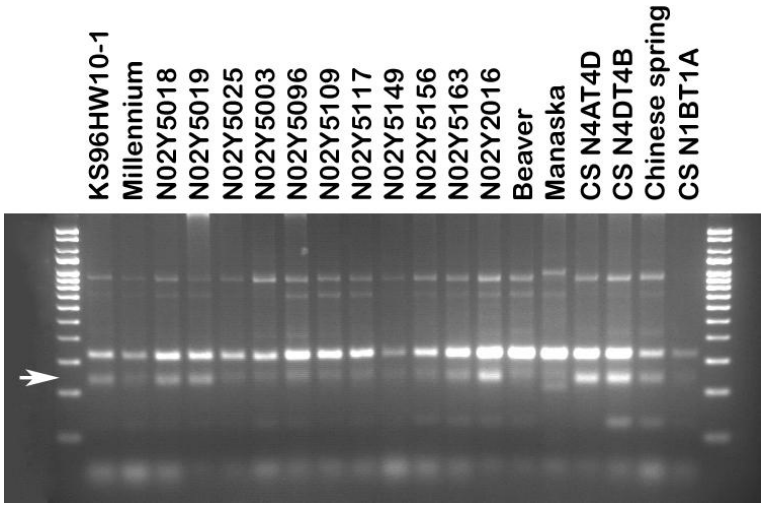


Figure 4.25: PCR amplification pattern of the *Xgwm1100* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates 227bp amplicons produced by lines with normal or small 1B alien fragments. On either side of the agarose gel (3%) is a DNA length marker Q-Step 2.

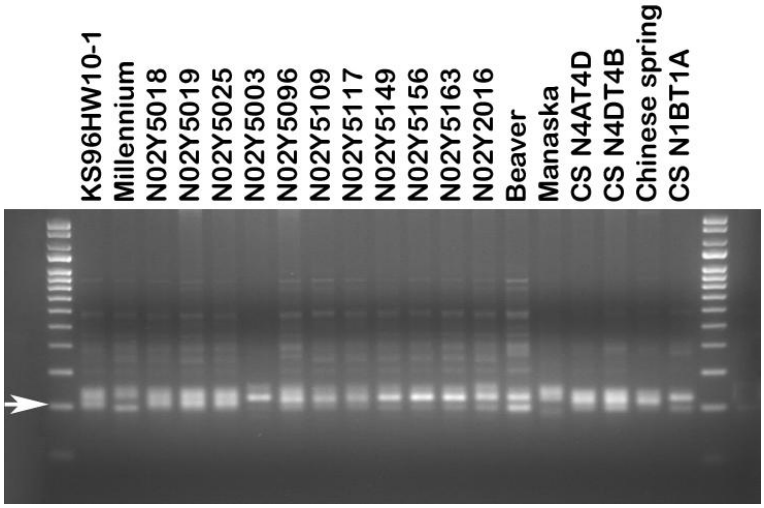


Figure 4.26: PCR amplification pattern of the *Xbarc119* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates 208bp amplicons, on either side of the agarose gel (3%) is a DNA length marker Q-Step 2.

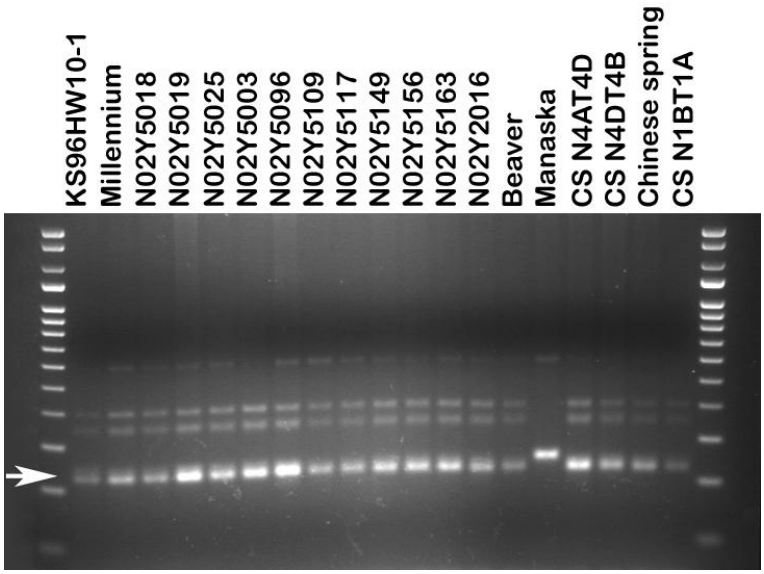


Figure 4.27: PCR amplification pattern of the *Xgwm3035* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates 225bp amplicons, on either side of the agarose gel (2%) is a DNA length marker Q-Step 2.

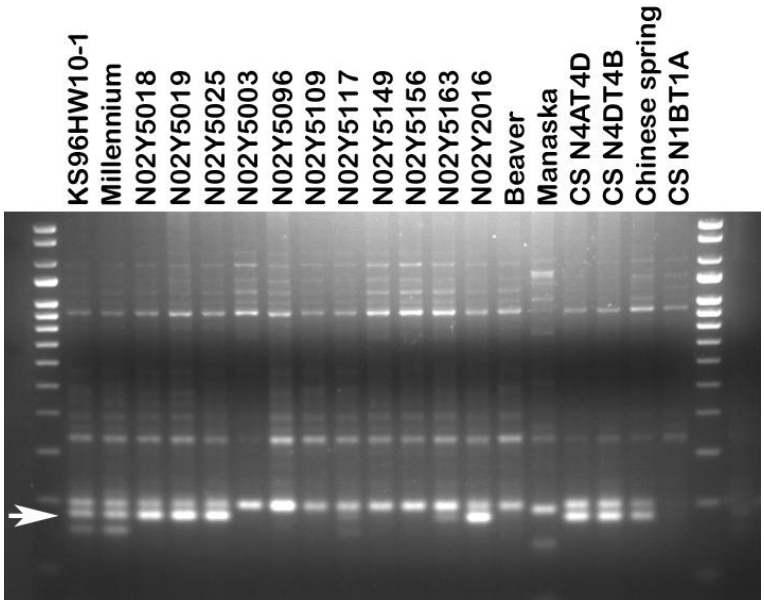


Figure 4.28: PCR amplification pattern of the *Xgpw363* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates ~200bp amplicons produced by lines with normal or small 1B alien fragments. On either side of the agarose gel (3%) is a DNA length marker Q-Step 2.

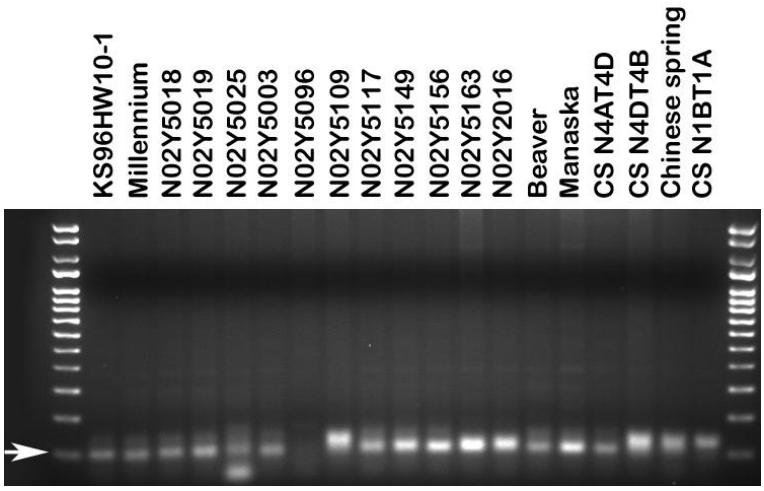


Figure 4.29: PCR amplification pattern of the *Xwmc329* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates 118bp amplicons, on either side of the agarose gel (3%) is a DNA length marker Q-Step 2.

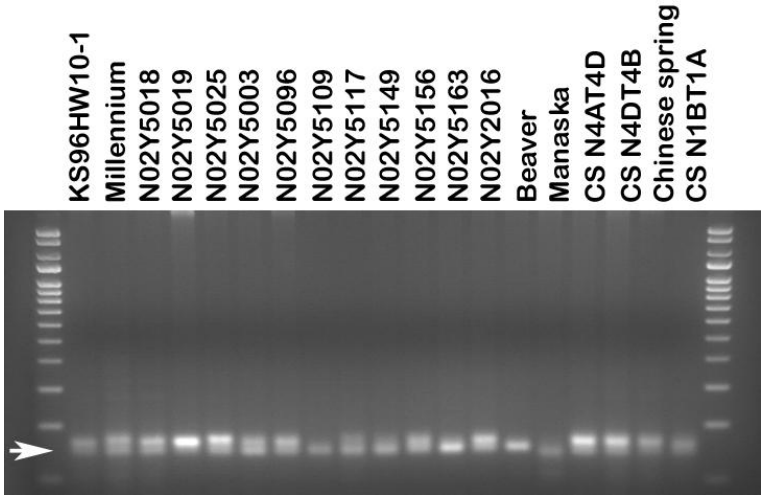


Figure 4.30: PCR amplification pattern of the *Xucr-8* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates 165bp amplicons, on either side of the agarose gel (3%) is a DNA length marker Q-Step 2.

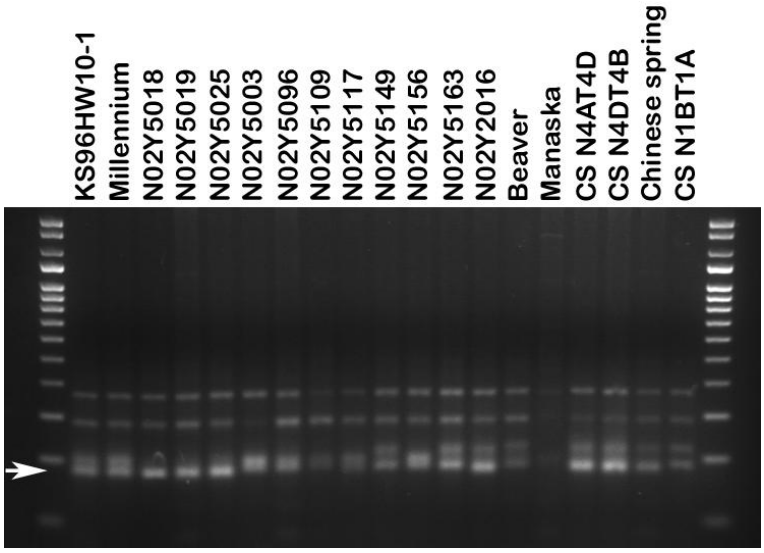


Figure 4.31: PCR amplification pattern of the *Xgwm374* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates 180bp amplicons, on either side of the agarose gel (3%) is a DNA length marker Q-Step 2.

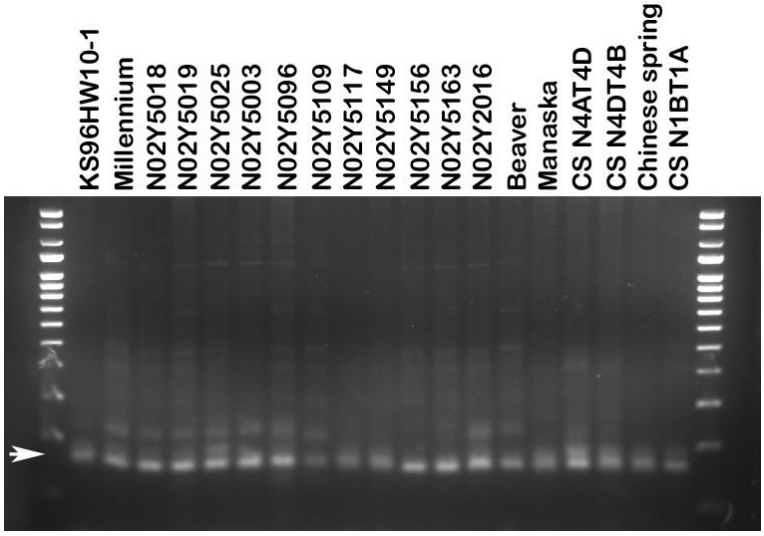


Figure 4.32: PCR amplification pattern of the *Xgwm264* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates 160bp amplicons, on either side of the agarose gel (3%) is a DNA length marker Q-Step 2.

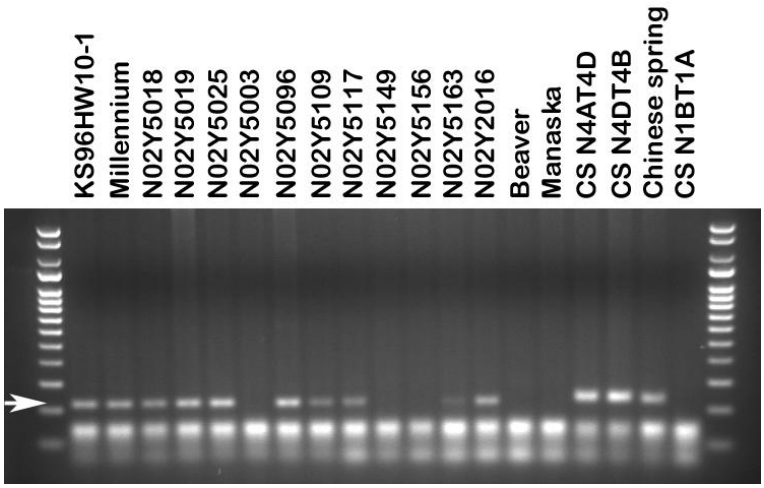


Figure 4.33: PCR amplification pattern of the *Xwmc406* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates 217bp amplicons produced by lines with normal or small 1B alien fragments. On either side of the agarose gel (3%) is a DNA length marker Q-Step 2.

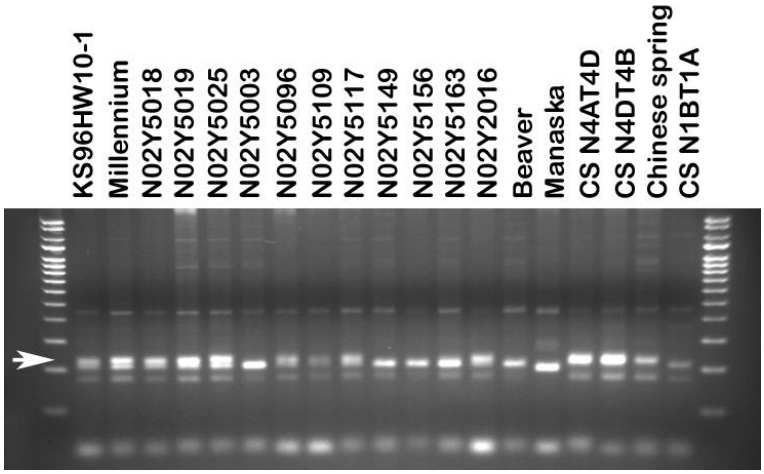


Figure 4.34: PCR amplification pattern of the *Xgpw7059* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates ~220bp amplicons produced by lines with normal or small 1B alien fragments. On either side of the agarose gel (3%) is a DNA length marker Q-Step 2.

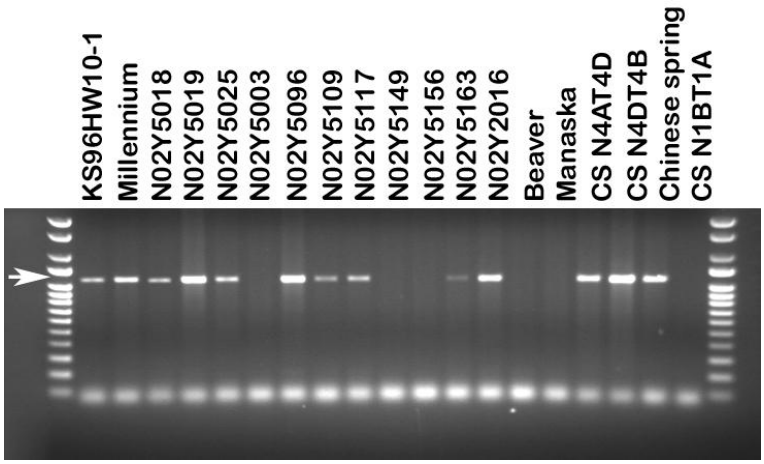


Figure 4.35: PCR amplification pattern of the *Xucr-6* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates 1100bp amplicons produced by lines with normal or small 1B alien fragments. On either side of the agarose gel (3%) is a DNA length marker Q-Step 2.

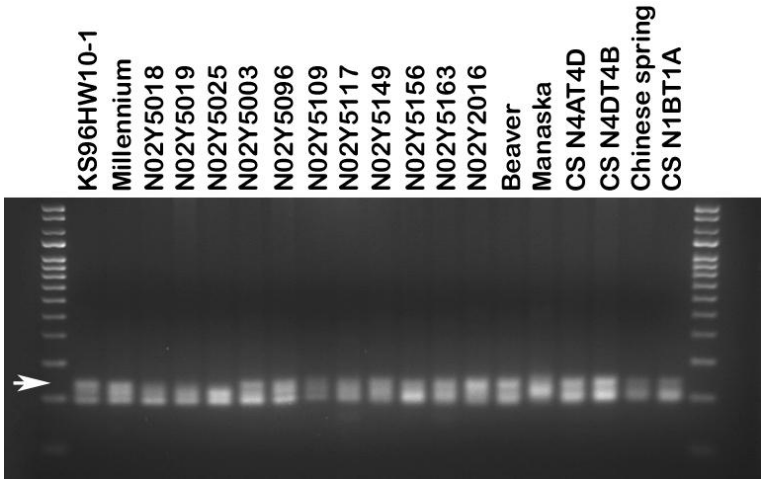


Figure 4. 36: PCR amplification pattern of the *Xbarc128* marker from wheat lines and *Th. intermedium* (Table 4.5). Arrow indicates 250bp amplicons, on either side of the agarose gel (3%) is a DNA length marker Q-Step 2.

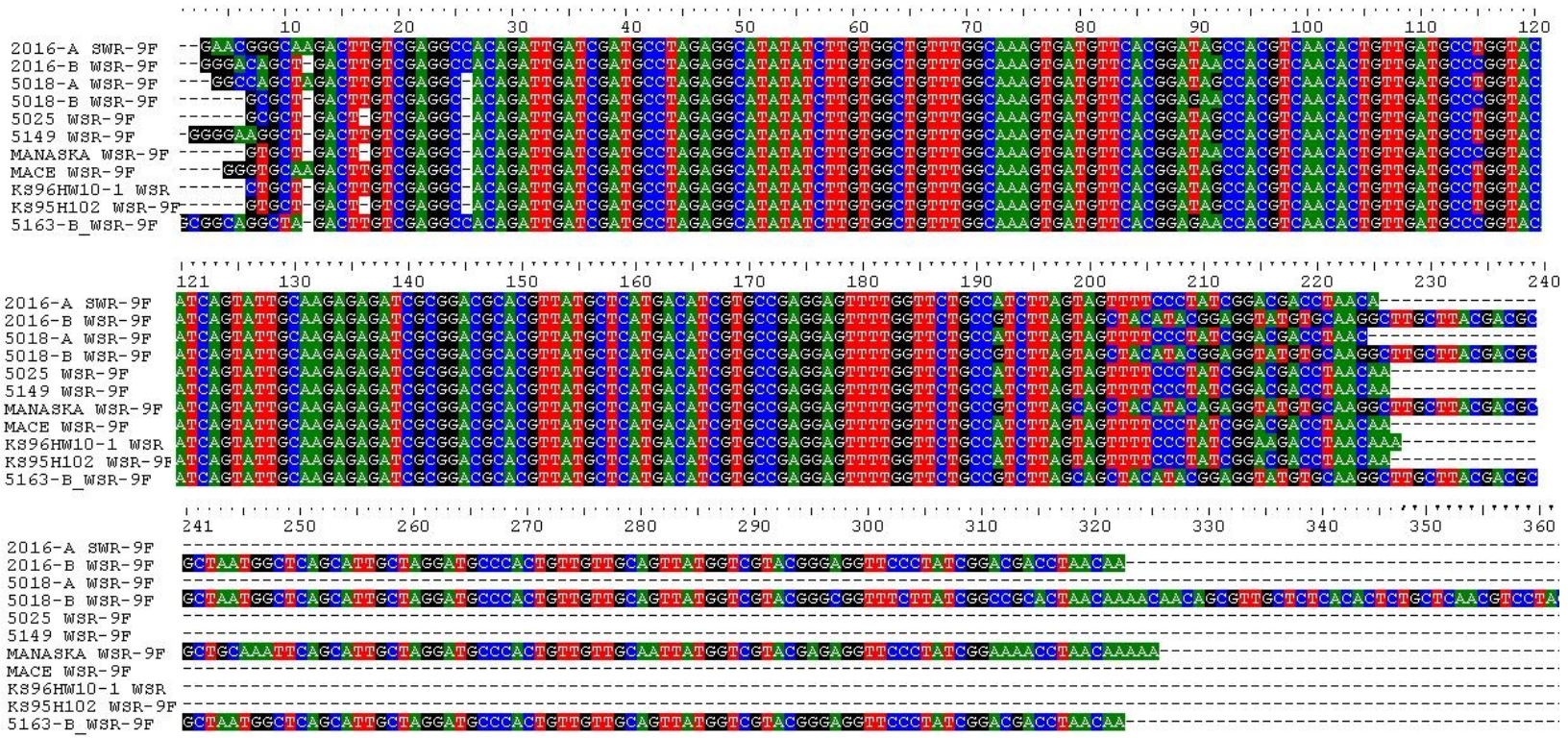


Figure 4.37: Multiple DNA sequence alignment of the WSR-9 marker sequence from hybrid wheat lines and *Th. intermedium* (Manaska). Sequence name of each species is followed by -A or -B and -9F, which indicates the amplicons amplified and sequenced from the recombinant 4D (given as A) or 1B recombinant chromosomes (given as B) respectively, both products were sequenced with WSR9 forward primer.

APPENDIX 5.1

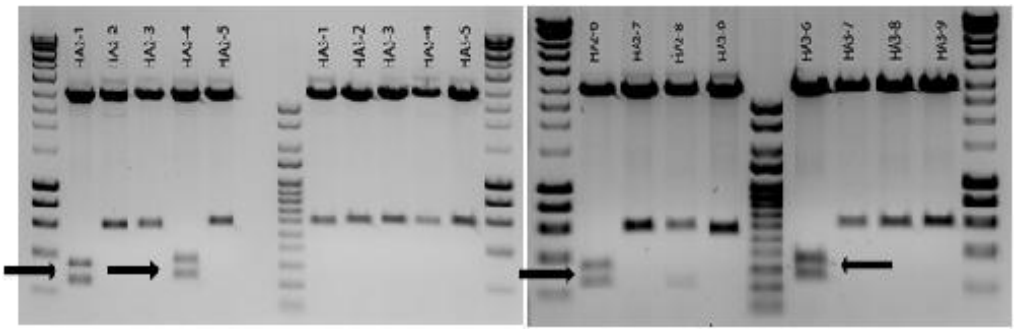


Figure 5.1: Inverse gel image of recombinant plasmid DNA digested with *Eco*RI restriction enzyme. Arrow indicates DNA fragment observed in few Afa sequence of *Th. intermedium*, indicative of the internal polymorphism. On either side of agarose gel (1.2%) is DNA length marker Q-Step 2.

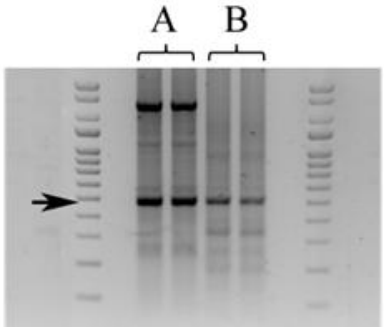


Figure 5.2: Inverse gel image of PCR amplification. Arrow indicates a DNA fragment of ~500bp produced by LTR6150 and Afa1-F primer pair. (Lanes A: 'Chinese Spring', B: *Th. intermedium*).

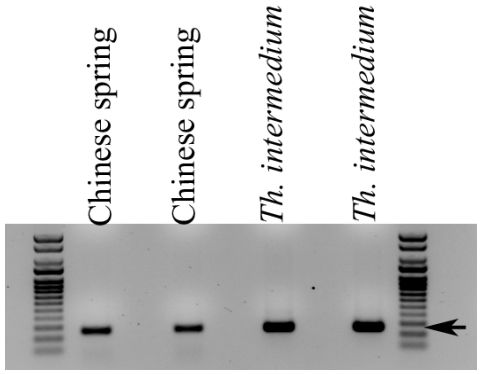


Figure 5.3: Inverse gel image of Afa-family sequences amplified with Nagaki *et al.*, (1995) primers. The arrow indicates a DNA fragment of 260bp.

Table 5.1: List of pSc119.2 homologous sequences in EMBL-EBI database (30 March, 2012). Consensus sequence of 118bp monomer units was used in BLASTN search.

Sr#	Accession	Description	Max score[*1]	Total score[*2]	Query coverage[*3]	E value[*4]	Max identity[*5]
1.	AJ517292.4	*Secale vavilovii* satellite DNA, p42-237	187	430	100%	2e-44	98%
2.	AJ517290.4	*Hordeum chilense* satellite DNA, p147-1716	172	493	100%	4e-40	96%
3.	AJ517288.3	*Hordeum bulbosum* satellite DNA, p147-3711	172	526	100%	4e-40	96%
4.	AJ517271.4	*Secale vavilovii* satellite DNA, p106208-204	170	291	100%	1e-39	92%
5.	AJ517289.4	*Hordeum chilense* satellite DNA, p147-4115	159	450	100%	2e-36	93%
6.	AJ517269.4	*Secale vavilovii* satellite DNA, p25208-182	152	258	100%	3e-34	96%
7.	AJ517276.4	*Aegilops umbellulata* satellite DNA, p25208-157	152	273	100%	3e-34	96%
8.	AJ517286.4	*Avena sativa* satellite DNA, p25208-2022	147	271	100%	1e-32	95%
9.	AJ517236.4	*Hordeum brachyantherum* satellite DNA, p10642-188	145	414	100%	5e-32	96%
10.	AJ517263.4	*Aegilops squ*arrosa satellite DNA, p25208-1517	143	271	97%	2e-31	94%
11.	AJ517277.4	*Aegilops umbellulata* satellite DNA, p25208-168	141	264	100%	6e-31	95%
12.	AJ517258.4	*Triticum monococcum* satellite DNA, p10642-133	140	254	100%	2e-30	96%
13.	AJ517261.4	*Aegilops squ*arrosa satellite DNA, p25208-1315	138	267	99%	8e-30	92%
14.	AJ517272.1	*Aegilops umbellulata* satellite DNA, p2542-091	138	138	79%	8e-30	93%
15.	AJ517260.4	*Secale cereale* satellite DNA, p25208-099	136	251	99%	3e-29	92%
16.	AJ517251.1	*Aegilops squ*arrosa satellite DNA, p25147-2726	136	179	98%	3e-29	96%
17.	AJ517253.4	*Triticum aestivum* satellite DNA, p25147-2322	134	262	99%	9e-29	90%
18.	AJ517239.1	*Hordeum bulbosum* satellite DNA, p25208-021	134	134	79%	9e-29	91%
19.	AJ517235.1	*Hordeum chilense* satellite DNA, p25147-1716	134	134	79%	9e-29	91%
20.	AJ517231.1	*Hordeum bulbosum* satellite DNA, p2542-134	134	134	79%	9e-29	91%
21.	AJ517264.4	*Triticum aestivum* satellite DNA, p25208-1618	132	265	99%	3e-28	92%
22.	AJ517259.4	*Triticum monococcum* satellite DNA, p10642-155	132	247	100%	3e-28	96%
23.	AJ517285.4	*Avena sativa* satellite DNA, p25208-1921	132	222	100%	3e-28	92%
24.	AJ517278.4	*Aegilops umbellulata* satellite DNA, p25208-179	132	247	99%	3e-28	92%
25.	AJ517249.4	*Aegilops squ*arrosa satellite DNA, p25147-2524	132	299	100%	3e-28	96%
26.	AJ517293.1	*Secale montanum* satellite DNA, p25147-081	131	173	100%	1e-27	92%
27.	AJ517270.4	*Secale vavilovii* satellite DNA, p25208-193	129	244	100%	4e-27	90%
28.	AJ517267.4	*Secale cereale* satellite DNA, p25208-2325	129	220	100%	4e-27	90%
29.	AJ517240.4	*Hordeum bulbosum* satellite DNA, p25208-032	129	249	100%	4e-27	90%
30.	AJ517262.4	*Aegilops squ*arrosa satellite DNA, p25208-1416	127	238	99%	1e-26	90%
31.	AJ517274.1	*Aegilops umbellulata* satellite DNA, p25147-135	127	127	78%	1e-26	90%
32.	AJ517291.4	*Secale vavilovii* satellite DNA, p106208-215	125	172	100%	5e-26	96%
33.	AJ517268.4	*Secale cereale* satellite DNA, p25208-2426	125	231	100%	5e-26	89%
34.	AJ517242.4	*Hordeum brachyantherum* satellite DNA, p25208-054	125	226	98%	5e-26	92%
35.	AJ517273.1	*Aegilops umbellulata* satellite DNA, p25147-124	125	164	100%	5e-26	96%
36.	Z75561.1	*Triticum* aestivum telomere-associated DNA (PSR2152)	125	342	100%	5e-26	84%
37.	AJ517243.4	*Hordeum brachyantherum* satellite DNA, p25208-066	123	215	100%	2e-25	90%
38.	AJ517241.4	*Hordeum bulbosum* satellite DNA, p25208-043	122	209	100%	6e-25	89%
39.	AJ517250.1	*Aegilops squ*arrosa satellite DNA, p25147-2625	122	122	76%	6e-25	90%
40.	AJ517266.4	*Triticum monvum* satellite DNA, p25208-1820	118	208	97%	7e-24	90%
41.	AJ517254.1	*Secale cereale* satellite DNA, p25147-1918	118	163	99%	7e-24	96%
42.	AJ517284.1	*Avena sativa* satellite DNA, p2542-3021	118	157	99%	7e-24	96%
43.	AJ517275.1	*Aegilops umbellulata* satellite DNA, p25147-146	118	118	78%	7e-24	88%
44.	AJ517233.1	*Hordeum bulbosum* satellite DNA, p25147-1413	116	155	100%	3e-23	96%
45.	AJ517282.4	*Secale cereale* satellite DNA, p25-315	114	200	97%	9e-23	89%
46.	AJ517287.4	*Avena sativa* satellite DNA, p25208-2123	114	208	100%	9e-23	88%
47.	AJ517238.1	*Hordeum bulbosum* satellite DNA, p25147-1312	113	155	100%	3e-22	93%
48.	AY551004.1	*Triticum aestivum* RAPD marker Pm6-X1	111	374	99%	1e-21	85%
49.	AJ517265.4	*Triticum aestivum* satellite DNA, p25208-1719	111	199	100%	1e-21	86%
50.	AJ517248.1	*Secale cereale* satellite DNA, p2542-3425	109	109	77%	4e-21	87%
51.	AJ517283.1	*Avena sativa* satellite DNA, p2542-2920	109	109	79%	4e-21	86%
52.	AJ517247.1	*Secale cereale* satellite DNA, p2542-3324	107	107	79%	1e-20	85%

Sr#	Accession	Description	Max score[*1]	Total score[*2]	Query coverage[*3]	E value[*4]	Max identity[*5]
53.	AJ517234.1	*Hordeum chilense* satellite DNA, p25147-1514	107	107	79%	1e-20	85%
54.	AF227454.1	*Bromus tectorum* clone 15 microsatellite sequence	105	105	72%	5e-20	88%
55.	AM285293.1	*Secale cereale* AFLP marker E-ACT/M-CAA/303	102	306	100%	6e-19	90%
56.	AJ517280.1	*Aegilops umbellulata* satellite DNA, p106208-1911	102	102	77%	6e-19	85%
57.	AJ517245.1	*Leymus mollis* satellite DNA, p2542-167	100	179	100%	2e-18	97%
58.	AJ517281.1	*Aegilops umbellulata* satellite DNA, p106208-2012	100	139	96%	2e-18	96%
59.	AJ517279.1	*Aegilops umbellulata* satellite DNA, p106208-1810	100	139	96%	2e-18	96%
60.	AJ517256.1	*Secale cereale satellite* DNA, p25147-123	98.7	98.7	77%	7e-18	84%
61.	AJ517246.1	*Leymus mollis* satellite DNA, p2542-189	98.7	172	99%	7e-18	97%
62.	AJ517229.1	*Hordeum bulbosum* satellite DNA, p2542-101	95.1	163	98%	8e-17	96%
63.	AJ517255.1	*Secale cereale satellite* DNA, p25147-3231	93.3	132	100%	3e-16	96%
64.	AJ517244.1	*Leymus mollis* satellite DNA, p2542-156	93.3	93.3	79%	3e-16	82%
65.	AJ517230.1	*Hordeum bulbosum* satellite DNA, p2542-123	87.8	170	100%	1e-14	93%
66.	AJ517227.1	*Hordeum brachyantherum* satellite DNA, p2542-2213	87.8	168	97%	1e-14	95%
67.	AJ517232.1	*Hordeum chilense* satellite DNA, p2542-145	84.2	159	97%	1e-13	92%
68.	AJ517228.1	*Hordeum brachyantherum* satellite DNA, p2542-2415	84.2	168	97%	1e-13	93%
69.	AJ517257.1	*Aegilops squ*arrosa satellite DNA, p10642-177	84.2	166	96%	1e-13	94%
70.	AJ517237.1	*Hordeum brachyantherum* satellite DNA, p2542-2112	82.4	163	100%	5e-13	90%
71.	EF455902.1	*Triticum aestivum* AFLP BHW15-21 genomic sequence	75.2	116	97%	8e-11	90%
72.	EF455889.1	*Triticum aestivum* AFLP BHW9-2 genomic sequence	75.2	121	97%	8e-11	89%
73.	EF455887.1	*Triticum aestivum* AFLP BHW8-2 genomic sequence	75.2	117	97%	8e-11	86%
74.	EF455886.1	*Triticum aestivum* AFLP BHW8-1 genomic sequence	75.2	75.2	81%	8e-11	77%
75.	EF455882.1	*Triticum aestivum* AFLP BHW17-22 genomic sequence	75.2	121	97%	8e-11	91%
76.	EF455883.1	*Triticum aestivum* AFLP BHW17-23 genomic sequence	71.6	117	97%	9e-10	89%
77.	AJ517252.1	*Triticum aestivum* satellite DNA, p25147-2120	71.6	110	100%	9e-10	96%
78.	EF455900.1	*Triticum aestivum* AFLP BHW14-(4)-1 genomic sequence	69.8	112	97%	3e-09	86%
79.	EF455888.1	*Triticum aestivum* AFLP BHW9-1 genomic sequence	69.8	112	97%	3e-09	86%
80.	HQ213958.1	*Hordeum vulgare* clone pHv-1457 repeat region	68.0	212	90%	1e-08	88%
81.	EF455915.1	*Triticum aestivum* AFLP BHW31-4 genomic sequence	68.0	130	61%	1e-08	88%
82.	EF614977.1	*Triticum aestivum* AFLP BHW50-2 genomic sequence	66.2	66.2	81%	4e-08	75%
83.	EF455898.1	*Triticum aestivum* AFLP BHW14-(3)-1 genomic sequence	66.2	108	97%	4e-08	86%
84.	EF455894.1	*Triticum aestivum* AFLP BHW15-22 genomic sequence	66.2	112	97%	4e-08	89%
85.	HM536205.1	*Hordeum vulgare* clone pHv-961 repeat region	53.6	105	86%	3e-04	72%
86.	X16097.1	*Barley relic* DNA HVT06, tandemly repeated seq	51.8	51.8	78%	9e-04	72%
87.	Z68784.1	*Hordeum compressum* satellite DNA (ID pCOM2_2)	46.4	46.4	33%	0.037	85%
88.	X16095.1	Barley relic DNA HVT01, tandemly repeated seq	39.2	39.2	82%	5.5	70%

*1 Max score: refers to highest alignment score of a set of aligned sequences
*2 Total score: refers to sum of aligned scores of all sequences for the subject sequence
*3 Query length: refers to coverage of query sequence within homologous sequences
*4 E value: number of alignments expected, lower E value (high number following e-) indicates more reliable results
*5 Max identify: refers to the highest percent identity for a set of aligned sequences

Table 5.2: List of pSc119.2 homologous sequences from EMBL-EBI database (30 March, 2012). McIntyre *et al.*, (1990) sequence of 611bp was used in BLASTN search.

Sr.	Accession	Description	Max score [*1]	Total score [*2]	Query coverage [*3]	E value [*4]	Max identity [*5]
1.	AJ517288.3	*Hordeum bulbosum* satellite DNA, p147-3711	502	1875	72%	1e-138	96%
2.	AJ517290.4	*Hordeum chilense* satellite DNA, p147-1716	477	1711	72%	5e-131	96%
3.	AJ517289.4	*Hordeum chilense* satellite DNA, p147-4115	428	1614	71%	2e-116	93%
4.	AJ517236.4	*Hordeum brachyantherum* satellite DNA, p10642-188	385	1477	70%	2e-103	95%
5.	AJ517292.4	*Secale vavilovii* satellite DNA, p42-237	374	1454	72%	4e-100	98%
6.	AY551004.1	*Triticum aestivum* RAPD marker Pm6-X1	343	1330	71%	7e-91	85%
7.	Z75561.1	*Triticum aestivum* telomere-associated DNA (clone PSR2152)	327	1234	70%	5e-86	87%
8.	AJ517271.4	*Secale vavilovii* satellite DNA, p106208-204	304	1155	72%	6e-79	91%
9.	AJ517249.4	*Aegilops squarrosa* satellite DNA, p25147-2524	295	1018	72%	3e-76	93%
10.	AJ517253.4	*Triticum aestivum* satellite DNA, p25147-2322	291	1010	72%	4e-75	93%
11.	AJ517286.4	*Avena sativa* satellite DNA, p25208-2022	269	978	72%	1e-68	92%
12.	AJ517276.4	*Aegilops umbellulata* satellite DNA, p25208-157	269	971	72%	1e-68	92%
13.	AJ517261.4	*Aegilops squarrosa* satellite DNA, p25208-1315	269	960	72%	1e-68	92%
14.	AJ517291.4	*Secale vavilovii* satellite DNA, p106208-215	266	899	72%	1e-67	87%
15.	AJ517264.4	*Triticum aestivum* satellite DNA, p25208-1618	260	958	72%	6e-66	92%
16.	AJ517277.4	*Aegilops umbellulata* satellite DNA, p25208-168	260	974	72%	6e-66	95%
17.	AJ517269.4	*Secale vavilovii* satellite DNA, p25208-182	255	911	72%	3e-64	91%
18.	AJ517240.4	*Hordeum bulbosum* satellite DNA, p25208-032	255	908	72%	3e-64	91%
19.	AJ517263.4	*Aegilops squarrosa* satellite DNA, p25208-1517	255	942	71%	3e-64	92%
20.	AJ517260.4	*Secale cereale* satellite DNA, p25208-099	253	927	72%	9e-64	90%
21.	AJ517258.4	*Triticum monococcum* satellite DNA, p10642-133	244	908	72%	5e-61	89%
22.	AJ517270.4	*Secale vavilovii* satellite DNA, p25208-193	242	857	72%	2e-60	89%
23.	AJ517278.4	*Aegilops umbellulata* satellite DNA, p25208-179	242	877	72%	2e-60	92%
24.	AJ517262.4	*Aegilops squarrosa* satellite DNA, p25208-1416	242	844	72%	2e-60	89%
25.	AJ517259.4	*Triticum monococcum* satellite DNA, p10642-155	237	890	72%	7e-59	89%
26.	AJ517268.4	*Secale cereale* satellite DNA, p25208-2426	228	825	72%	4e-56	89%
27.	AJ517242.4	*Hordeum brachyantherum* satellite DNA, p25208-054	223	799	72%	2e-54	96%
28.	AJ517243.4	*Hordeum brachyantherum* satellite DNA, p25208-066	214	760	72%	8e-52	96%
29.	AJ517285.4	*Avena sativa* satellite DNA, p25208-1921	212	778	72%	3e-51	92%
30.	AJ517287.4	*Avena sativa* satellite DNA, p25208-2123	210	753	72%	1e-50	88%
31.	AJ517266.4	*Triticum aestivum* satellite DNA, p25208-1820	206	733	71%	1e-49	93%
32.	AJ517267.4	*Secale cereale* satellite DNA, p25208-2325	205	765	72%	4e-49	88%
33.	AJ517282.4	*Secale cereale* satellite DNA, p25-315	197	716	71%	6e-47	94%
34.	AJ517265.4	*Triticum aestivum* satellite DNA, p25208-1719	197	684	72%	6e-47	84%
35.	AJ517241.4	*Hordeum bulbosum* satellite DNA, p25208-043	197	720	72%	6e-47	90%
36.	AJ517245.1	*Leymus mollis* satellite DNA, p2542-167	181	641	72%	5e-42	94%
37.	AJ517251.1	*Aegilops squarrosa* satellite DNA, p25147-2726	181	637	71%	5e-42	96%
38.	AJ517293.1	*Secale montanum* satellite DNA, p25147-081	178	621	71%	6e-41	93%
39.	AJ517230.1	*Hordeum bulbosum* satellite DNA, p2542-123	178	589	67%	6e-41	93%
40.	AJ517231.1	*Hordeum bulbosum* satellite DNA, p2542-134	172	567	66%	3e-39	98%
41.	AJ517272.1	*Aegilops umbellulata* satellite DNA, p2542-091	170	571	67%	9e-39	92%
42.	AJ517246.1	*Leymus mollis* satellite DNA, p2542-189	168	610	72%	3e-38	93%
43.	AJ517273.1	*Aegilops umbellulata* satellite DNA, p25147-124	168	546	66%	3e-38	92%
44.	AJ517254.1	*Secale cereale satellite* DNA, p25147-1918	167	581	71%	1e-37	96%
45.	AJ517228.1	*Hordeum brachyantherum* satellite DNA, p2542-2415	167	564	65%	1e-37	93%
46.	AJ517227.1	*Hordeum brachyantherum* satellite DNA, p2542-2213	167	571	66%	1e-37	95%
47.	AJ517235.1	*Hordeum chilense satellite* DNA, p25147-1716	163	533	66%	1e-36	92%
48.	AJ517274.1	*Aegilops umbellulata* satellite DNA, p25147-135	163	531	66%	1e-36	91%
49.	AJ517257.1	*Aegilops squarrosa* satellite DNA, p10642-177	163	562	66%	1e-36	94%
50.	AJ517237.1	*Hordeum brachyantherum* satellite DNA, p2542-2112	161	553	67%	5e-36	90%
51.	AJ517233.1	*Hordeum bulbosum* satellite DNA, p25147-1413	159	533	68%	2e-35	90%
52.	AJ517250.1	*Aegilops squarrosa* satellite DNA, p25147-2625	159	529	66%	2e-35	93%

Sr.	Accession	Description	Max score [*1]	Total score[*2]	Query coverage [*3]	E value[*4]	Max identity[*5]
53.	AJ517238.1	*Hordeum bulbosum* satellite DNA, p25147-1312	158	558	71%	6e-35	93%
54.	AJ517232.1	*Hordeum chilense* satellite DNA, p2542-145	158	528	65%	6e-35	90%
55.	AJ517284.1	*Avena sativa* satellite DNA, p2542-3021	158	533	68%	6e-35	91%
56.	AM285293.1	*Secale cereale* AFLP marker E-ACT/M-CAA/303	156	1052	72%	2e-34	89%
57.	AJ517275.1	*Aegilops umbellulata* satellite DNA, p25147-146	154	501	66%	7e-34	89%
58.	AJ517229.1	*Hordeum bulbosum* satellite DNA, p2542-101	152	538	67%	2e-33	96%
59.	AJ517239.1	*Hordeum bulbosum* satellite DNA, p25208-021	149	497	65%	3e-32	89%
60.	AJ517234.1	*Hordeum chilense* satellite DNA, p25147-1514	145	459	66%	3e-31	87%
61.	AJ517283.1	*Avena sativa* satellite DNA, p2542-2920	143	484	66%	1e-30	91%
62.	AJ517281.1	*Aegilops umbellulata* satellite DNA, p106208-2012	143	511	70%	1e-30	88%
63.	AJ517279.1	*Aegilops umbellulata* satellite DNA, p106208-1810	143	511	70%	1e-30	88%
64.	AJ517280.1	*Aegilops umbellulata* satellite DNA, p106208-1911	140	504	70%	1e-29	88%
65.	HQ213958.1	*Hordeum vulgare* clone pHv-1457 repeat region	136	629	67%	2e-28	70%
66.	AJ517256.1	*Secale cereale* satellite DNA, p25147-123	136	434	63%	2e-28	89%
67.	AJ517255.1	*Secale cereale* satellite DNA, p25147-3231	136	428	63%	2e-28	94%
68.	AF227454.1	*Bromus tectorum* clone 15 microsatellite sequence	134	472	68%	6e-28	88%
69.	HM536205.1	*Hordeum vulgare* clone pHv-961 repeat region	131	724	67%	8e-27	75%
70.	AJ517248.1	*Secale cereale* satellite DNA, p2542-3425	127	416	65%	9e-26	85%
71.	AJ517247.1	*Secale cereale* satellite DNA, p2542-3324	127	410	66%	9e-26	84%
72.	AJ517244.1	*Leymus mollis* satellite DNA, p2542-156	127	394	62%	9e-26	96%
73.	AJ517252.1	*Triticum aestivum* satellite DNA, p25147-2120	114	360	63%	6e-22	94%
74.	EF455889.1	*Triticum aestivum* AFLP BHW9-2 genomic sequence	113	385	71%	2e-21	79%
75.	X16096.1	Barley relic DNA HVT02, tandemly repeated seq	113	281	60%	2e-21	71%
76.	EF455887.1	*Triticum aestivum* AFLP BHW8-2 genomic sequence	107	371	71%	9e-20	78%
77.	EF455882.1	*Triticum aestivum* AFLP BHW17-22 genomic sequence	107	374	71%	9e-20	79%
78.	EF455900.1	*Triticum aestivum* AFLP BHW14-(4)-1 genomic sequence	104	353	71%	1e-18	77%
79.	EF455894.1	*Triticum aestivum* AFLP BHW15-22 genomic sequence	104	358	71%	1e-18	78%
80.	EF455888.1	*Triticum aestivum* AFLP BHW9-1 genomic sequence	104	358	71%	1e-18	77%
81.	EF455883.1	*Triticum aestivum* AFLP BHW17-23 genomic sequence	104	364	71%	1e-18	78%
82.	EF455915.1	*Triticum aestivum* AFLP BHW31-4 genomic sequence	98.7	467	43%	5e-17	88%
83.	EF455902.1	*Triticum aestivum* AFLP BHW15-21 genomic sequence	98.7	349	71%	5e-17	77%
84.	EF455898.1	*Triticum aestivum* AFLP BHW14-(3)-1 genomic sequence	98.7	338	71%	5e-17	76%
85.	EF455886.1	*Triticum aestivum* AFLP BHW8-1 genomic sequence	98.7	353	71%	5e-17	77%
86.	AY642926.1	*Hordeum vulgare* BAC CC24_14, complete sequence	98.7	285	67%	5e-17	70%
87.	EF614977.1	*Triticum aestivum* AFLP BHW50-2 genomic sequence	95.1	329	71%	6e-16	76%
88.	FN564430.1	*Triticum aestivum* chromosome 3B-specific BAC library, contig ctg0464b	89.7	155	24%	2e-14	75%
89.	DQ257591.1	*Hordeum vulgare* subsp. vulgare clone 29-2-LB flanking T-DNA insertion sequence	84.2	209	62%	1e-12	70%
90.	DQ175913.1	*Hordeum vulgare* subsp. vulgare clone 29-22A-LB flanking T-DNA insertion sequence	84.2	209	62%	1e-12	70%
91.	AY188331.1	*Triticum monococcum* DV92 chromosome 5AL BAC 231A16, complete sequence	80.6	80.6	26%	1e-11	74%
92.	AF354658.1	*Triticum aestivum* isolate AGT-CAGT8 scab resistance-linked AFLP fragment gene sequence	80.6	207	33%	1e-11	86%
93.	AY485644.1	*Triticum monococcum* phosphatidylserine decarboxylase, ZCCT2, ZCCT1, and SNF2P genes, complete cds, nucellin pseudogene, complete sequence, putative transposase, phosphatidylinositol phosphatidylcholine transfer protein sec14 cytosolic-like protein, and phytochrome P450-like protein genes, complete cds, and unknown genes	78.8	78.8	26%	4e-11	73%
94.	FN564426.1	*Triticum aestivum* chromosome 3B-specific BAC library, contig ctg0005b	77.0	184	24%	1e-10	80%
95.	GQ184456.1	*Triticum monococcum* clone BAC AM10001, complete sequence	75.2	75.2	24%	5e-10	73%
96.	AF326781.1	*Triticum monococcum* actin (ACT-1) gene, partial cds, putative chromosome condensation factor (CCF), putative resistance protein (RGA-2), putative resistance protein	75.2	222	26%	5e-10	74%

Sr.	Accession	Description	Max score [*1]	Total score[*2]	Query coverage [*3]	E value[*4]	Max identity[*5]
		(RGA2) and putative nodulin-like-like protein (NLL) gene, complete cds, and retrotransposons Josephine, Angela-2, Angela-4, Heidi, Greti, Angela-3, Fatima, Erika-1, Angela-6, Angela-5, Barbara, Isabelle, Erika-2, and Claudia					
97.	X16095.1	Barley relic DNA HVT01, tandemly repeated seq	75.2	128	48%	5e-10	70%
98.	DQ904440.1	*Triticum monococcum* subsp. aegilopoides clone BAC TbBAC5, complete sequence	73.4	141	26%	2e-09	73%
99.	AY054376.1	*Hordeum vulgare* Sukkula retrotransposon long terminal repeat, partial and complete sequences	66.2	66.2	14%	3e-07	80%
100.	Z68784.1	*Hordeum compressum* satellite DNA (ID pCOM2_2)					

*1 Max score: refers to highest alignment score of a set of aligned sequences
*2 Total score: refers to sum of aligned scores of all sequences for the subject sequence
*3 Query length: refers to coverage of query sequence within homologous sequences
*4 E value: number of alignments expected, lower E value (high number following e-) indicates more reliable results
*5 Max identify: refers to the highest percent identity for a set of aligned sequences

Table 5.3: List of species with total number of pSc119.2 sequences in EMBL-EBI database (30 March, 2012). Consensus sequence of 118bp monomer units was used in BLASTN search.

Sr#	**Name of species**	**Copies**
1	*Secale vavilovii*	05
2	*Secale cerale*	10
3	*Secale montanum*	01
4	*Hordeum chilense*	05
5	*Hordeum bulbosum*	09
6	*Hordeum brachyantherum*	06
7	*Hordeum vulgare*	04
8	*H. compressum*	01
9	*Aegilops squarrosa*	07
10	*Aegilops umbellulata*	10
11	*Triticum monococcum*	02
12	*Triticum aestivum*	18
13	*Triticum monvum*	01
14	*Avena sativa*	05
15	*Bromus tectorum*	01
16	*Leymus mollis*	03
Total		88

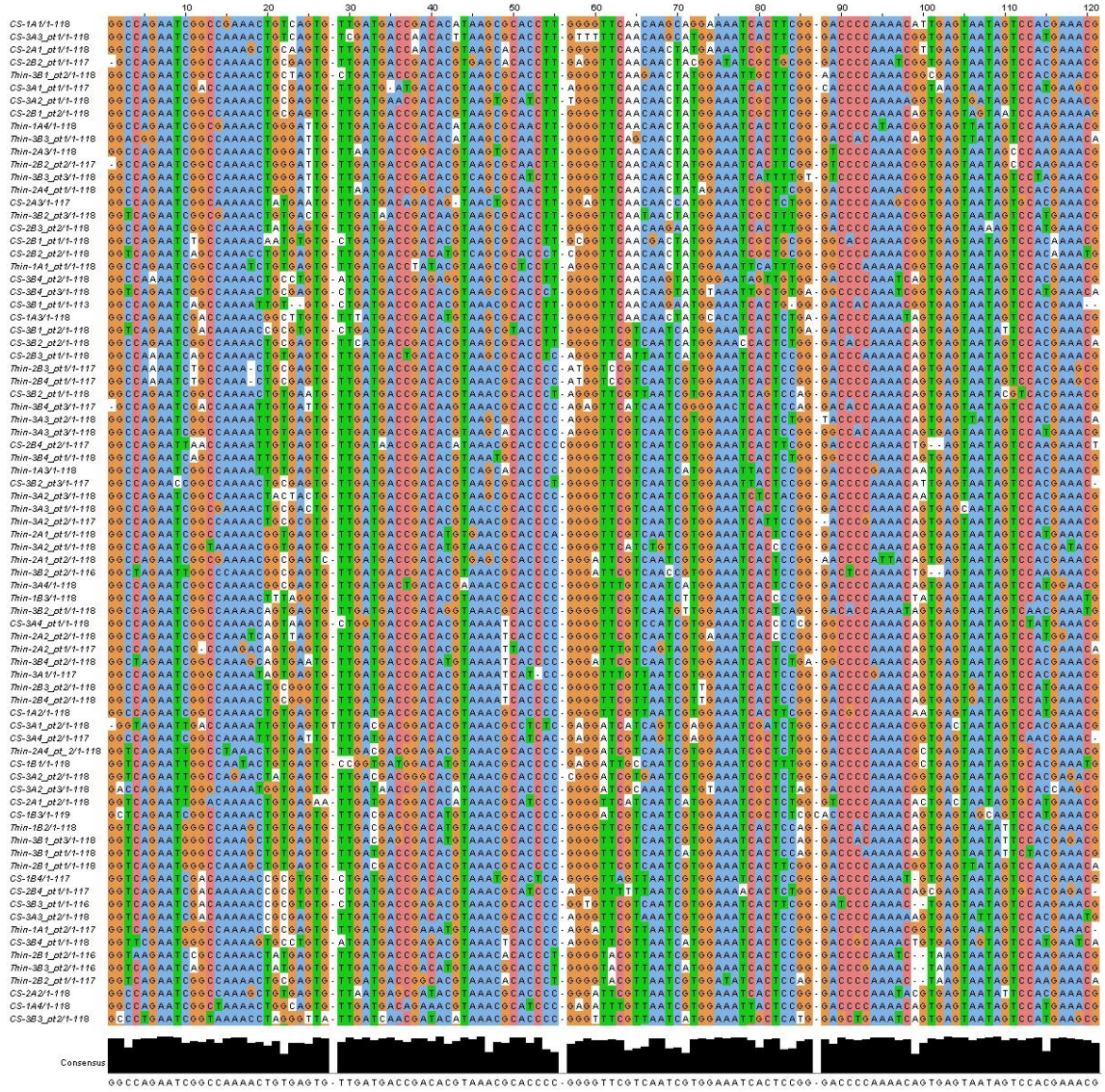


Figure 5.4: Multiple DNA sequence alignment of pSc119.2 sequences isolated from 'Chinese Spring' and *Th. intermedium* using default settings of the Jalview Multiple Alignment Editor.

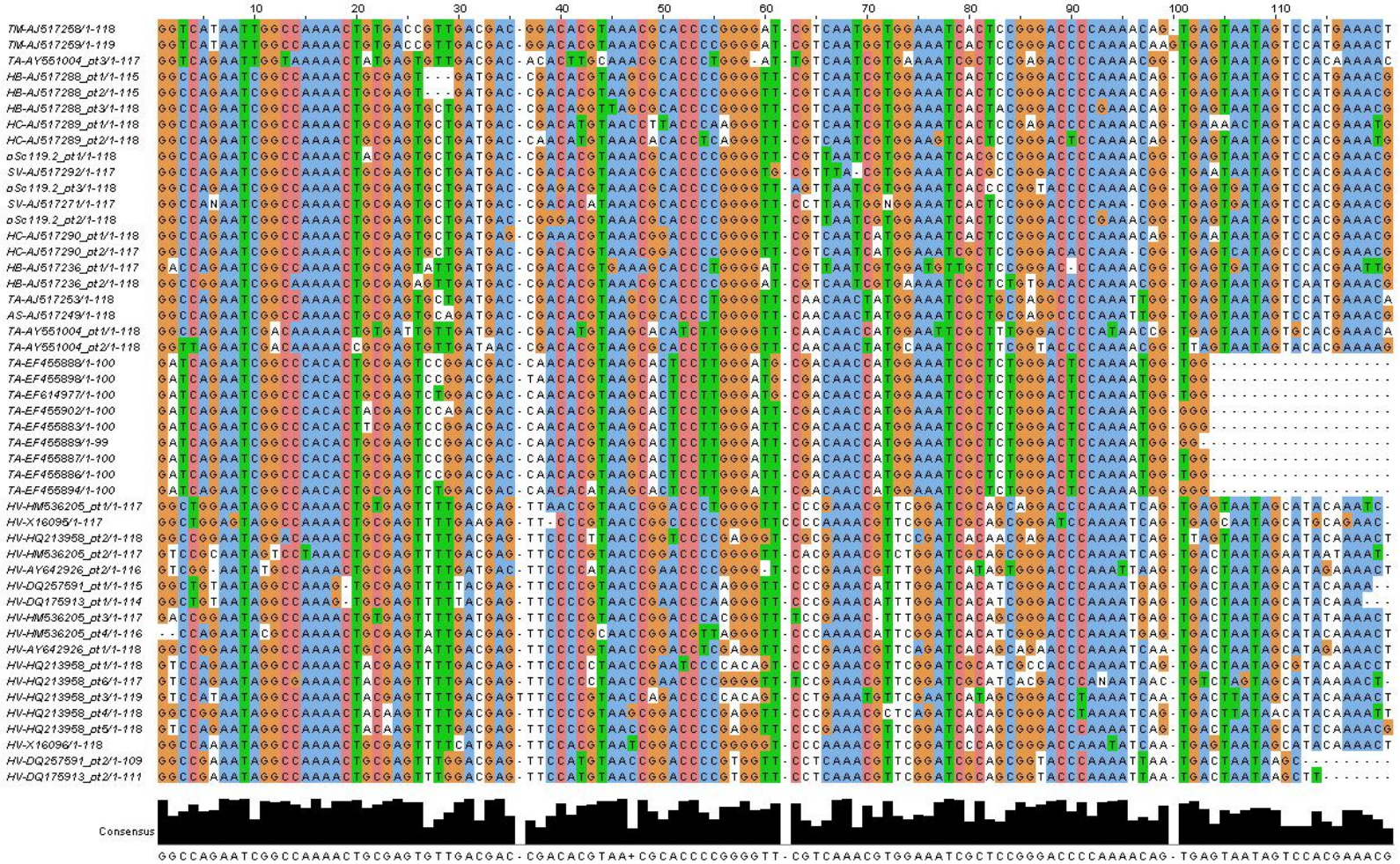


Figure 5.5: Multiple DNA sequence alignment of pSc119. 2 sequences (downloaded from EMBL-EBI database) using default settings of the Jalview Multiple Alignment Editor.

Table 5.4: List of Afa-family sequences downloaded from EMBL-EBI database (30 March, 2013). The 339bp of consensus Afa family sequence was used in BLASTN search.

	Accession	Description	Max score[*1]	Total score[*2]	Query coverage[*3]	E value[*4]	Max identity[*5]
1.	AB003692.1	*Elymus trachycaulus* DNA, species specific tandem repeat sequence	502	1525	100%	6e-139	96%
2.	EF567062.1	*Triticum aestivum* cultivar Glenlea clone BAC 1648_464 disease resistance protein (Lr1) genomic region	491	911	100%	1e-135	92%
3.	JF758493.1	*Triticum aestivum* clone 1144N5 genomic sequence	489	1845	100%	4e-135	92%
4.	EF081031.1	*Triticum turgidum* subsp. durum clone BAC 466G24 genomic sequence	489	1257	100%	4e-135	92%
5.	EF081030.1	*Triticum urartu* clone BAC 404H6 genomic sequence	489	1257	100%	4e-135	92%
6.	FM242578.1	*Triticum aestivum*, storage protein activator (spa) locus region, D genome, clone BAC Ren2409K09	466	846	100%	4e-128	95%
7.	DQ249273.1	*Hordeum vulgare* subsp. vulgare cultivar Morex BAC 631P8, complete sequence	466	4693	100%	4e-128	94%
8.	D30736.1	*Aegilops squarrosa* repetitive DNA sequence	464	1341	100%	2e-127	91%
9.	FJ477093.1	*Hordeum vulgare* cultivar Cepada capa Rym4 and MCT-1 genes, complete cds	462	1413	100%	5e-127	90%
10.	FJ477092.1	*Hordeum vulgare* subsp. vulgare cultivar Haruna Nijo Rym4 and MCT-1 genes, complete cds	455	4759	100%	8e-125	90%
11.	AF446141.1	*Aegilops tauschii* LZ-NBS-LRR class RGA, NBS-LRR class RGA, HCBT-like putative defense response protein, and putative alliin lyase genes, complete cds, and unknown genes	452	2114	100%	1e-123	95%
12.	AY643843.1	*Hordeum vulgare* subsp. vulgare clones BAC 519K7 and 799C8 hardness locus region	450	2310	100%	3e-123	91%
13.	AF474072.1	*Hordeum vulgare* sp. vulgare cultivar Morex BAC clone 773k14, complete sequence	450	1068	100%	3e-123	93%
14.	AY661558.1	*Hordeum vulgare* subsp. vulgare eIF4E gene locus, complete sequence	450	1.265e+04	100%	3e-123	91%
15.	FJ436983.1	*Triticum aestivum* cultivar Chinese Spring hexose carrier, LR34, cytochrome P450, lectin receptor kinases, and cytochrome P450 genes, complete cds	448	2086	100%	1e-122	91%
16.	FJ436985.1	*Triticum aestivum* cultivar Renan Lr34 locus, partial sequence	448	1336	100%	1e-122	91%
17.	FJ436984.1	*Triticum aestivum* cultivar Glenlea Lr34 locus, partial sequence	448	2086	100%	1e-122	91%
18.	HQ213964.1	*Hordeum vulgare* clone pHv-1631 repeat region	446	983	100%	4e-122	90%
19.	AF474373.1	*Hordeum vulgare* subsp. vulgare BAC 259I16, complete sequence	446	1624	100%	4e-122	93%
20.	AF427791.1	*Hordeum vulgare* Mla locus, complete sequence	446	3851	100%	4e-122	90%
21.	FJ436986.1	*Aegilops tauschii* Lr34 locus, partial sequence	443	1345	100%	5e-121	91%
22.	AY268139.1	*Hordeum vulgare* BAC 184G9, complete sequece	443	635	100%	5e-121	90%
23.	AY853252.1	*Hordeum vulgare* telomeric chromosome 7H region, complete sequence	441	3580	100%	2e-120	93%
24.	HQ213965.1	*Hordeum vulgare* clone pHv-1982 repeat region	439	1028	100%	6e-120	90%
25.	HQ213961.1	*Hordeum vulgare* clone pHv-874 repeat region	439	873	100%	6e-120	91%
26.	EU812563.1	*Hordeum vulgare* subsp. vulgare Rpg4 gene, complete sequence, RGA1 (RGA1) gene, complete cds, Rpg5 gene, complete sequence, PP2C (PP2C) gene, complete cds, and ADF3 gene, complete sequence	439	1669	100%	6e-120	92%
27.	AF488415.1	*Triticum monococcum* chromosome 7Am BAC 5K14 complete sequence	439	1343	100%	6e-120	93%

	Accession	Description	Max score[*1]	Total score[*2]	Query coverage[*3]	E value[*4]	Max identity[*5]
28.	HQ213962.1	*Hordeum vulgare* clone pHv-1390 repeat region	435	829	100%	7e-119	91%
29.	HQ213963.1	*Hordeum vulgare* clone pHv-1468 repeat region	434	970	100%	3e-118	88%
30.	HM536207.1	*Hordeum vulgare* clone pHv-1123 repeat region	434	1061	100%	3e-118	89%
31.	FN564434.1	*Triticum aestivum* chromosome 3B-specific BAC library, contig ctg0954b	434	4085	100%	3e-118	93%
32.	AB022728.1	*Leymus racemosus* DNA, tandem repetitive Afa-family sequence, clone:pLrAfa3	430	545	98%	3e-117	97%
33.	FN564431.1	*Triticum aestivum* chromosome 3B-specific BAC library, contig ctg0528b	426	964	98%	4e-116	96%
34.	AB022731.1	*Leymus racemosus* DNA, tandem repetitive Afa-family sequence, clone: pLrAfa6	426	541	97%	4e-116	97%
35.	AY663392.1	*Triticum aestivum* cultivar Renan clone BAC 930H14, complete sequence	425	802	100%	1e-115	90%
36.	AM932685.1	*Triticum aestivum* 3B chromosome, clone BAC TA3B95F5	421	698	100%	2e-114	88%
37.	AB022727.1	*Leymus racemosus* DNA, tandem repetitive Afa-family sequence, clone:pLrAfa2	417	543	98%	2e-113	96%
38.	AB022725.1	*Psathyrostachys juncea* DNA, tandem repetitive Afa-family sequence, clone:pPjAfa3	417	539	98%	2e-113	96%
39.	AB022723.1	*Psathyrostachys juncea* DNA, tandem repetitive Afa-family sequence, clone:pPjAfa1	417	538	98%	2e-113	96%
40.	HQ213959.1	*Hordeum vulgare* clone pHv-496 repeat region	416	901	100%	7e-113	89%
41.	AY943294.1	*Hordeum vulgare* subsp. vulgare clone BAC 673I14, complete sequence	416	919	100%	7e-113	88%
42.	AB022730.1	*Leymus racemosus* DNA, tandem repetitive Afa-family sequence, clone: pLrAfa5	416	527	97%	7e-113	96%
43.	FN564430.1	*Triticum aestivum* chromosome 3B-specific BAC library, contig ctg0464b	414	779	100%	2e-112	87%
44.	AB022726.1	*Leymus racemosus* DNA, tandem repetitive Afa-family sequence, clone:pLrAfa1	412	530	98%	8e-112	96%
45.	FN645450.1	*Triticum aestivum* chromosome 3B-specific BAC library, contig ctg0011b	407	1919	100%	4e-110	94%
46.	AP009567.1	*Hordeum vulgare* subsp. vulgare genes for putative iron-deficiency specific 4 protein and putative ethylene-responsive transcription factor, complete cds	407	867	100%	4e-110	90%
47.	AB022729.1	*Leymus racemosus* DNA, tandem repetitive Afa-family sequence, clone:pLrAfa4	405	547	98%	1e-109	100%
48.	AM932689.1	*Triticum aestivum* 3B chromosome, clone BAC TA3B63N2, 3 unordered pieces	392	3026	99%	8e-106	90%
49.	Z21645.1	*Hordeum chilense* genome-specific DNA	383	426	100%	4e-103	89%
50.	EU660892.1	*Triticum aestivum* clone BAC 1354M21 cytosolic acetyl-CoA carboxylase (Acc-2) and putative amino acid permeases genes, complete cds	381	709	100%	1e-102	91%
51.	AY642926.1	*Hordeum vulgare* BAC CC24_14, complete sequence	376	376	83%	6e-101	89%
52.	CT009625.1	*Aegilops tauschii*	369	718	99%	9e-99	93%
53.	CR626926.1	*Aegilops tauschii*	369	718	99%	9e-99	93%
54.	EU626553.1	*Triticum urartu* clone BAC 261N5, complete sequence	367	1087	99%	3e-98	85%
55.	AB022724.1	*Psathyrostachys juncea* DNA, tandem repetitive Afa-family sequence, clone:pPjAfa2	367	500	98%	3e-98	97%
56.	HQ213960.1	*Hordeum vulgare* clone pHv-580 repeat region	365	731	100%	1e-97	91%
57.	FM242576.1	*Triticum aestivum*, storage protein activator (spa) locus region, B genome, clone BAC Ren0871J20	365	699	100%	1e-97	93%

	Accession	Description	Max score[*1]	Total score[*2]	Query coverage[*3]	E value[*4]	Max identity[*5]
58.	AB003259.1	*Triticum urartu* DNA, tandem repetitive Afa-family sequence, clone pTuAfa1	354	487	98%	2e-94	97%
59.	FN564435.1	*Triticum aestivum* chromosome 3B-specific BAC library, contig ctg1030b	352	420	82%	7e-94	90%
60.	AY146587.2	*Triticum turgidum* subsp. durum Pm3 locus, genomic sequence	352	1126	99%	7e-94	91%
61.	AB003261.1	*Triticum urartu* DNA, tandem repetitive Afa-family sequence, clone pTuAfa3	351	480	97%	2e-93	97%
62.	AY485644.1	*Triticum monococcum* phosphatidylserine decarboxylase, ZCCT2, ZCCT1, and SNF2P genes, complete cds, nucellin pseudogene, complete sequence, putative transposase, phosphatidylinositol phosphatidylcholine transfer protein sec14 cytosolic-like protein, and phytochrome P450-like protein genes, complete cds, and unknown genes	345	345	74%	1e-91	91%
63.	AY951945.1	*Triticum monococcum* TmBAC 60J11 FR-Am2 locus, genomic sequence	343	343	72%	4e-91	91%
64.	AB003264.1	*Triticum urartu* DNA, tandem repetitive Afa-family sequence, clone pTuAfa6	343	442	98%	4e-91	90%
65.	AB003256.1	*Aegilops triuncialis* var. triuncialis DNA, Afa-family tandem repeat sequence, clone: pAsAfa2	340	458	98%	4e-90	94%
66.	AB003260.1	*Triticum urartu* DNA, tandem repetitive Afa-family sequence, clone pTuAfa2	336	455	95%	5e-89	94%
67.	AB003258.1	*Aegilops triuncialis* var. triuncialis DNA, Afa-family tandem repeat sequence, clone: pAsAfa3	336	460	98%	5e-89	95%
68.	AB003257.1	*Aegilops triuncialis* var. triuncialis DNA, Afa-family tandem repeat sequence, clone: pAsAfa3	336	460	98%	5e-89	95%
69.	AB003262.1	*Triticum urartu* DNA, tandem repetitive Afa-family sequence, clone pTuAfa4	331	464	98%	2e-87	97%
70.	AB003255.1	*Aegilops triuncialis* var. triuncialis DNA, Afa-family tandem repeat sequence, clone: pAsAfa1	331	449	98%	2e-87	94%
71.	AB003229.1	*Secale cereale* DNA, tandem repetitive Afa-family sequence, clone Afa-cer4	315	315	62%	2e-82	93%
72.	X76300.1	*Triticum aestivum* (Chinese spring) tandemly repeated DNA sequence	313	363	82%	6e-82	97%
73.	AB003263.1	*Triticum urartu* DNA, tandem repetitive Afa-family sequence, clone pTuAfa5	302	424	91%	1e-78	95%
74.	AB003247.1	*Triticum aestivum* DNA, tandem repetitive Afa-family sequence, clone Afa-TCS3	297	297	62%	5e-77	92%
75.	AB003245.1	*Triticum aestivum* DNA, tandem repetitive Afa-family sequence, clone Afa-TCS1	295	295	62%	2e-76	91%
76.	AB003242.1	*Aegilops speltoides* DNA, tandem repetitive Afa-family sequence, clone Afa-spe3	293	293	61%	6e-76	91%
77.	AB003243.1	*Aegilops speltoides* DNA, tandem repetitive Afa-family sequence, clone Afa-spe4	289	289	61%	7e-75	90%
78.	D82989.1	*Triticum monococcum* DNA, tandem repetitive Afa-family sequence, clone Afa-mon3	289	289	62%	7e-75	90%
79.	AB003228.1	*Secale cereale* DNA, tandem repetitive Afa-family sequence, clone Afa-cer3	288	288	62%	2e-74	90%
80.	AB003252.1	*Hordeum vulgare* DNA, tandem repetitive Afa-family sequence, clone Afa-vur2	286	286	61%	8e-74	90%
81.	AB003222.1	*Triticum turgidum* subsp. durum DNA, Afa-family tandem sequence, clone: Afa-6DCSL3	286	286	62%	8e-74	90%
82.	AB003217.1	*Triticum turgidum* subsp. durum DNA, Afa-family tandem sequence, clone: Afa-5DCSL1	286	286	62%	8e-74	90%
83.	AB003212.1	*Triticum turgidum* subsp. durum DNA, Afa-family tandem sequence, clone: Afa-4DCSL5	286	286	62%	8e-74	90%
84.	Z54373.1	*Hordeum vulgare* repetitive DNA	286	435	96%	8e-74	90%
85.	AB003235.1	*Triticum turgidum* subsp. durum DNA, Afa-family tandem repeat sequence, clone: Afa-dur1	280	280	62%	4e-72	89%
86.	AB003206.1	*Triticum turgidum* subsp. durum DNA, Afa-family tandem sequence, clone: Afa-3DCSL4	280	280	62%	4e-72	89%

	Accession	Description	Max score[*1]	Total score[*2]	Query coverage[*3]	E value[*4]	Max identity[*5]
87.	AB003205.1	*Triticum turgidum* subsp. durum DNA, Afa-family tandem sequence, clone: Afa-3DCSL3	280	280	62%	4e-72	89%
88.	AB003201.1	*Triticum turgidum* subsp. durum DNA, Afa-family tandem sequence, clone: Afa-2DCSL3	280	280	62%	4e-72	89%
89.	EU934206.1	*Hordeum vulgare* subsp. vulgare cultivar Vairogs 1H(5) Mla region 538P8 locus genomic sequence	279	438	97%	1e-71	90%
90.	EU934204.1	*Hordeum vulgare* subsp. vulgare cultivar Sencis 1H(5) Mla region 538P8 locus genomic sequence	279	435	97%	1e-71	90%
91.	EU934199.1	*Hordeum vulgare* subsp. vulgare cultivar Malva 1H(5) Mla region 538P8 locus genomic sequence	279	435	97%	1e-71	90%
92.	EU934189.1	*Hordeum vulgare* subsp. vulgare cultivar Dzintars 1H(5) Mla region 538P8 locus genomic sequence	279	438	97%	1e-71	90%
93.	EU934188.1	*Hordeum vulgare* subsp. vulgare cultivar Druvis 1H(5) Mla region 538P8 locus genomic sequence	279	435	97%	1e-71	90%
94.	AB003223.1	*Triticum turgidum* subsp. durum DNA, Afa-family tandem sequence, clone: Afa-7DCSL1	279	279	62%	1e-71	89%
95.	AB003215.1	*Triticum turgidum* subsp. durum DNA, Afa-family tandem sequence, clone: Afa-4DCSL8	279	279	62%	1e-71	90%
96.	AB003199.1	*Triticum turgidum* subsp. durum DNA, Afa-family tandem sequence, clone: Afa-2DCSL1	279	279	63%	1e-71	89%
97.	D82991.1	*Triticum monococcum* DNA, tandem repetitive Afa-family sequence, clone Afa-mon5	279	279	61%	1e-71	90%
98.	Z54374.1	*Hordeum vulgare* repetitive DNA	279	417	96%	1e-71	90%
99.	AB003226.1	*Secale cereale* DNA, tandem repetitive Afa-family sequence, clone Afa-cer1	277	277	62%	4e-71	89%
100.	AB003225.1	*Triticum turgidum* subsp. durum DNA, Afa-family tandem sequence, clone: Afa-7DCSL3	277	277	62%	4e-71	89%

*1 Max score: refers to highest alignment score of a set of aligned sequences
*2 Total score: refers to sum of aligned scores of all sequences for the subject sequence
*3 Query length: refers to coverage of query sequence within homologous sequences
*4 E value: number of alignments expected, lower E value (high number following e-) indicates more reliable results
*5 Max identify: refers to the highest percent identity for a set of aligned sequences

Total 5.5: List of species with total number of Afa sequences in EMBL-EBI database (30 March, 2012). The 339bp Afa repeat unit was used in BLASTN search.

Sr#	Species	copies
1.	*Triticum durum*	*(34)*
2.	*Triticum urartu*	*(10)*
3.	*Triticum aestivum*	*(6)*
4.	*Triticum monococcum*	*(6)*
5.	*Hordeum vulgare*	*(6)*
6.	*Leymus racemosus*	*(6)*
7.	*Hordeum vulgare* subsp. vulgare	*(5)*
8.	*Aegilops speltoides*	*(5)*
9.	*Aegilops comosa*	*(5)*
10.	*Secale cereale*	*(4)*
11.	*Aegilops triuncialis* var. triuncialis	*(4)*
12.	*Psathyrostachys juncea*	*(3)*
13.	*Aegilops tauschii*	*(1)*
14.	*Elymus trachycaulus*	*(1)*

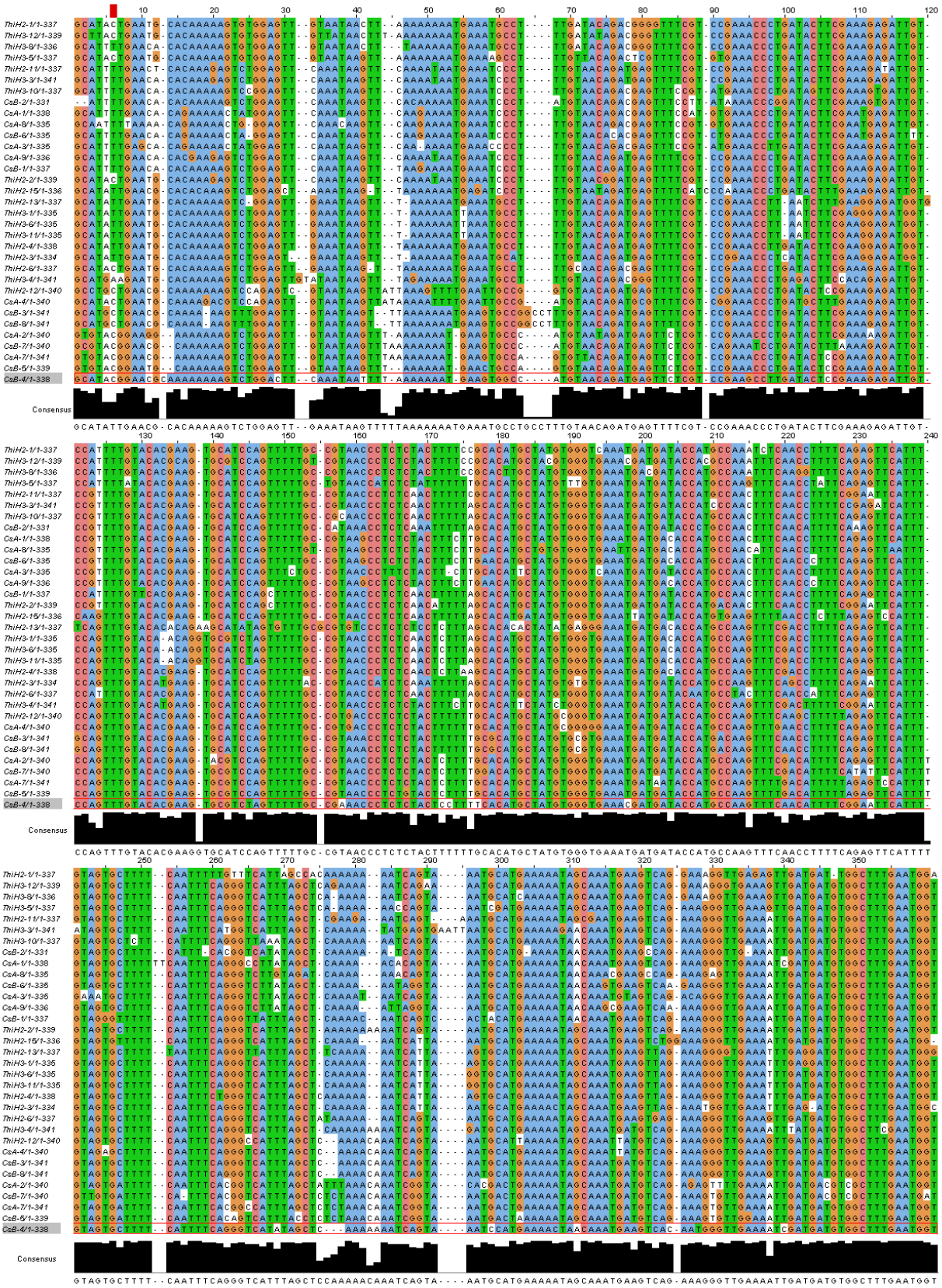


Figure 5.6: Multiple DNA sequence alignment of Afa-family sequences isolated from ‘Chinese Spring’ wheat and *Th. intermedium* using default settings of the Jalview Multiple Alignment Editor.

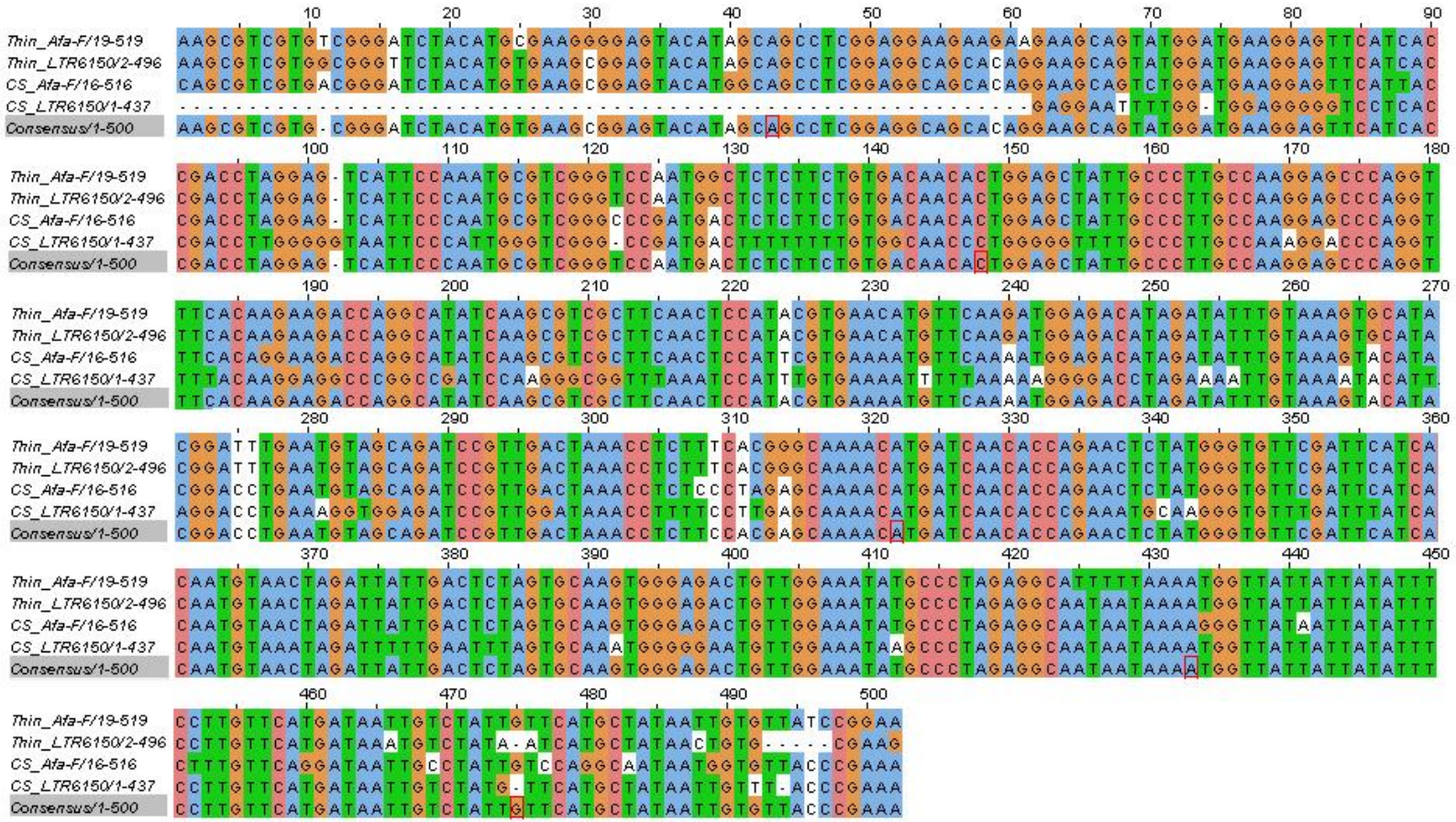


Figure 5.7: Multiple DNA sequence alignment of the LTR sequences isolated from 'Chinese Spring' wheat and *Th. intermedium*, with LTR specific and Afa-1F primer combination. Sequences were aligned using default settings of the Jalview Multiple Alignment Editor.

REFERENCES

Aalfs JD, Kingston RE. 2000. What does 'chromatin remodeling' mean? *Trends in Biochemical Sciences,* 25: 548-555.

Adams KL, Wendel JF. 2005a. Novel patterns of gene expression in polyploid plants. *Trends Genet,* 21: 539-43.

Adams KL, Wendel JF. 2005b. Polyploidy and genome evolution in plants. *Curr Opin Plant Biol,* 8: 135-41.

Adams MD, Kelley JM, Gocayne JD, Dubnick M, Polymeropoulos MH, Xiao H, Merril CR, Wu A, Olde B, Moreno RF, Kerlavage AR, McCombie WR, Venter JC. 1991. Complementary DNA sequencing: Expressed sequence tags and human genome project. *Science,* 252: 1651-1656.

Ågren JA, Wright SI. 2011. Co-evolution between transposable elements and their hosts: A major factor in genome size evolution? *Chromosome Research,* 19: 777-786.

Akhunov ED, Akhunova AR, Anderson OD, Anderson JA, Blake N, Clegg MT, Coleman-Derr D, Conley EJ, Crossman CC, Deal KR, Dubcovsky J, Gill BS, Gu YQ, Hadam J, Heo H, Huo N, Lazo GR, Luo M, Ma YQ, Matthews DE, McGuire PE, Morrell PL, Qualset CO, Renfro J, Tabanao D, Talbert LE, Tian C, Toleno DM, Warburton ML, You FM, Zhang W, Dvorak J. 2010. Nucleotide diversity maps reveal variation in diversity among wheat genomes and chromosomes. *Bmc Genomics,* 11.

Alexandrov I, Kazakov A, Tumeneva I, Shepelev V, Yurov Y. 2001. Alpha-satellite DNA of primates: old and new families. *Chromosoma,* 110: 253-66.

Altieri MA. 2000. The ecological impacts of transgenic crops on agroecosystem health. *Ecosystem Health,* 6: 13-23.

Ammiraju JSS, Zuccolo A, Yu Y, Song X, Piegu B, Chevalier F, Walling JG, Ma J, Talag J, Brar DS, SanMiguel PJ, Jiang N, Jackson SA, Panaud O, Wing RA. 2007. Evolutionary dynamics of an ancient retrotransposon family provides insights into evolution of genome size in the genus Oryza. *Plant Journal,* 52: 342-351.

Anamthawat-Jónsson K, Heslop-Harrison JS. 1993. Isolation and characterization of genome-specific DNA sequences in *Triticeae* species. *Mol Gen Genet,* 240: 151-158.

Atkinson TG, Grant MN. 1967. An evaluation of streak mosaic losses in winter wheat

Phytopathology 57: 188-92.

Ayala-Navarrete L, Bariana HS, Singh RP, Gibson JM, Mechanicos AA, Larkin PJ. 2007. Trigenomic chromosomes by recombination of *Thinopyrum intermedium* and *Th. ponticum* translocations in wheat. *Theoretical and Applied Genetics*, 116: 63-75.

Ayala-Navarrete L, Tourton E, Mechanicos AA, Larkin PJ. 2009. Comparison of *Thinopyrum intermedium* derivatives carrying barley yellow dwarf virus resistance in wheat. *Genome*, 52: 537-546.

Ayala L, Henry M, González-de-León D, Van Ginkel M, Mujeeb-Kazi A, Keller B, Khairallah M. 2001. A diagnostic molecular marker allowing the study of *Th. intermedium*-derived resistance to BYDV in bread wheat segregating populations. *Theoretical and Applied Genetics*, 102: 942-949.

Badaeva ED, Friebe B, Zoshchuk SA, Zelenin AV, Gill BS. 1998. Molecular cytogenetic analysis of tetraploid and hexaploid *Aegilops crassa*. *Chromosome Res*, 6: 629-37.

Baker B, Zambryski P, Staskawicz B, Dinesh-Kumar SP. 1997. Signaling in plant-microbe interactions. *Science*, 276: 726-733.

Baley GJ, Talbert LE, Martin JM, Young MJ, Habernicht DK, Kushnak GD, Berg JE, Lanning SP, Bruckner PL. 2001. Agronomic and end-use qualities of *Wheat streak mosaic virus* resistant spring wheat. *Crop Science*, 41: 1779-1784.

Bao Yg, Wang S, Wang Xq, Wang Yh, Li Xf, Wang L, Wang Hg. 2009. Heterosis and combining ability for major yield traits of a new wheat germplasm shannong 0095 derived from *Thinopyrum intermedium*. *Agricultural Sciences in China*, 8: 753-760.

Barton KA, Brill WJ. 1983. Prospects in plant genetic engineering. *Science*, 219: 671-676.

Bedbrook JR, Jones J, O'Dell M, Thompson RD, Flavell RB. 1980. A molecular description of telomeric heterochromatin in Secale species. *Cell*, 19: 545-60.

Belyaev ND, Houben A, Baranczewski P, Schubert I. 1997. Histone H4 acetylation in plant heterochromatin is altered during the cell cycle. *Chromosoma*, 106: 193-197.

Bender J. 2004. DNA methylation and epigenetics. *Annual Review of Plant Biology.*

Bennett MD, Leitch IJ. 1995a. Nuclear DNA amounts in angiosperms. *Annals of Botany*, 76: 113-176.

Bennett MD, Leitch IJ. 1995b. Nuclear DNA Amounts in Angiosperms. *Ann Bot,* 76: 113-176.

Bennett MD, Leitch IJ. 2011. Nuclear DNA amounts in angiosperms: Targets, trends and tomorrow. *Annals of Botany,* 107: 467-590.

Bennetzen JL. 2000. The many hues of plant heterochromatin. *Genome Biol,* 1: 107.1-107.4.

Bennetzen JL. 2005. Transposable elements, gene creation and genome rearrangement in flowering plants. *Current Opinion in Genetics and Development,* 15: 621-627.

Bennetzen JL, Sanmiguel P, Chen M, Tikhonov A, Francki M, Avramova Z. 1998. Grass genomes. *Proceedings of the National Academy of Sciences of the United States of America,* 95: 1975-1978.

Bentham G. 1882. Notes on Gramineae. Journal of the Linnean Society, Botany 18: 14–134.

Berger SL. 2002. Histone modifications in transcriptional regulation. *Current Opinion in Genetics and Development,* 12: 142-148.

Bestor TH, Verdine GL. 1994. DNA methyltransferases. *Current Opinion in Cell Biology,* 6: 380-389.

Biagetti M, Vitellozzi F, Ceoloni C. 1999. Physical mapping of wheat-*Aegilops longissima* breakpoints in mildew-resistant recombinant lines using FISH with highly repeated and low-copy DNA probes. *Genome,* 42: 1013-1019.

Bird AP, Southern EM. 1978. Use of restriction enzymes to study eukaryotic DNA methylation: I. The methylation pattern in ribosomal DNA from *Xenopus laevis*. *Journal of Molecular Biology,* 118: 27-47.

Bockus WW, Appel JA, Bowden RL, Fritz AK, Gill BS, Joe Martin T, Sears RG, Seifers DL, Brown-Guedira GL, Eversmeyer MG. 2001. Success stories: Breeding for wheat disease resistance in Kansas. *Plant Disease,* 85: 453-460.

Bodvarsdottir SK, Anamthawat-Jonsson K. 2003. Isolation, characterization, and analysis of *Leymus*-specific DNA sequences. *Genome,* 46: 673-682.

Borlaug NE. 1983. Contributions of conventional plant breeding to food production. *Science,* 219: 689-693.

Brown AHD. 2010. Variation under domestication in plants: 1859 and today. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 365: 2523-2530.

Bryan GJ, Collins AJ, Stephenson P, Orry A, Smith JB, Gale MD. 1997. Isolation and characterisation of microsatellites from hexaploid bread wheat. *Theoretical and Applied Genetics,* 94: 557-563.

Buckler Iv ES, Thornsberry JM, Kresovich S. 2001. Molecular diversity, structure and domestication of grasses. *Genetical Research,* 77: 213-218.

Caldwell KS, Langridge P, Powell W. 2004. Comparative sequence analysis of the region harboring the hardness locus in barley and its colinear region in rice. *Plant Physiology,* 136: 3177-3190.

Carvalho A, MartÃ-n A, Heslop-Harrison JS, Guedes-Pinto H, Lima-Brito J. 2009. Identification of the spontaneous 7BS/7RL intergenomic translocation in one F1 multigeneric hybrid from the *Triticeae* tribe. *Plant Breeding,* 128: 105-108.

Castilho A, Heslop-Harrison JS. 1995. Physical mapping of 5S and 18S-25S rDNA and repetitive DNA sequences in *Aegilops umbellulata*. *Genome,* 38: 91-96.

Cato SA, Gardner RC, Kent J, Richardson TE. 2001. A rapid PCR-based method for genetically mapping ESTs. *Theoretical and Applied Genetics,* 102: 296-306.

Chakraborty S, Newton AC. 2011. Climate change, plant diseases and food security: An overview. *Plant Pathology,* 60: 2-14.

Charles M, Belcram H, Just J, Huneau C, Viollet A, Couloux A, Segurens B, Carter M, Huteau V, Coriton O, Appels R, Samain S, Chalhoub B. 2008. Dynamics and Differential Proliferation of Transposable Elements During the Evolution of the B and A Genomes of Wheat. *Genetics,* 180: 1071-1086.

Charlesworth B, Sniegowski P, Stephan W. 1994. The evolutionary dynamics of repetitive DNA in eukaryotes. *Nature,* 371: 215-20.

Charmet G. 2011. Wheat domestication: Lessons for the future. *Comptes Rendus - Biologies,* 334: 212-220.

Chee HT, Siang HT, Chai LH, Faridah QZ, Othman YR, Heslop-Harrison JS, Kalendar R, Schulman AH. 2005. Genome constitution and classification using retrotransposon-based markers in the orphan crop banana. *Journal of Plant Biology,* 48: 96-105.

Chen Q. 2005. Detection of alien chromatin introgression from *Thinopyrum* into wheat using S genomic DNA as a probe - A landmark approach for *Thinopyrum* genome research. *Cytogenetic and Genome Research,* 109: 350-359.

Chen Q, Conner RL, Laroche A. 1996. Molecular characterization of *Haynaldia villosa* chromatin in wheat lines carrying resistance to wheat curl mite colonization. *Theoretical and Applied Genetics*, 93: 679-684.

Chen Q, Conner RL, Laroche A, Fedak G, Thomas JB. 1999a. Genomic origins of *Thinopyrum* chromosomes specifying resistance to *Wheat streak mosaic virus* and its vector, Aceria tosichella. *Genome*, 42: 289-295.

Chen Q, Conner RL, Li HJ, Graf R, Laroche A, Li HY, Ahmad F. 2003a. Genomic characterization of new sources of resistance to both Wheat streak mosaic virus and wheat curl mite in wheat-*Thinopyrum* partial amphiploids. *Journal of Genetics and Breeding*, 57: 155-164.

Chen Q, Conner RL, Li HJ, Sun SC, Ahmad F, Laroche A, Graf RJ. 2003b. Molecular cytogenetic discrimination and reaction to *Wheat streak mosaic viru*s and the wheat curl mite in Zhong series of wheat-*Thinopyrum intermedium* partial amphiploids. *Genome*, 46: 135-145.

Chen Q, Friebe B, Conner RL, Laroche A, Thomas JB, Gill BS. 1998a. Molecular cytogenetic characterization of *Thinopyrum intermedium*-derived wheat germplasm specifying resistance to wheat streak mosaic virus. *Theoretical and Applied Genetics*, 96: 1-7.

Chen ZJ. 2007. Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids.

Chinnusamy V, Zhu JK. 2009. Epigenetic regulation of stress responses in plants. *Current Opinion in Plant Biology*, 12: 133-139.

Choi IR, Horken KM, Stenger DC, French R. 2005. An internal RNA element in the P3 cistron of Wheat streak mosaic virus revealed by synonymous mutations that affect both movement and replication. *Journal of General Virology*, 86: 2605-2614.

Choi IR, Stenger DC, French R. 2000. Multiple interactions among proteins encoded by the mite-transmitted *Wheat streak mosaic tritimovirus*. *Virology*, 267: 185-198.

Christian ML, Willis WG. 1993. Survival of *Wheat streak mosaic-virus* in grass hosts in Kansas from wheat harvest to fall wheat emergence. *Plant Disease*, 77: 239-242.

Cohen-Karni D, Xu D, Apone L, Fomenkov A, Sun Z, Davis PJ, Morey Kinney SR, Yamada-Mabuchi M, Xu SY, Davis T, Pradhan S, Roberts RJ, Zheng Y. 2011. The MspJI family of modification-dependent restriction endonucleases for

epigenetic studies. *Proceedings of the National Academy of Sciences of the United States of America,* 108: 11040-11045.

Collard BCY, Mackill DJ. 2008. Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 363: 557-572.

Comai L, Madlung A, Josefsson C, Tyagi A. 2003. Do the different parental 'heteromes' cause genomic shock in newly formed allopolyploids? *Philos Trans R Soc Lond B Biol Sci,* 358: 1149-55.

Contento A, Ceccarelli M, Gelati T, Maggini F, Baldoni L, Cionini G. 2002. Diversity of Olea genotypes and the origin of cultivated olives. *Theor Appl Genet,* 104: 1229-1238.

Contento A, Heslop-Harrison JS, Schwarzacher T. 2005. Diversity of a major repetitive DNA sequence in diploid and polyploid *Triticeae*. *Cytogenet Genome Res,* 109: 34-42.

Coutts BA, Strickland GR, Kehoe MA, Severtson DL, Jones RAC. 2008. The epidemiology of *Wheat streak mosaic virus* in Australia: case histories, gradients, mite vectors, and alternative hosts. *Australian Journal of Agricultural Research,* 59: 844-853.

Cox CM, Murray TD, Jones SS. 2002. Perennial wheat germ plasm lines resistant to eyespot, *Cephalosporium* stripe, and *Wheat streak mosaic*. *Plant Disease,* 86: 1043-1048.

Cuadrado A, Jouve N. 1995. Fluorescent in situ hybridization and C-banding analyses of highly repetitive DNA sequences in the heterochromatin of rye (*Secale montanum* Guss.) and wheat incorporating *S. montanum* chromosome segments. *Genome,* 38: 795-802.

Curtis BC, Rajaram S, Gómez Macpherson H. 2002. *Bread wheat Improvement and production* Plant Production and Protection Series 567.

Darwin C. 1905. The variation of animals and plants under domestication. John Murray, London (popular edition in two volumes)

De Bodt S, Maere S, Van De Peer Y. 2005. Genome duplication and the origin of angiosperms. *Trends in Ecology and Evolution,* 20: 591-597.

De Boer JG, Yazawa R, Davidson WS, Koop BF (2007) Bursts and horizontal evolution of DNA transposons in the speciation of *Pseudotetraploid salmonids. Bmc Genomics 8.*

De Waard MA, Georgopoulos SG, Hollomon DW, Ishii H, Leroux P, Ragsdale NN, Schwinn FJ. 1993. Chemical control of plant diseases: Problems and prospects. *Annual Review of Phytopathology,* 31: 403-421.

Devos KM. 2010. Grass genome organization and evolution. *Current Opinion in Plant Biology,* 13: 139-145.

Devos KM, Atkinson MD, Chinoy CN, Liu CJ, Gale MD. 1992. RFLP-based genetic map of the homoeologous group 3 chromosomes of wheat and rye. *Theoretical and Applied Genetics,* 83: 931-939.

Devos KM, Gale MD. 2000. Genome relationships: the grass model in current research. *Plant Cell,* 12: 637-46.

Dewannieux M, Esnault C, Heidmann T. 2003. LINE-mediated retrotransposition of marked Alu sequences. *Nature Genetics,* 35: 41-48.

Diamond J. 2002. Evolution, consequences and future of plant and animal domestication. *Nature,* 418: 700-707.

Divis LA, Graybosch RA, Peterson CJ, Baenziger PS, Hein GL, Beecher BB, Martin TJ. 2006. Agronomic and quality effects in winter wheat of a gene conditioning resistance to *Wheat streak mosaic virus*. *Euphytica,* 152: 41-49.

Dong F, Miller JT, Jackson SA, Wang GL, Ronald PC, Jiang J. 1998. Rice (*Oryza sativa*) centromeric regions consist of complex DNA. *Proc Natl Acad Sci U S A,* 95: 8135-40.

Doyle JJ, Doyle JL. 1990. Isolation of plant DNA from fresh tissue. *Focus* 12: 13-15.

Dubcovsky J, Dvorak J. 2007. Genome plasticity a key factor in the success of polyploid wheat under domestication. *Science,* 316: 1862-1866.

Dvorak J, Akhunov ED, Akhunov AR, Deal KR, Luo MC. 2006. Molecular characterization of a diagnostic DNA marker for domesticated tetraploid wheat provides evidence for gene flow from wild tetraploid wheat to hexaploid wheat. *Molecular Biology and Evolution,* 23: 1386-1396.

Dvorak J, Luo MC, Akhunov ED. 2011. N.I. Vavilov's theory of centres of diversity in the light of current understanding of wheat diversity, domestication and evolution. *Czech Journal of Genetics and Plant Breeding,* 47: S20-S27.

Dwyer GI, Gibbs MJ, Gibbs AJ, Jones RAC. 2007. *Wheat streak mosaic virus* in Australia: Relationship to isolates from the Pacific Northwest of the USA and its dispersion via seed transmission. *Plant Disease,* 91: 164-170.

Eckardt NA. 2010. Evolution of domesticated bread wheat. *Plant Cell,* 22: 993.

Elena SF, Sanjuán R. 2005. Adaptive value of high mutation rates of RNA viruses: Separating causes from consequences. *Journal of Virology,* 79: 11555-11558.

Ellis MH, Rebetzke GJ, Mago R, Chu P. 2003. First report of *Wheat streak mosaic virus* in Australia. *Australasian Plant Pathology,* 32: 551-553.

Endo TR, Gill BS. 1996. The deletion stocks of common wheat. *Journal of Heredity,* 87: 295-307.

Fahim M, Ayala-Navarrete L, Millar AA, Larkin PJ. 2010b. Hairpin RNA derived from viral NIa gene confers immunity to *Wheat streak mosaic virus* infection in transgenic wheat plants. *Plant Biotechnology Journal,* 8: 821-834.

Fahim M, Dove H, Kelman WM, Ayala-Navarrete L, Larkin PJ. 2010a. Does grazing of infected wheat by sheep result in salivary transmission of *Wheat streak mosaic virus*? *Crop and Pasture Science,* 61: 247-254.

Fahim M, Mechanicos A, Ayala-Navarrete L, Haber S, Larkin PJ. 2011. Resistance to *Wheat streak mosaic virus*- a survey of resources and development of molecular markers. *Plant Pathology*.

Fahim M, Millar AA, Wood CC, Larkin PJ. 2012. Resistance to *Wheat streak mosaic virus* generated by expression of an artificial polycistronic microRNA in wheat. *Plant Biotechnology Journal,* 10: 150-163.

Fans JD, Laddomada B, Gill BS. 1998. Molecular mapping of segregation distortion loci in *Aegilops tauschii*. *Genetics,* 149: 319-327.

Fedak G, Chen Q, Conner RL, Laroche A, Comeau A, St-Pierre CA. 2001. Characterization of wheat-*Thinopyrum* partial amphiploids for resistance to *Barley yellow dwarf virus*. *Euphytica,* 120: 373-378.

Feldman M, Kislev ME. 2007. Domestication of emmer wheat and evolution of free-threshing tetraploid wheat. *Israel Journal of Plant Sciences,* 55: 207-221.

Feldman M, Levy AA. 2005a. Allopolyploidy--a shaping force in the evolution of wheat genomes. *Cytogenet Genome Res,* 109: 250-8.

Feldman M, Levy AA. 2005b. Allopolyploidy - A shaping force in the evolution of wheat genomes. *Cytogenetic and Genome Research,* 109: 250-258.

Feldman M, Levy AA. 2009. Genome evolution in allopolyploid wheat-a revolutionary reprogramming followed by gradual changes. *Journal of Genetics and Genomics,* 36: 511-518.

Feldman M, Lupton FGH, Miller TE. 1995. *Wheats. In Evolution of Crop Plants,* J. Smartt and N.W. Simmonds, eds (London: Longman Scientific).

Feldman M, Sears ER. 1981. The wild gene resources of wheat *Sci Am* 244: 102-112.

Fellers J, Wan J, Hong Y, Collins GB, Hunt AG. 1998. In vitro interactions between a potyvirus-encoded, genome-linked protein and RNA-dependent RNA polymerase. *Journal of General Virology,* 79: 2043-2049.

Felsenstein J. 1985. Confidence-limits on phylogenies - an approach using the bootstrap. *Evolution,* 39: 783-791.

Feng S, Cokus SJ, Zhang X, Chen PY, Bostick M, Goll MG, Hetzel J, Jain J, Strauss SH, Halpern ME, Ukomadu C, Sadler KC, Pradhan S, Pellegrini M, Jacobsen SE. 2010. Conservation and divergence of methylation patterning in plants and animals. *Proceedings of the National Academy of Sciences of the United States of America,* 107: 8689-8694.

Feschotte C, Jiang N, Wessler SR. 2002. Plant transposable elements: Where genetics meets genomics. *Nature Reviews Genetics,* 3: 329-341.

Feuillet C, Keller B. 2002a. Comparative genomics in the grass family: molecular characterization of grass genome structure and evolution. *Ann Bot (Lond),* 89: 3-10.

Finnegan DJ. 1989. Eukaryotic transposable elements and genome evolution. *Trends in Genetics,* 5: 103-107.

Finnegan EJ, Genger RK, Peacock WJ, Dennis ES. 1998. DNA methylation in plants. *Annual Review of Plant Physiology and Plant Molecular Biology,* 49: 223-247.

Finnegan EJ, Kovac KA. 2000a. Plant DNA methyltransferases. *Plant Molecular Biology,* 43: 189-201.

Finnegan EJ, Kovac KA. 2000b. Plant DNA methyltransferases. *Plant Mol Biol,* 43: 189-201.

Finnegan EJ, Peacock WJ, Dennis ES. 1996. Reduced DNA methylation in *Arabidopsis thaliana* results in abnormal plant development. *Proc Natl Acad Sci U S A,* 93: 8449-54.

Finnegan EJ, Peacock WJ, Dennis ES. 2000. DNA methylation, a key regulator of plant development and other processes. *Curr Opin Genet Dev,* 10: 217-23.

Flaus A, Owen-Hughes T. 2011. Mechanisms for ATP-dependent chromatin remodelling: The means to the end. *Febs Journal,* 278: 3579-3595.

Flavell RB. 1986. Repetitive DNA and chromosome evolution in plants. *Philos Trans R Soc Lond B Biol Sci,* 312: 227-42.

Flavell RB, Bennett MD, Smith JB, Smith DB. 1974a. Genome size and the proportion of repeated nucleotide sequence DNA in plants. *Biochem Genet,* 12: 257-69.

Flavell RB, Bennett MD, Smith JB, Smith DB. 1974b. Genome size and the proportion of repeated nucleotide sequence DNA in plants. *Biochemical Genetics,* 12: 257-269.

Fojtova M, Kovarik A, Matyasek R. 2001. Cytosine methylation of plastid genome in higher plants. Fact or artefact? *Plant Sci,* 160: 585-593.

Forsström PO, Merker A, Schwarzacher T. 2002. Characterisation of mildew resistant wheat-rye substitution lines and identification of an inverted chromosome by fluorescent in situ hybridisation. *Heredity,* 88: 349-355.

Foulkes MJ, Slafer GA, Davies WJ, Berry PM, Sylvester-Bradley R, Martre P, Calderini DF, Griffiths S, Reynolds MP. 2011. Raising yield potential of wheat. III. Optimizing partitioning to grain while maintaining lodging resistance. *Journal of Experimental Botany,* 62: 469-486.

Friebe B, Gill KS, Tuleen NA, Gill BS. 1996a. Transfer of *wheat streak mosaic virus* resistance from *Agropyron intermedium* into wheat. *Crop Science,* 36: 857-861.

Friebe B, Jiang J, Raupp WJ, McIntosh RA, Gill BS. 1996b. Characterization of wheat-alien translocations conferring resistance to diseases and pests: Current status. *Euphytica,* 91: 59-87.

Friebe B, Mukai Y, Dhaliwal HS, Martin TJ, Gill BS. 1991. Identification of alien chromatin specifying resistance to *Wheat streak mosaic* and greenbug in wheat-germ plasm by c-banding and in situ hybridization *Theoretical and Applied Genetics,* 81: 381-389.

Friebe B, Qi LL, Wilson DL, Chang ZJ, Selfers DL, Martin TJ, Fritz AK, Gill BS. 2009. Wheat-*Thinopyrum intermedium* recombinants resistant to *Wheat streak mosaic virus* and *Triticum mosaic virus*. *Crop Science,* 49: 1221-1226.

Friso S, Choi SW, Girelli D, Mason JB, Dolnikowski GG, Bagley PJ, Olivieri O, Jacques PF, Rosenberg IH, Corrocher R, Selhub J. 2002. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. *Proceedings of the National Academy of Sciences of the United States of America,* 99: 5606-5611.

Frommer M, McDonald LE, Millar DS, Collis CM, Watt F, Grigg GW, Molloy PL, Paul CL. 1992. A genomic sequencing protocol that yields a positive display of 5-

methylcytosine residues in individual DNA strands. *Proceedings of the National Academy of Sciences of the United States of America,* 89: 1827-1831.

Fu YB, Somers DJ. 2009. Genome-wide reduction of genetic diversity in wheat breeding. *Crop Science,* 49: 161-168.

Fuchs J, Demidov D, Houben A, Schubert I. 2006. Chromosomal histone modification patterns - from conservation to diversity. *Trends in Plant Science,* 11: 199-208.

Fuchs J, Jasencakova Z, Schubert I. 2005. On chromatin modification and heterochromatin. *Chromosome Res,* 13: 8-9.

Fulnecek J, Matyasek R, Kovarik A. 2002. Distribution of 5-methylcytosine residues in 5S rRNA genes in *Arabidopsis thaliana* and *Secale cereale*. *Mol Genet Genomics,* 268: 510-7.

Fulneček J, Matyášek R, Kovařík A. 2009. Faithful inheritance of cytosine methylation patterns in repeated sequences of the allotetraploid tobacco correlates with the expression of DNA methyltransferase gene families from both parental genomes. *Molecular Genetics and Genomics,* 281: 407-420.

Gadaleta A, Giancaspro A, Giove SL, Zacheo S, Mangini G, Simeone R, Signorile A, Blanco A. 2009. Genetic and physical mapping of new EST-derived SSRs on the A and B genome chromosomes of wheat. *Theoretical and Applied Genetics,* 118: 1015-1025.

Gaeta RT, Pires JC, Iniguez-Luy F, Leon E, Osborn TC. 2007. Genomic changes in resynthesized *Brassica napus* and their effect on gene expression and phenotype. *Plant Cell,* 19: 3403-3417.

Ganal MW, Röder MS. 2007. Microsatellite and SNP markers in wheat breeding. . *In: Varshney RK, Tuberosa R (eds) Genomics assisted crop improvement* vol. 2 Genomics applications in crops. Springer, Berlin.

Gao L, Ma Q, Liu Y, Xin Z, Zhang Z. 2009. Molecular characterization of the genomic region harboring the BYDV-resistance gene Bdv2 in wheat. *Journal of Applied Genetics,* 50: 89-98.

Gao LF, Jing RL, Huo NX, Li Y, Li XP, Zhou RH, Chang XP, Tang JF, Ma ZY, Jia JZ. 2004. One hundred and one new microsatellite loci derived from ESTs (EST-SSRs) in bread wheat. *Theoretical and Applied Genetics,* 108: 1392-1400.

Gaut BS. 2002. Evolutionary dynamics of grass genomes. *New Phytologist,* 154: 15-28.

Gepts P, Papa R. 2002. Evolution during domestication. *Encyclopedia of Life Sciences.* London: Nature Publishing Group Macmillan Publishers Ltd, Nature Publishing Group.

Gerlach WL, Bedbrook JR. 1979. Cloning and characterization of ribosomal RNA genes from wheat and barley. *Nucleic Acids Res,* 7: 1869-1885.

Gerlach WL, Dyer TA. 1980. Sequence organization of the repeating units in the nucleus of wheat which contain 5S rRNA genes. *Nucleic Acids Res,* 8: 4851-65.

Gill BS, Friebe B, Endo TR. 1991. Standard karyotype and nomenclature system for description of chromosome bands and structural-aberrations in wheat (*Triticum aestivum*). *Genome,* 34: 830-839.

Gill BS, Friebe BR, White FF. 2011. Alien introgressions represent a rich source of genes for crop improvement. *Proceedings of the National Academy of Sciences of the United States of America,* 108: 7657-7658.

Gill BS, Raupp WJ. 1987. Direct gene transfer from *Aegilops squarrosa* L. to hexaploid wheat. *Crop Science,* 27.

Goncharov NP. 2011. Genus Triticum L. taxonomy: The present and the future. *Plant Systematics and Evolution,* 295: 1-11.

Gowher H, Leismann O, Jeltsch A. 2000. DNA of *Drosophila melanogaster* contains 5-methylcytosine. *Embo J,* 19: 6918-23.

Grafi G, Zemach A, Pitto L. 2007. Methyl-CpG-binding domain (MBD) proteins in plants. *Biochimica Et Biophysica Acta-Gene Structure and Expression,* 1769: 287-294.

Graybosch RA, Peterson CJ, Baenziger PS, Baltensperger DD, Nelson LA, Jin Y, Kolmer J, Seabourn B, French R, Hein G, Martin TJ, Beecher B, Schwarzacher T, Heslop-Harrison P. 2009. Registration of 'Mace' Hard Red Winter Wheat. *Journal of Plant Registrations,* 3: 51-56.

Griffiths S, Sharp R, Foote TN, Bertin I, Wanous M, Reader S, Colas I, Moore G. 2006. Molecular characterization of *Ph1* as a major chromosome pairing locus in polyploid wheat. *Nature,* 439: 749-752.

Gustafson P, Raskina O, Ma X, Nevo E. 2009. Wheat evolution, domestication, and improvement. In: (ed.) C ed., Wheat: Science and Trade. Wiley-Blackwell.

Guyomarc'h H, Sourdille P, Charmet G, Edwards KJ, Bernard M. 2002. Characterisation of polymorphic microsatellite markers from *Aegilops tauschii* and transferability

to the D-genome of bread wheat. *Theoretical and Applied Genetics,* 104: 1164-1172.

Haber S, Pradhan M, Somers D. 2007. Breaking the linkage between the *Wsm1* gene for resistance to *Wheat streak mosaic virus* and the alien chromatin of its origin. *Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie,* 29: 215-216.

Hackel E. 1887. *Gramineae. In: Engler, A. and K. Prantl (eds.) Die Natürlichen Pflanzenfamilien* Engelmann, Leipzig, Germany.

Hacques MF, Muller S, De Murcia G, Van Regenmortel MHV, Marion C. 1990. Accessibility and structural role of histone domains in chromatin. Biophysical and immunochemical studies of progressive digestion with immobilized proteases. *Journal of Biomolecular Structure and Dynamics,* 8: 619-641.

Hagras AAA, Kishii M, Tanaka H, Sato K, Tsujimoto H. 2005. Genomic differentiation of *Hordeum chilense* from *H. vulgare* as revealed by repetitive and EST sequences. *Genes & Genetic Systems,* 80: 147-159.

Haley SD, Johnson JJ, Peairs FB, Stromberger JA, Heaton EE, Seifert SA, Kottke RA, Rudolph JB, Martin TJ, Bai G, Chen X, Bowden RL, Jin Y, Kolmer JA, Seifers DL, Chen M, Seabourn BW. 2011. Registration of 'Snowmass' Wheat. *Journal of Plant Registrations,* 5: 87-90.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. . *Nucl Acids Symp Ser* 41.

Han FP, Fedak G, Ouellet T, Liu B. 2003. Rapid genomic changes in interspecific and intergeneric hybrids and allopolyploids of *Triticeae*. *Genome,* 46: 716-723.

Hao M, Luo J, Yang M, Zhang L, Yan Z, Yuan Z, Zheng Y, Zhang H, Liu D, Gustafson P. 2011. Comparison of homoeologous chromosome pairing between hybrids of wheat genotypes Chinese Spring *ph1b* and Kaixian-luohanmai with rye. *Genome*, 54: 959-964.

Harper J, Armstead I, Thomas A, James C, Gasior D, Bisaga M, Roberts L, King I, King J. 2011. Alien introgression in the grasses *Lolium perenne* (perennial ryegrass) and *Festuca pratensis* (meadow fescue): The development of seven monosomic substitution lines and their molecular and cytological characterization. *Annals of Botany,* 107: 1313-1321.

Harvey TL, Martin TJ, Seifers DL. 2003. Resistance to the wheat curl mite (Acari : Eriophyidae) prevents loss in wheat yield. *Journal of Agricultural and Urban Entomology*, 20: 7-10.

Harvey TL, Seifers DL, Martin TJ. 2001. Host range differences between two strains of wheat curl mites (Acari: Eriophyidae). *Journal of Agricultural and Urban Entomology*, 18: 35-41.

Harvey TL, Seifers DL, Martin TJ, Michaud JP. 2005. Effect of resistance to *Wheat streak mosaic virus* on transmission efficiency of wheat curl mites. *Journal of Agricultural and Urban Entomology*, 22: 1-6.

Hein GL, . 2010. Wheat curl mite. In: W. W. Bockus RLB, R. L. Hunger, W. L. Morril, T. D. Murray, and R. W. Smiley (eds.), ed. *Compendium of Wheat Diseases and Pests, 3rd ed. APS Press, St.*

Heitkam T, Schmidt T. 2009. BNR - A line family from *Beta vulgaris*- contains a rrm domain in open reading frame 1 and defines a l1 sub-clade present in diverse plant genomes. *Plant Journal*, 59: 872-882.

Henikoff S. 2000. Heterochromatin function in complex genomes. *Biochim Biophys Acta*, 1470: O1-8.

Heslop-Harrison JS. 2000a. RNA, genes, genomes and chromosomes: repetitive DNA sequences in plants. In: Olmo E, Redi CA eds. *Chromosomes Today, Vol 13.*

Heslop-Harrison JS. 2000b. Comparative genome organization in plants: From sequence and markers to chromatin and chromosomes. *Plant Cell*, 12: 617-635.

Heslop-Harrison JS. 2002. Exploiting novel germplasm. *Australian Journal of Agricultural Research*, 53: 873-879.

Heslop-Harrison JS. 2003. Tandemly repeated DNA sequences and centromeric chromosomal regions of *Arabidopsis* species. *Chromosome Research*, 11: 241-253.

Heslop-Harrison JS, Schwarzacher T. 2011b. Genetics and genomics of crop domestication. In: Plant biotechnology and agriculture: Prospects for the 21st century

Altman A, Hasegawa. PM eds. *Plant biotechnology and agriculture.* USA, In: Elsevier Inc.

Heslop-Harrison JSP. 2010. Genes in evolution: The control of diversity and speciation. *Annals of Botany*, 106: 437-438.

Heslop-Harrison JSP, Schwarzacher T. 2011a. Organisation of the plant genome in chromosomes. *Plant Journal,* 66: 18-33.

Heun M, Schäfer-Pregl R, Klawan D, Castagna R, Accerbi M, Borghi B, Salamini F. 1997. Site of einkorn wheat domestication identified by DNA fingerprinting. *Science,* 278: 1312-1314.

Horn PJ, Peterson CL. 2002. Molecular biology: Chromatin higher order folding: Wrapping up transcription. *Science,* 297: 1824-1827.

Houben A, Demidov D, Gernand D, Meister A, Leach CR, Schubert I. 2003. Methylation of histone H3 in euchromatin of plant chromosomes depends on basic nuclear DNA content. *Plant Journal,* 33: 967-973

Hovmøller MS, Sørensen CK, Walter S, Justesen AF. 2011. Diversity of *Puccinia striiformis* on cereals and grasses.

http://www.fao.org. Statistics Division, Statistical Yearbook 2009–2010, Food and Agricultural Organization, United Nations. United Nations, Rome.

http://www.wheatgenome.org/. International Wheat Genome Sequencing Consortium.

Hu LJ, Li GR, Zeng ZX, Chang ZJ, Liu C, Zhou JP, Yang ZJ. 2011. Molecular cytogenetic identification of a new wheat-*Thinopyrum* substitution line with stripe rust resistance. *Euphytica,* 177: 169-177.

Huang XQ, Wolf M, Ganal MW, Orford S, Koebner RMD, Röder MS. 2007. Did modern plant breeding lead to genetic erosion in European winter wheat varieties? *Crop Science,* 47: 343-349.

Huda A, Jordan IK. 2009. Epigenetic regulation of mammalian genomes by transposable elements.

Hull R. 2004. Mathew's Plant Virology. Academic Press, New York.

Hunger RM, Sherwood JL, Evans CK, Montana JR. 1992. Effects of planting date and inoculation date on severity of *Wheat streak mosaic* in hard red winter-wheat cultivars. *Plant Disease,* 76: 1056-1060.

Ilic K, SanMiguel PJ, Bennetzen JL. 2003. A complex history of rearrangement in an orthologous region of the maize, sorghum, and rice genomes. *Proceedings of the National Academy of Sciences of the United States of America,* 100: 12265-12270.

Irizarry RA, Ladd-Acosta C, Carvalho B, Wu H, Brandenburg SA, Jeddeloh JA, Wen B, Feinberg AP. 2008. Comprehensive high-throughput arrays for relative methylation (CHARM). *Genome Research,* 18: 780-790.

Islam AKMR, Shepherd KW (1980) Meiotic restitution in wheat-barley hybrids. Chromosoma 79:363-372

Jeltsch A. 2002. Beyond Watson and Crick: DNA methylation and molecular enzymology of DNA methyltransferases (Vol 3, pg 274, 2002). *Chembiochem,* 3: 382-382.

Jiang J, Gill BS. 1994. Different species-specific chromosome translocations in *Triticum timopheevii* and *T. turgidum* support the diphyletic origin of polyploid wheats. *Chromosome Res*, 2: 59-64.

Jiang W, Garrett KA, Peterson DE, Harvey TL, Bowden RL, Fang L. 2005. The window of risk for emigration of *Wheat streak mosaic virus* varies with host eradication method. *Plant Disease,* 89: 853-858.

Jiao Y, Wickett NJ, Ayyampalayam S, Chanderbali AS, Landherr L, Ralph PE, Tomsho LP, Hu Y, Liang H, Soltis PS, Soltis DE, Clifton SW, Schlarbaum SE, Schuster SC, Ma H, Leebens-Mack J, Depamphilis CW. 2011. Ancestral polyploidy in seed plants and angiosperms. *Nature,* 473: 97-100.

Jin W, Melo JR, Nagaki K, Talbert PB, Henikoff S, Dawe RK, Jiang J. 2004. Maize centromeres: Organization and functional adaptation in the genetic background of oat. *Plant Cell,* 16: 571-581.

Jones RAC, Coutts BA, Mackie AE, Dwyer GI. 2005. Seed transmission of *Wheat streak mosaic virus* shown unequivocally in wheat. *Plant Disease,* 89: 1048-1050.

Josefsson C, Dilkes B, Comai L. 2006. Parent-Dependent Loss of Gene Silencing during Interspecies Hybridization. *Current Biology,* 16: 1322-1328.

Kajikawa M, Okada N. 2002. LINEs mobilize SINEs in the eel through a shared 3′ sequence. *Cell,* 111: 433-444.

Kanno T, Bucher E, Daxinger L, Huettel B, Kreil DP, Breinig F, Lind M, Schmitt MJ, Simon SA, Gurazada SGR, Meyers BC, Lorkovic ZJ, Matzke AJM, Matzke M. 2010. RNA-directed DNA methylation and plant development require an IWR1-type transcription factor. *EMBO Reports,* 11: 65-71.

Kapitonov VV, Jurka J. 2008. A universal classification of eukaryotic transposable elements implemented in Repbase. *Nature Reviews Genetics,* 9: 411-412.

Kashkush K, Feldman M, Levy AA. 2002. Gene loss, silencing and activation in a newly synthesized wheat allotetraploid. *Genetics,* 160: 1651-1659.

Kashkush K, Feldman M, Levy AA. 2003. Transcriptional activation of retrotransposons alters the expression of adjacent genes in wheat. *Nature Genetics,* 33: 102-106.

Kato M, Miura A, Bender J, Jacobsen SE, Kakutani T. 2003. Role of CG and non-CG methylation in immobilization of transposons in *Arabidopsis*. *Current Biology*, 13: 421-426.

Kazazian J, H. H. 2004. Mobile Elements: Drivers of Genome Evolution. *Science,* 303: 1626-1632.

Kejnovsky E, Hobza R, Cermak T, Kubat Z, Vyskot B. 2009. The role of repetitive DNA in structure and evolution of sex chromosomes in plants. *Heredity*, 102: 533-541.

Kidwell MG, Lisch DR. 2000. Transposable elements and host genome evolution. *Trends in Ecology and Evolution,* 15: 95-99.

Kidwell MG, Lisch DR. 2001. Perspective: transposable elements, parasitic DNA, and genome evolution. *Evolution Int J Org Evolution,* 55: 1-24.

Kihara H. 1930. Karyologische studien an *Fragaria* mit besonderer Berucksichtigung der Geschlechtschromosomen. *Cytologia,* 1: 345-357.

Kihara H. 1944. Discovery of the DD-analyser, one of the ancestors of *Triticum vulgare*. *Agric Hortic (Tokyo)*19: 889–890.

Kihara H. 1954. Considerations on the evolution and distribution of *Aegilops* species based on the Analyzer method *Cytologia,* 19: 336-357.

Kihara H, Nishiyama I. 1930. Genomanalyse bei *Triticum* und *Aegilops* (von KIHARA). Genomaffinitiiten in trio, tetra- und pentaploiden WeizenBastarden. *Cytologia,* 1: 263-284.

King IP, Forster BP, Law CC, Cant KA, Orford SE, Gorham J, Reader S, Miller TE. 1997a. Introgression of salt-tolerance genes from *Thinopyrum bessarabicum* into wheat. *New Phytologist,* 137: 75-81.

King IP, Law CN, Cant KA, Orford SE, Reader SM, Miller TE. 1997b. Tritipyrum, a potential new salt-tolerant cereal. *Plant Breeding,* 116: 127-132.

King IP, Purdie KA, Miller TE, Law CN, Rogers WJ. 1992. Exploitation of chromosome 4s1, from *Aegilops sharonensis*, for the production of stable 44-chromosome wheat lines. *Heredity,* 69: 160-165.

King IP, Purdie KA, Rezanoor HN, Koebner RMD, Miller TE, Reader SM, Nicholson P. 1993. Characterization of *Thinopyrum bessarabicum* chromosome segments in

wheat using random amplified polymorphic DNAs (RAPDs) and genomic in situ hybridization. *Theoretical and Applied Genetics,* 86: 895-900.

Kishii M, Nagaki K, Tsujimoto H. 2001. A tandem repetitive sequence located in the centromeric region of common wheat (*Triticum aestivum*) chromosomes. *Chromosome Res,* 9: 417-28.

Kishii M, Nagaki K, Tsujimoto H, Sasakuma T. 1999. Exclusive localization of tandem repetitive sequences in subtelomeric heterochromatin regions of *Leymus racemosus* (*Poaceae, Triticeae*). *Chromosome Res,* 7: 519-29.

Kishii M, Tsujimoto H. 2002. Genus-specific localization of the TaiI family of tandem-repetitive sequences in either the centromeric or subtelomeric regions in *Triticeae* species (*Poaceae*) and its evolution in wheat. *Genome,* 45: 946-55.

Kong L, Anderson JM, Ohm HW. 2009. Segregation distortion in common wheat of a segment of *Thinopyrum intermedium* chromosome 7E carrying Bdv3 and development of a Bdv3 marker. *Plant Breeding,* 128: 591-597.

Kornberg RD, Lorch Y. 1999. Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. *Cell,* 98: 285-94.

Korzun V. 2002. Use of molecular markers in cereal breeding. *Cellular and Molecular Biology Letters,* 7: 811-820.

Krattinger SG, Lagudah ES, Wicker T, Risk JM, Ashton AR, Selter LL, Matsumoto T, Keller B. 2011. Lr34 multi-pathogen resistance ABC transporter: Molecular analysis of homoeologous and orthologous genes in hexaploid wheat and other grass species. *Plant Journal,* 65: 392-403.

Kubis SE, Castilho AM, Vershinin AV, Heslop-Harrison JS. 2003a. Retroelements, transposons and methylation status in the genome of oil palm (*Elaeis guineensis*) and the relationship to somaclonal variation. *Plant Mol Biol,* 52: 69-79.

Kubis SE, Castilho AMMF, Vershinin AV, Heslop-Harrison JS. 2003b. Retroelements, transposons and methylation status in the genome of oil palm (*Elaeis guineensis*) and the relationship to somaclonal variation. *Plant Molecular Biology,* 52: 69-79.

Kubis SE, Schmidt T, Heslop-Harrison JS. 1998. Repetitive DNA elements as a major component of plant genomes. *Ann Bot,* 82: 45-55.

Kubota S, Ishibashi T, Kohno S. 1997. A germline restricted, highly repetitive DNA sequence in Paramyxine atami: An interspecifically conserved, but somatically eliminated, element. *Molecular and General Genetics,* 256: 252-256.

Kudela O, Kudelova M, Novakova S, Glasa M. 2008. First report of *Wheat streak mosaic virus* in Slovakia. *Plant Disease,* 92: 1365-1365.

Kuhn GCS, Heslop-Harrison JS. 2011. Characterization and genomic organization of PERI, a repetitive DNA in the *Drosophila buzzatii* cluster related to DINE-1 transposable elements and highly abundant in the sex chromosomes. *Cytogenetic and Genome Research,* 132: 79-88.

Kuhn GCS, Schwarzacher T, Heslop-Harrison JS. 2007. Long-range organisation of two non-homologous satellite DNAs from the *Drosophila buzzatii* cluster: Mutual relative arrangements and molecular drive. *Chromosome Research,* 15: 35-36.

Kuhn GCS, Schwarzacher T, Heslop-Harrison JS. 2010. The non-regular orbit: Three satellite DNAs in *Drosophila martensis* (buzzatii complex, repleta group) followed three different evolutionary pathways. *Molecular Genetics and Genomics,* 284: 251-262.

Kuhn GCS, Sene FM. 2005. Evolutionary turnover of two pBuM satellite DNA subfamilies in the *Drosophila buzzatii* species cluster (repleta group): From alpha to alpha/beta arrays. *Gene,* 349: 77-85.

Kuhn GCS, Sene FM, Moreira-Filho O, Schwarzacher T, Heslop-Harrison JS. 2008. Sequence analysis, chromosomal distribution and long-range organization show that rapid turnover of new and old pBuM satellite DNA repeats leads to different patterns of variation in seven species of the *Drosophila buzzatii* cluster. *Chromosome Research,* 16: 307-324.

Kuhn GCS, Teo CH, Schwarzacher T, Heslop-Harrison JS. 2009. Evolutionary dynamics and sites of illegitimate recombination revealed in the interspersion and sequence junctions of two nonhomologous satellite DNAs in cactophilic *Drosophila* species. *Heredity,* 102: 453-464.

Kumar A, Bennetzen JL. 1999. Plant retrotransposons. *Annu Rev Genet,* 33: 479-532.

Kumar A, Sharma M. 2011. Wheat genome phylogeny and improvement. *Australian Journal of Crop Science,* 5: 1120-1126.

Lander E, L. LM, Bruce B, Chad N. 2001. Erratum: Initial sequencing and analysis of the human genome: International Human Genome Sequencing Consortium *Nature,* 409:860-921.

Langdon T, Seago C, Mende M, Leggett M, Thomas H, Forster JW, Jones RN, Jenkins G. 2000. Retrotransposon evolution in diverse plant genomes. *Genetics,* 156: 313-325.

Langham MAC, Doxtader DC, Haley SD, Kalsbeck S, Little RS, Ibrahim AM. 2001. Yield and growth reductions in winter wheat infected with *Wheat streak mosaic virus*. *Phytopathology* 91: S52

Lanoiselet VM, Hind-Lanoiselet TL, Murray GM. 2008. Studies on the seed transmission of *Wheat streak mosaic virus*. *Australasian Plant Pathology,* 37: 584-588.

Lans H, Marteijn JA, Vermeulen W. 2012. ATP-dependent chromatin remodeling in the DNA-damage response. *Epigenetics and Chromatin,* 5.

Larkin PJ, Banks PM, Lagudah ES, Appels R, Xiao C, Zhiyong X, Ohm HW, McIntosh RA. 1995. Disomic *Thinopyrum intermedium* addition lines in wheat with *Barley yellow dwarf virus* resistance and with rust resistances. *Genome,* 38: 385-394.

Law JA, Jacobsen SE. 2010. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nature Reviews Genetics,* 11: 204-220.

Lev-Yadun S, Gopher A, Abbo S. 2000. The cradle of agriculture. *Science,* 288: 1602-1603.

Levy AA, Feldman M. 2002. The impact of polyploidy on grass genome evolution. *Plant Physiol,* 130: 1587-93.

Levy AA, Feldman M. 2004. Genetic and epigenetic reprogramming of the wheat genome upon allopolyploidization. *Biological Journal of the Linnean Society,* 82: 607-613.

Li D, Yap KS. 2011. Climate change and its impact on food and nutrition security and food safety in China.

Li H, Conner RL, Chen Q, Graf RJ, Laroche A, Ahmad F, Kuzyk AD. 2005a. Promising genetic resources for resistance to *Wheat streak mosaic virus* and the wheat curl mite in wheat-*Thinopyrum* partial amphiploids and their derivatives. *Genetic Resources and Crop Evolution*, 51: 827-835.

Li H, Wang X. 2009. *Thinopyrum ponticum* and *Th. intermedium*: the promising source of resistance to fungal and viral diseases of wheat. *Journal of Genetics and Genomics*, 36: 557-565.

Li HJ, Arterburn M, Jones SS, Murray TD. 2005b. Resistance to eyespot of wheat, caused by *Tapesia yallundae*, derived from *Thinopyrum intermedium* homoeologous group 4 chromosome. *Theoretical and Applied Genetics*, 111: 932-940.

Li Q, Chen XM, Wang MN, Jing JX. 2011. Yr45, a new wheat gene for stripe rust resistance on the long arm of chromosome 3D. *Theoretical and Applied Genetics*, 122: 189-197.

Lisch D. 2009. Epigenetic regulation of transposable elements in plants. *Annual Review of Plant Biology*, 60: 43-66.

Liu B, Brubaker CL, Mergeai G, Cronn RC, Wendel JF. 2001. Polyploid formation in cotton is not accompanied by rapid genomic changes. *Genome*, 44: 321-30.

Liu B, Vega JM, Feldman M. 1998a. Rapid genomic changes in newly synthesized amphiploids of *Triticum* and *Aegilops*. II. Changes in low-copy coding DNA sequences. *Genome*, 41: 535-42.

Liu B, Vega JM, Segal G, Abbo S, Rodova M, Feldman M. 1998b. Rapid genomic changes in newly synthesized amphiploids of *Triticum* and *Aegilops*. I. Changes in low-copy non-coding DNA sequences. *Genome*, 41: 272-277.

Liu B, Wendel JF. 2003. Epigenetic phenomena and the evolution of plant allopolyploids. *Mol Phylogenet Evol*, 29: 365-79.

Liu W, Rouse M, Friebe B, Jin Y, Gill B, Pumphrey MO. 2011. Discovery and molecular mapping of a new gene conferring resistance to stem rust, Sr53, derived from *Aegilops geniculata* and characterization of spontaneous translocation stocks with reduced alien chromatin. *Chromosome Research*, 19: 669-682.

Liu W, Seifers DL, Qi LL, Friebe B, Gill BS. 2011. A compensating wheat-*Thinopyrum intermedium* Robertsonian translocation conferring resistance to *Wheat streak mosaic virus* and *Triticum mosaic virus*. Crop Science 51:2382-2390

López-Moya JJ, Wang RY, Pirone TP. 1999. Context of the coat protein DAG motif affects potyvirus transmissibility by aphids. *Journal of General Virology*, 80: 3281-3288.

Lu H, Price J, Devkota R, Rush C, Rudd J. 2011. A dominant gene for resistance to *Wheat streak mosaic virus* in winter wheat line CO960293-2. *Crop Science*, 51: 5-12.

Luger K, Mäder AW, Richmond RK, Sargent DF, Richmond TJ. 1997. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature*, 389: 251-260.

Lukaszewski AJ. 2000. Manipulation of the 1RS.1BL translocation in wheat by induced homoeologous recombination. *Crop Science*, 40: 216-225.

Lukaszewski AJ, Lapinski B, Rybka K. 2005. Limitations of in situ hybridization with total genomic DNA in routine screening for alien introgressions in wheat. *Cytogenetic and Genome Research,* 109: 373-377.

Luo MC, Deal KR, Akhunov ED, Akhunova AR, Anderson OD, Anderson JA, Blake N, Clegg MT, Coleman-Derr D, Conley EJ, Crossman CC, Dubcovsky J, Gill BS, Gu YQ, Hadam J, Heo HY, Huo N, Lazo G, Ma Y, Matthews DE, McGuire PE, Morrell PL, Qualset CO, Renfro J, Tabanao D, Talbert LE, Tian C, Toleno DM, Warburton ML, You FM, Zhang W, Dvorak J. 2009. Genome comparisons reveal a dominant mechanism of chromosome number reduction in grasses and accelerated genome evolution in *Triticeae*. *Proceedings of the National Academy of Sciences of the United States of America,* 106: 15780-15785.

Ma J, Wing RA, Bennetzen JL, Jackson SA. 2007. Plant centromere organization: a dynamic structure with conserved functions. *Trends in Genetics,* 23: 134-139.

Ma XF, Fang P, Gustafson JP. 2004. Polyploidization-induced genome variation in triticale. *Genome,* 47: 839-48.

Ma XF, Gustafson JP. 2005. Genome evolution of allopolyploids: a process of cytological and genetic diploidization. *Cytogenet Genome Res,* 109: 236-49.

Maeshima K, Hihara S, Eltsov M. 2010. Chromatin structure: Does the 30-nm fibre exist in vivo? *Current Opinion in Cell Biology,* 22: 291-297.

Mangini G, Taranto F, Giove SL, Gadaleta A, Blanco A. 2010. Identification of durum wheat cultivars by a minimum number of microsatellite markers. *Cereal Research Communications,* 38: 155-162.

Manole V, Bazgă B. 2011. The impact of climate changes on agriculture and food security. *Quality - Access to Success,* 12: 93-99.

Martin TJ, Fritz AK, Seifers DL, Shroyer JP. 2007. RonL hard white wheat. *Kansas State Univ., Agricultural Experiment Station and Cooperative Extension Service Release L-926*.

Martin TJ, Harvey TL, Bender CG, Seifers DL. 1984. Control of *Wheat streak mosaic-virus* with vector resistance in wheat. *Phytopathology*, 74: 963-964.

Matsuoka Y. 2011. Evolution of polyploid triticum wheats under cultivation: The role of domestication, natural hybridization and allopolyploid speciation in their diversification. *Plant and Cell Physiology,* 52: 750-764.

Matzke M, Kanno T, Daxinger L, Huettel B, Matzke AJ. 2009. RNA-mediated chromatin-based silencing in plants. *Current Opinion in Cell Biology,* 21: 367-376.

McClintock B. 1984. The significance of responses of the genome to challenge. *Science,* 226: 792-801.

McIntosh RA, Dubcovsky J, Rogers JW, Morris C, Appels R, Xia XC. 2010. Catalogue of gene symbols for wheat: MacGene 2010. http://www.shigen.nig.ac.jp/wheat/komugi/genes/download.jsp.

McIntyre CL, Pereira S, Moran LB, Appels R. 1990. New Secale cereale (rye) DNA derivatives for the detection of rye chromosome segments in wheat. *Genome,* 33: 635-40.

Mckinney HH. 1937. Mosaic diseases of wheat and related cereals. *USDA circular no. 422.*

McNeil JE, French R, Hein GL, Baenziger PS, Eskridge KM. 1996. Characterization of genetic variability among natural populations of *Wheat streak mosaic virus*. *Phytopathology,* 86: 1222-1227.

Melderis A. 1980. *Tribe Triticeae. In: Tutin, T.G., V.H. Heywood, N.A. Burges, D.M. Moore, D.H. Valentine, S.M. Walters and D.A. Webb (eds.) Flora Europaea* Cambridge University Press, Cambridge, UK.

Meštrović N, Plohl M, Mravinac B, Ugarković D. 1998. Evolution of satellite DNAs from the genus Palorus - Experimental evidence for the 'library' hypothesis. *Molecular Biology and Evolution,* 15: 1062-1068.

Mette MF, Kanno T, Aufsatz W, Jakowitsch J, Winden JVD, Matzke MA, Matzke AJM. 2002. Endogenous viral sequences and their potential contribution to heritable virus resistance in plants. *Embo Journal,* 21: 461-469.

Miller JT, Dong F, Jackson SA, Song J, Jiang J. 1998. Retrotransposon-related DNA sequences in the centromeres of grass chromosomes. *Genetics,* 150: 1615-23.

Mirik M, Michels Jr GJ, Kassymzhanova-Mirik S, Elliott NC, Bowling R. 2006. Hyperspectral spectrometry as a means to differentiate uninfested and infested winter wheat by greenbug (*Hemiptera: Aphididae*). *Journal of Economic Entomology,* 99: 1682-1690.

Mochida K, Shinozaki K. 2010. Genomics and bioinformatics resources for crop improvement. *Plant and Cell Physiology,* 51: 497-523.

Morgante M, Hanafey M, Powell W. 2002. Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. *Nature Genetics,* 30: 194-200.

Moyzis RK, Buckingham JM, Cram LS, Dani M, Deaven LL, Jones MD, Meyne J, Ratliff RL, Wu JR. 1988. A highly conserved repetitive DNA sequence, (TTAGGG)(n), present at the telomeres of human chromosomes. *Proceedings of the National Academy of Sciences of the United States of America,* 85: 6622-6626.

Mujeeb-Kazi A, Hettel GP. 1995. Utilising wild grass biodiversity in wheat improvement: 15 years of wide cross research at CIMMYT. *CIMMYT Research Report No. 2. Mexico, DF, CIMMYT*.

Mukai Y, Nakara Y, Yamamoto M. 1993. Simultaneus discrimination of three genomes in hexaploid wheat by multicolor fluorescence in situ hybridization using total genomic and highly repetitive DNA probes. *Genome,* 36: 489-494.

Murat F, Xu JH, Tannier E, Abrouk M, Guilhot N, Pont C, Messing J, Salse J. 2010. Ancestral grass karyotype reconstruction unravels new mechanisms of genome shuffling as a source of plant evolution. *Genome Research,* 20: 1545-1557.

Murata M, Heslop-Harrison JS, Motoyoshi F. 1997. Physical mapping of the 5S ribosomal RNA genes in *Arabidopsis thaliana* by multi-color fluorescence in situ hybridization with cosmid clones. *Plant J,* 12: 31-7.

Mutti JS, Sandhu D, Sidhu D, Gill KS. 2010. Dynamic nature of a wheat centromere with a functional gene. *Molecular Breeding,* 26: 177-187.

Mutti JS, Stephen Baenziger P, Graybosch RA, French R, Gill KS. 2011. Registration of seven winter wheat germplasm lines carrying the wsm1 gene for *Wheat streak mosaic virus* resistance. *Journal of Plant Registrations*, 5: 414-417.

Nagaki K, Kishii M, Tsujimoto H, Sasakuma T. 1999. Tandem repetitive Afa-family sequences from *Leymus racemosus* and *Psathyrostachys juncea* (Poaceae). *Genome*, 42: 1258-1260.

Nagaki K, Tsujimoto H, Isono K, Sasakuma T. 1995. Molecular characterization of a tandem repeat, Afa family, and its distribution among *Triticeae*. *Theor Appl Genet,* 38: 479-486.

Nagaki K, Tsujimoto H, Sasakuma T. 1998a. Dynamics of tandem repetitive Afa-family sequences in *Triticeae*, wheat-related species. *J Mol Evol,* 47: 183-9.

Nagaki K, Tsujimoto H, Sasakuma Y. 1998b. H genome specific repetitive sequence, pEt2, of *Elymus trachycaulus* in part of Afa family of *Triticeae*. *Genome,* 41: 134-136.

Navia D, Truol G, Mendonca RS, Sagadin M. 2006. *Aceria tosichella* Keifer (Acari : Eriophyidae) from *Wheat Streak Mosaic Virus*-infected wheat plants in Argentina. *International Journal of Acarology,* 32: 189-193.

Nellemann C, MacDevette M, Manders T, Eickhout B, Svihus B, Prins AG, Kaltenborn BP. 2009. The environmental food crisis: the environment's role in averting future food crises. A UNEP rapid response assessment.GRID-Arendal: UN Environment Programme. Norway.

Okamoto M. 1957. A synaptic effect of chromosome V *Wheat Inf. Serv,* 5 6.

Oldfield GN. 1970. Mite transmission of plant viruses. *Annual Review of Entomology,* 15: 343-&.

Orgel LE, Crick FH. 1980. Selfish DNA: the ultimate parasite. *Nature,* 284: 604-7.

Otto SP, Whitton J. 2000. Polyploid incidence and evolution.

Ozkan H, Brandolini A, Pozzi C, Effgen S, Wunder J, Salamini F. 2005. A reconsideration of the domestication geography of tetraploid wheats. *Theoretical and Applied Genetics,* 110: 1052-1060.

Ozkan H, Levy AA, Feldman M. 2001. Allopolyploidy-induced rapid genome evolution in the wheat (*Aegilops-Triticum*) group. *Plant Cell,* 13: 1735-47.

Panne D, Müller SA, Wirtz S, Engel A, Bickle TA. 2001. The McrBCx restriction endonuclease assembles into a ring structure in the presence of G nucleotides. *EMBO Journal,* 20: 3210-3217.

Panne D, Raleigh EA, Bickle TA. 1999. The McrBC endonuclease translocates DNA in a reaction dependent on GTP hydrolysis. *Journal of Molecular Biology,* 290: 49-60.

Parra F, Casas A, Peñaloza-Ramírez JM, Cortés-Palomec AC, Rocha-Ramírez V, González-Rodríguez A. 2010. Evolution under domestication: Ongoing artificial selection and divergence of wild and managed *Stenocereus pruinosus* (*Cactaceae*) populations in the Tehuacán Valley, Mexico. *Annals of Botany,* 106: 483-496.

Paterson AH, Bowers JE, Bruggmann R, Dubchak I, Grimwood J, Gundlach H, Haberer G, Hellsten U, Mitros T, Poliakov A, Schmutz J, Spannagl M, Tang H, Wang X, Wicker T, Bharti AK, Chapman J, Feltus FA, Gowik U, Grigoriev IV, Lyons E,

Maher CA, Martis M, Narechania A, Otillar RP, Penning BW, Salamov AA, Wang Y, Zhang L, Carpita NC, Freeling M, Gingle AR, Hash CT, Keller B, Klein P, Kresovich S, McCann MC, Ming R, Peterson DG, Mehboob Ur R, Ware D, Westhoff P, Mayer KFX, Messing J, Rokhsar DS. 2009. The *Sorghum bicolor* genome and the diversification of grasses. *Nature,* 457: 551-556.

Pedersen C, Langridge P. 1997. Identification of the entire chromosome complement of bread wheat by two-colour FISH. *Genome,* 40: 589-593.

Peleg Z, Fahima T, Korol AB, Abbo S, Saranga Y. 2011. Genetic analysis of wheat domestication and evolution under domestication. *Journal of Experimental Botany,* 62: 5051-5061.

Peng JH, Lapitan NLV. 2005. Characterization of EST-derived microsatellites in the wheat genome and development of eSSR markers. *Functional and Integrative Genomics,* 5: 80-96.

Peng JH, Sun D, Nevo E. 2011. Domestication evolution, genetics and genomics in wheat. *Molecular Breeding,* 28: 281-301.

Peng JH, Zadeh H, Lazo GR, Gustafson JP, Chao S, Anderson OD, Qi LL, Echalier B, Gill BS, Dilbirligi M, Sandhu D, Gill KS, Greene RA, Sorrells ME, Akhunov ED, Dvořák J, Linkiewicz AM, Dubcovsky J, Hossain KG, Kalavacharla V, Kianian SF, Mahmoud AA, Miftahudin, Conley EJ, Anderson JA, Pathan MS, Nguyen HT, McGuire PE, Qualset CO, Lapitan NLV. 2004. Chromosome bin map of expressed sequence tags in homoeologous group 1 of hexaploid wheat and homoeology with rice and *Arabidopsis*. *Genetics,* 168: 609-623.

Pérez-Gutiérrez MA, Suárez-Santiago VN, López-Flores I, Romero AT, Garrido-Ramos MA. 2012. Concerted evolution of satellite DNA in *Sarcocapnos*: A matter of time. *Plant Molecular Biology,* 78: 19-29.

Perry DJ. 2004. Identification of Canadian durum wheat varieties using a single PCR. *Theoretical and Applied Genetics,* 109: 55-61.

Pires JC, Zhao J, Schranz ME, Leon EJ, Quijada PA, Lukens L, Osborn TC. 2004. Flowering time divergence and genomic rearrangements in resynthesized polyploids (Brassica). *2004,* 82: 675-688.

Prasad M, Varshney RK, Roy JK, Balyan HS, Gupta PK. 2000. The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. *Theoretical and Applied Genetics,* 100: 584-592.

Price JA, Workneh F, Evett SR, Jones DC, Arthur J, Rush CM. 2010. Effects of *Wheat streak mosaic virus* on root development and water-use efficiency of hard red winter wheat. *Plant Disease,* 94: 766-770.

Prins R, Marais GF, Pretorius ZA, Janse BJH, Marais AS. 1997. A study of modified forms of the Lr19 translocation of common wheat. *Theoretical and Applied Genetics*, 95: 424-430.

Purugganan MD, Fuller DQ. 2009. The nature of selection during plant domestication. *Nature,* 457: 843-848.

Purugganan MD, Fuller DQ. 2011. Archaeological data reveal slow rates of evolution during plant domestication. *Evolution,* 65: 171-183.

Qi L, Echalier B, Friebe B, Gill BS. 2003. Molecular characterization of a set of wheat deletion stocks for use in chromosome bin mapping of ESTs. *Functional and Integrative Genomics,* 3: 39-55.

Qi L, Friebe B, Zhang P, Gill BS. 2007. Homoeologous recombination, chromosome engineering and crop improvement. *Chromosome Research,* 15: 3-19.

Qi L, Friebe B, Zhang P, Gill BS. 2009. A molecular-cytogenetic method for locating genes to pericentromeric regions facilitates a genomewide comparison of synteny between the centromeric regions of wheat and rice. *Genetics,* 183: 1235-1247.

Qi LL, Echalier B, Chao S, Lazo GR, Butler GE, Anderson OD, Akhunov ED, Dvořák J, Linkiewicz AM, Ratnasiri A, Dubcovsky J, Bermudez-Kandianis CE, Greene RA, Kantety R, La Rota CM, Munkvold JD, Sorrells SF, Sorrells ME, Dilbirligi M, Sidhu D, Erayman M, Randhawa HS, Sandhu D, Bondareva SN, Gill KS, Mahmoud AA, Ma XF, Miftahudin, Gustafson JP, Conley EJ, Nduati V, Gonzalez-Hernandez JL, Anderson JA, Peng JH, Lapitan NLV, Hossain KG, Kalavacharla V, Kianian SF, Pathan MS, Zhang DS, Nguyen HT, Choi DW, Fenton RD, Close TJ, McGuire PE, Qualset CO, Gill BS. 2004. A chromosome bin map of 16,000 expressed sequence tag loci and distribution of genes among the three genomes of polyploid wheat. *Genetics*, 168: 701-712.

Rayburn AL, Gill BS. 1986. Isolation of a D-genome specific repeated DNA sequence from *Aegilops squarrosa*. *Plant Molecular Biology Reporter,* 4: 102-109.

Reddy l, Friesen Tl, Meinhardt SW, Chao S, Faris JD. 2008. Genomic analysis of the Snn1 locus on wheat chromosome arm 1BS and the identification of candidate genes. *Plant Genome,* 1: 55–66.

Ribeiro-Carvalho C, Guedes-Pinto H, Igrejas G, Stephenson P, Schwarzacher T, Heslop-Harrison JS. 2004. High levels of genetic diversity throughout the range of the Portuguese wheat landrace 'Barbela'. *Annals of Botany*, 94: 699-705.

Richardson AD, Aikens M, Berlyn GP, Marshall P. 2004. Drought stress and paper birch (Betula Papyrifera) seedlings: Effects of an organic biostimulant on plant health and stress tolerance, and detection of stress effects with instrument-based, noninvasive methods. *Journal of Arboriculture*, 30: 52-61.

Richmond TJ, Finch JT, Rushton B, Rhodes D, Klug A. 1984. Structure of the nucleosome core particle at 7 resolution. *Nature*, 311: 532-537.

Riedell WE, Blackmer TM. 1999. Leaf reflectance spectra of cereal aphid-damaged wheat. *Crop Science*, 39: 1835-1840.

Robinson PJJ, Fairall L, Huynh VAT, Rhodes D. 2006. EM measurements define the dimensions of the "30-nm" chromatin fiber: Evidence for a compact, interdigitated structure. *Proceedings of the National Academy of Sciences of the United States of America*, 103: 6506-6511.

Röder MS, Korzun V, Gill BS, Ganal MW. 1998b. The physical mapping of microsatellite markers in wheat. *Genome*, 41: 278-283.

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW. 1998a. A microsatellite map of wheat. *Genetics*, 149: 2007-2023.

Roder MS, Lapitan NL, Sorrells ME, Tanksley SD. 1993. Genetic and physical mapping of barley telomeres. *Mol Gen Genet*, 238: 294-303.

Ross-Ibarra J, Morrell PL, Gaut BS. 2007. Plant domestication, a unique opportunity to identify the genetic basis of adaptation. *Proceedings of the National Academy of Sciences of the United States of America*, 104: 8641-8648.

Roussel V, Leisova L, Exbrayat F, Stehno Z, Balfourier F. 2005. SSR allelic diversity changes in 480 European bread wheat varieties released from 1840 to 2000. *Theoretical and Applied Genetics*, 111: 162-170.

Saeidi H, Rahiminejad MR, Heslop-Harrison JS. 2008. Retroelement insertional polymorphisms, diversity and phylogeography within diploid, D-genome *Aegilops tauschii* (*Triticeae, Poaceae*) sub-taxa in Iran. *Annals of Botany*, 101: 855-861.

Sáenz P, Cervera MT, Dallot S, Quiot L, Quiot JB, Riechmann JL, García JA. 2000. Identification of a pathogenicity determinant of Plum pox virus in the sequence

encoding the C-terminal region of protein P3+6K1. *Journal of General Virology,* 81: 557-566.

Sakuma S, Salomon B, Komatsuda T. 2011. The domestication syndrome genes responsible for the major changes in plant form in the *Triticeae* crops. *Plant and Cell Physiology,* 52: 738-749.

Salamini F, Özkan H, Brandolini A, Schäfer-Pregl R, Martin W. 2002. Genetics and geography of wild cereal domestication in the Near East. *Nature Reviews Genetics,* 3: 429-441.

Salina EA, Lim KY, Badaeva ED, Shcherban AB, Adonina IG, Amosova AV, Samatadze TE, Vatolina TY, Zoshchuk SA, Leitch AR. 2006. Phylogenetic reconstruction of *Aegilops* section Sitopsis and the evolution of tandem repeats in the diploids and derived wheat polyploids. *Genome,* 49: 1023-1035.

Salina EA, Sergeeva EM, Adonina IG, Shcherban AB, Belcram H, Huneau C, Chalhoub B. 2011. The impact of Ty3-gypsy group LTR retrotransposons Fatima on B-genome specificity of polyploid wheats. *BMC Plant Biology,* 11.

Salmon A, Ainouche ML, Wendel JF. 2005. Genetic and epigenetic consequences of recent hybridization and polyploidy in Spartina (Poaceae). *Mol Ecol,* 14: 1163-75.

Salse J, Chague V, Bolot S, Magdelenat G, Huneau C, Pont C, Belcram H, Couloux A, Gardais S, Evrard A, Segurens B, Charles M, Ravel C, Samain S, Charmet G, Boudet N, Chalhoub B. 2008. New insights into the origin of the B genome of hexaploid wheat: Evolutionary relationships at the SPA genomic region with the S genome of the diploid relative *Aegilops speltoides*. *Bmc Genomics,* 9.

Sanchez-Sanchez H, Henry M, Cardenas-Soriano E, Alvizo-Villasana HF. 2001. Identification of *Wheat streak mosaic virus* and its vector *Aceria tosichella* in Mexico. *Plant Disease,* 85: 13-17.

Sanford JC, Johnston SA. 1985. The concept of parasite-derived resistance: Deriving resistance genes from the parasite's own genome. *Journal of Theoretical Biology,* 113: 395-405.

Schmidt T, Heslop-Harrison JS. 1998a. Genomes, genes and junk: The large-scale organization of plant chromosomes. *Trends in Plant Science,* 3: 195-199.

Schmidt T, Heslop-Harrison JS. 1998b. Genomes, genes and junk: the large-scale organization of plant chromosomes. *Trends Plant Sci,* 3: 195-199.

Scholz M, Ruge-Wehling B, Habeku A, Schrader O, Pendinen G, Fischer K, Wehling P. 2009. Ryd4 Hb: a novel resistance gene introgressed from *Hordeum bulbosum* into barley and conferring complete and dominant resistance to the *Barley yellow dwarf virus*. *Theoretical and Applied Genetics,* 119: 837-849.

Schwarzacher T. 1996. The physical organization of *Triticeae* chromosomes. *The Society of Experimental Biology*: 71-76.

Schwarzacher T. 2003a. Meiosis, recombination and chromosomes: a review of gene isolation and fluorescent *in situ* hybridization data in plants. *Journal of Experimental Botany,* 54: 11-23.

Schwarzacher T. 2003b. DNA, chromosomes, and *in situ* hybridization. *Genome,* 46: 953-962.

Schwarzacher T. 2008. Chromosomes, recombination and proteins at meiosis - A tribute to Peter Moens (1931-2008). *Chromosome Research,* 16: 679-682.

Schwarzacher T, Ali N, Chaudhary HK, Graybosch R, Kapalande HV, Kinski E, Heslop-Harrison JS. 2011. Fluorescent *in situ* hybridization as a genetic technology to analyzing chromosomal organization of alien wheat recombinant lines. *IAEA-TECDOC-1664,* 90: 121-128

Schwarzacher T, Anamthawatjonsson K, Harrison GE, Islam A, Jia JZ, King IP, Leitch AR, Miller TE, Reader SM, Rogers WJ, Shi M, Heslopharrison JS. 1992. Genomic *in situ* hybridization to identify alien chromosomes and chromosome segments in wheat. *Theoretical and Applied Genetics,* 84: 778-786.

Schwarzacher T, Heslop-Harrison JS. 1991. *In situ* hybridization to plant telomeres using synthetic oligomers. *Genome,* 34: 317-323.

Schwarzacher T, Heslop-Harrison. JS. 2000. Practical *in situ* hybridization. *Oxford: Bios*: 203+xii pp.

Schwarzacher T, Leitch AR, Bennett MD, Heslopharrison JS. 1989. *In situ* localization of parental genomes in a wide hybrid. *Annals of Botany,* 64: 315-324.

Sears ER. 1966. Nullisomictetrasomic combinations in hexaploid wheat. In: RILEY R, LEWIS KR eds. *Chromosome manipulations and plant genetics.* Oliver and Boyd.

See DR, Brooks S, Nelson JC, Brown-Guedira G, Friebe B, Gill BS. 2006. Gene evolution at the ends of wheat chromosomes. *Proceedings of the National Academy of Sciences of the United States of America,* 103: 4162-4167.

Sehgal SK, Kaur S, Gupta S, Sharma A, Kaur R, Bains NS. 2011. A direct hybridization approach to gene transfer from *Aegilops tauschii* Coss. to *Triticum aestivum* L. *Plant Breeding,* 130: 98-100.

Seifers DL, Harvey TL, Martin TJ, Jensen SG. 1998. A partial host range of the high plains virus of corn and wheat. *Plant Disease,* 82: 875-879.

Seifers DL, Martin TJ, Harvey TL, Fellers JP, Michaud JP. 2009a. Identification of the Wheat Curl Mite as the Vector of *Triticum mosaic virus*. *Plant Disease,* 93: 25-29.

Seifers DL, Martin TJ, Harvey TL, Gill BS. 1995. Temperature sensitivity and efficacy of *Wheat streak mosaic-virus* resistance derived from *Agropyron intermedium*. *Plant Disease,* 79: 1104-1106.

Seifers DL, Martin TJ, Harvey TL, Haber S. 2007. Temperature-sensitive *Wheat streak mosaic virus* resistance identified in KS03HW12 wheat. *Plant Disease,* 91: 1029-1033.

Seifers DL, Martin TJ, Harvey TL, Haber S, Haley SD. 2006. Temperature sensitivity and efficacy of *Wheat streak mosaic virus* resistance derived from CO960293 wheat. *Plant Disease,* 90: 623-628.

Seifers DL, Martin TJ, Harvey TL, Haber S, Krokhin O, Spicer V, Ying S, Standing KG. 2009b. Identification of Variants of the High Plains virus Infecting Wheat in Kansas. *Plant Disease,* 93: 1265-1274.

Sergeeva EM, Salina EA, Adonina IG, Chalhoub B. 2010. Evolutionary analysis of the CACTA DNA-transposon Caspar across wheat species using sequence comparison and in situ hybridization. *Molecular Genetics and Genomics*, 284: 11-23.

Shaked H, Kashkush K, Ozkan H, Feldman M, Levy AA. 2001. Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. *Plant Cell,* 13: 1749-59.

Shapiro JA. 2005. A 21st century view of evolution: Genome system architecture, repetitive DNA, and natural genetic engineering. *Gene,* 345: 91-100.

Sharma HC, Gill BS. 1983. Current status of wide hybridization in wheat. *Euphytica*, 32: 17-31.

Sharma S, Bhat PR, Ehdaie B, Close TJ, Lukaszewski AJ, Waines JG. 2009. Integrated genetic map and genetic analysis of a region associated with root traits on the

short arm of rye chromosome 1 in bread wheat. *Theoretical and Applied Genetics*, 119: 783-793.

Sharp GL, Martin JM, Lanning SP, Blake NK, Brey CW, Sivamani E, Qu R, Talbert LE. 2002. Field evaluation of Transgenic and classical sources of W*heat streak mosaic virus* resistance. *Crop Science,* 42: 105-110.

Sibikeeva YE, Sibikeev SN, Krupnov VA. 2004. The effect of Lr19-translocation on in vitro androgenesis and inheritance of leaf-rust resistance in DH3 lines and F2 hybrids of common wheat. *Russian Journal of Genetics,* 40: 1003-1006.

Singal R, Ginder GD. 1999. DNA methylation. *Blood,* 93: 4059-70.

Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Njau P, Wanyera R, Herrera-Foessel SA, Ward RW. 2008. Will stem rust destroy the world's wheat crop?

Singh RP, Mujeeb-Kazi A, Huerta-Espino J. 1998. *Lr46*: A gene conferring slow-rusting resistance to leaf rust in wheat. *Phytopathology,* 88: 890-894.

Sivamani E, Brey CW, Dyer WE, Talbert LE, Qu R. 2000. Resistance to *Wheat streak mosaic virus* in transgenic wheat expressing the viral replicase (NIb) gene. *Molecular Breeding,* 6: 469-477.

Sivamani E, Brey CW, Talbert LE, Young MA, Dyer WE, Kaniewski WK, Qu R. 2002. Resistance to *Wheat streak mosaic virus* in transgenic wheat engineered with the viral coat protein gene. *Transgenic Research,* 11: 31-41.

Slotkin RK, Martienssen R. 2007. Transposable elements and the epigenetic regulation of the genome. *Nature Reviews Genetics,* 8: 272-285.

Slotkin RK, Vaughn M, Borges F, Tanurdžić M, Becker JD, Feijó JA, Martienssen RA. 2009. Epigenetic Reprogramming and Small RNA Silencing of Transposable Elements in Pollen. *Cell,* 136: 461-472.

Slykhuis JT. 1955. *Aceria tulipae* Keifer (Acarina: Eryophydae) in relation to the spread of *Wheat streak mosaic*. *Phytopathology* 45: 116-128.

Smith DB, Flavell RB. 1975. Characterisation of the wheat genome by renaturation kinetics. *Chromosoma,* 50: 223-242.

Soltis DE, Albert VA, Leebens-Mack J, Bell CD, Paterson AH, Zheng C, Sankoff D, DePamphilis CW, Wall PK, Soltis PS. 2009. Polyploidy and angiosperm diversification. *American Journal of Botany,* 96: 336-348.

Soltis DE, Soltis PS. 1999. Polyploidy: recurrent formation and genome evolution. *Trends in Ecology and Evolution,* 14: 348-352.

Soltis PS, Soltis DE. 2000. The role of genetic and genomic attributes in the success of polyploids. *Proc Natl Acad Sci U S A,* 97: 7051-7.

Somers DJ, Isaac P, Edwards K. 2004. A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics,* 109: 1105-1114.

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB. 2005. Development and mapping of microsatellite (SSR) markers in wheat. *Theoretical and Applied Genetics,* 110: 550-560.

Sourdille P, Singh S, Cadalen T, Brown-Guedira GL, Gay G, Qi L, Gill BS, Dufour P, Murigneux A, Bernard M. 2004a. Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). *Functional and Integrative Genomics,* 4: 12-25.

Sourdille P, Tavaud M, Charmet G, Bernard M. 2001. Transferability of wheat microsatellites to diploid *Triticeae* species carrying the A, B and D genomes. *Theoretical and Applied Genetics,* 103: 346-352.

Spetz C, Valkonen JPT. 2004. Potyviral 6K2 protein long-distance movement and symptom-induction functions are independent and host-specific. *Molecular Plant-Microbe Interactions,* 17: 502-510.

Stein N. 2007. *Triticeae* genomics: Advances in sequence analysis of large genome cereal crops. *Chromosome Research,* 15: 21-31.

Stenger D, Young B, Qu F, Morris T, French R. 2007a. *Wheat streak mosaic virus* P1, not HC-Pro, facilitates disease synergism and suppression of post-transcriptional gene silencing. *Phytopathology,* 97: S111-S111.

Stenger DC, Hall JS, Choi IR, French R. 1998. Phylogenetic relationships within the family Potyviridae: *Wheat streak mosaic virus* and *Brome streak mosaic virus* are not members of the genus *Rymovirus*. *Phytopathology,* 88: 782-787.

Stenger DC, Seifers DL, French R. 2002. Patterns of polymorphism in *Wheat streak mosaic virus*: Sequence space explored by a Clade of closely related viral genotypes rivals that between the most divergent strains. *Virology,* 302: 58-70.

Stenger DC, Young BA, Qu F, Morris TJ, French R. 2007b. *Wheat streak mosaic virus* lacking helper component-proteinase is competent to produce disease synergism in double infections with Maize chlorotic mottle virus. *Phytopathology,* 97: 1213-1221.

Stewart FJ, Panne D, Bickle TA, Raleigh EA. 2000. Methyl-specific DNA binding by *Mcr*BC, a modification-dependent restriction enzyme. *Journal of Molecular Biology*, 298: 611-622.

Stewart FJ, Raleigh EA. 1998. Dependence of *Mcr*BC cleavage on distance between recognition elements. *Biological Chemistry*, 379: 611-616.

Stokstad E. 2007. Plant pathology: deadly wheat fungus threatens world's breadbaskets *Science*, 315: 1786-1787.

Stukenbrock EH, McDonald BA. 2008. The origins of plant pathogens in agro-ecosystems.

Sutton T, Whitford R, Baumann U, Dong C, Able JA, Langridge P. 2003. The Ph2 pairing homoeologous locus of wheat (*Triticum aestivum*): Identification of candidate meiotic genes using a comparative genetics approach. *Plant Journal*, 36: 443-456.

Suzuki MM, Bird A. 2008. DNA methylation landscapes: Provocative insights from epigenomics. *Nature Reviews Genetics*, 9: 465-476.

Takeda S, Matsuoka M. 2008. Genetic approaches to crop improvement: Responding to environmental and population changes. *Nature Reviews Genetics*, 9: 444-457.

Taketa S, Ando H, Takeda K, Harrison GE, Heslop-Harrison JS. 2000. The distribution, organization and evolution of two abundant and widespread repetitive DNA sequences in the genus *Hordeum*. *Theoretical and Applied Genetics*, 100: 169-176.

Talbert LE, Bruckner PL, Smith LY, Sears R, Martin TJ. 1996. Development of PCR markers linked to resistance to *Wheat streak mosaic virus* in wheat. *Theoretical and Applied Genetics*, 93: 463-467.

Tamura K. 1992. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content biases. *Molecular Biology and Evolution*, 9: 678-687.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28: 2731-2739.

Tang ZX, Fu SL, Ren ZL, Zhang T, Zou YT, Yang ZJ, Li GR, Zhou JP, Zhang HQ, Yan BJ, Zhang HY, Tan FQ, Gustafson P. 2011. Diversity and evolution of four dispersed repetitive DNA sequences in the genus *Secale*. *Genome*, 54: 285-300.

Tatineni S, Graybosch RA, Hein GL, Wegulo SN, French R. 2010. Wheat cultivar-specific disease synergism and alteration of virus accumulation during co-infection with *Wheat streak mosaic* virus and *Triticum mosaic virus*. *Phytopathology,* 100: 230-238.

Tatineni S, Van Winkle DH, French R. 2011. The N-terminal region of *Wheat streak mosaic virus* coat protein is a host- and strain-specific long-distance transport factor. *Journal of Virology,* 85: 1718-1731.

Thomas JA, Hein GL. 2003. Influence of volunteer wheat plant condition on movement of the wheat curl mite, *Aceria tosichella*, in winter wheat. *Experimental and Applied Acarology,* 31: 253-268.

Thomas JB, Conner RL, Graf RJ. 2004. Comparison of different sources of vector resistance for controlling *Wheat streak mosaic* in winter wheat. *Crop Science*, 44: 125-130.

Todorovska E, Abumhadi N, Kamenarova K, Jeleva D, Kostova A, Christov N, Alexandrova N, Jacquemin JM, Anzai H, Nakamura C, Atanassov A. 2005. Biotechnological approaches for cereal crops improvement part II: Use of molecular markers in cereal breeding. *Biotechnology and Biotechnological Equipment,* 19: 91-104.

Todorovska EG, Alexandrova NA, Marinova EI, Atanassov AI. 2001. Application of molecular markers in Bulgarian breeding programmes of cereals. *Biotechnology and Biotechnological Equipment,* 15: 14-16.

Tsitsin NV. 1965. Remote hybridisation as a method of creating new species and varieties of plants. *Euphytica,* 14: 326-330.

Tsujimoto H, Mukai Y, Akagawa K, Nagaki K, Fujigaki J, Yamamoto M, Sasakuma T. 1997. Identification of individual barley chromosomes based on repetitive sequences: Conservative distribution of Afa-family repetitive sequences on the chromosomes of barley and wheat. *Genes & Genetic Systems,* 72: 303-309.

Tsukahara S, Kobayashi A, Kawabe A, Mathieu O, Miura A, Kakutani T. 2009. Bursts of retrotransposition reproduced in *Arabidopsis*. *Nature,* 461: 423-426.

Turner BM. 2009. Epigenetic responses to environmental change and their evolutionary implications. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 364: 3403-3418.

Turner BM, Birley AJ, Lavender J. 1992. Histone H4 isoforms acetylated at specific lysine residues define individual chromosomes and chromatin domains in *Drosophila* polytene nuclei. *Cell,* 69: 375-384.

Van de Wouw M, van Hintum T, Kik C, van Treuren R, Visser B. 2010. Genetic diversity trends in twentieth century crop cultivars: A meta analysis. *Theoretical and Applied Genetics,* 120: 1241-1252.

Varshney RK, Dubey A. 2009. Novel genomic tools and modern genetic and breeding approaches for crop improvement. *Journal of Plant Biochemistry and Biotechnology,* 18: 127-138.

Vaughan DA, Balázs E, Heslop-Harrison JS. 2007. From crop domestication to super-domestication. *Annals of Botany,* 100: 893-901.

Velandia M, Rejesus RM, Jones DC, Price JA, Workneh F, Rush CM. 2010. Economic impact of *Wheat streak mosaic virus* in the Texas High Plains. *Crop Protection,* 29: 699-703.

Vershinin A, Svitashev S, Gummesson PO, Salomon B, von Bothmer R, Bryngelsson T. 1994. Characterization of a family of tandemly repeated DNA sequence in *Triticeae*. *Theor Appl Genet,* 89: 217-225.

Vershinin AV, Alkhimova EG, Heslop-Harrison JS. 1996. Molecular diversification of tandemly organized DNA sequences and heterochromatic chromosome regions in some *Triticeae* species. *Chromosome Res,* 4: 517-25.

Vershinin AV, Heslop-Harrison JS. 1998. Comparative analysis of the nucleosomal structure of rye, wheat and their relatives. *Plant Mol Biol,* 36: 149-61.

Vershinin AV, Schwarzacher T, Heslop-Harrison JS. 1995. The large-scale genomic organization of repetitive DNA families at the telomeres of rye chromosomes. *Plant Cell,* 7: 1823-1833.

Vogel JPGarvin DFMockler TCSchmutz JRokhsar DBevan MWBarry KLucas SHarmon-Smith MLail KTice HGrimwood JMcKenzie NHuo NXGu YQLazo GRAnderson ODYou FMLuo MCDvorak JWright JFebrer MIdziak DHasterok RLindquist EWang MFox SEPriest HDFilichkin SAGivan SABryant DWChang JHWu HYWu WHsia APSchnable PSKalyanaraman ABarbazuk BMichael TPHazen SPBragg JNLaudencia-Chingcuanco DWeng YQHaberer GSpannagl MMayer KRattei TMitros TLee SJRose JKCMueller LAYork TLWicker TBuchmann JPTanskanen JSchulman AHGundlach Hde Oliveira ACMaia LDBelknap WJiang NLai JSZhu LCMa JXSun CPritham ESalse JMurat

FAbrouk MBruggmann RMessing JFahlgren NSullivan CMCarrington JCChapman EJMay GDZhai JXGanssmann MGurazada SGRGerman MMeyers BCGreen PJTyler LWu JJThomson JChen SScheller HVHarholt JUlvskov PKimbrel JABartley LECao PJJung KHSharma MKVega-Sanchez MRonald PDardick CDDe Bodt SVerelst WInze DHeese MSchnittger AYang XHKalluri UCTuskan GAHua ZHVierstra RDCui YOuyang SHSun QXLiu ZYYilmaz AGrotewold ESibout RHematy KMouille GHofte HPelloux JO'Connor DSchnable JRowe SHarmon FCass CLSedbrook JCByrne MEWalsh SHiggins JLi PHBrutnell TUnver TBudak HBelcram HCharles MChalhoub BBaxter I, Int Brachypodium I. 2010. Genome sequencing and analysis of the model grass *Brachypodium distachyon*. *Nature,* 463: 763-768.

Wagner I, Capesius I. 1981. Determination of 5-methylcytosine from plant DNA by high-performance liquid chromatography. *Biochem Biophys Acta,* 654: 52-56.

Wang L, Ma J, Zhou R, Wang X, Jia J. 2002. Molecular tagging of the yellow rust resistance gene Yr10 in common wheat, P.I.178383 (*Triticum aestivum* L.). *Euphytica,* 124: 71-73.

Wang MJ, Zhang Y, Lin ZS, Ye XG, Yuan YP, Ma W, Xin ZY. 2010. Development of EST-PCR markers for *Thinopyrum intermedium* chromosome 2Ai#2 and their application in characterization of novel wheat-grass recombinants. *Theoretical and Applied Genetics,* 121: 1369-1380.

Waterhouse PM, Wang MB, Finnegan EJ. 2001. Role of short RNAs in gene silencing. *Trends Plant Sci,* 6: 297-301.

Wegulo SN, Hein GL, Klein RN, French RC, . 2008. Managing *Wheat streak mosaic*. University of Nebraska - Lincoln Extension. EC1871.

Wells DG, Kota RS, Sandhu HS, Gardner WS, Finney KF. 1982. Registration of one disomic substitution line and 5 translocation lines of winter-wheat germplasm resistant to *Wheat streak mosaic-virus*. *Crop Science,* 22: 1277-1278.

Wells DG, Wong RSC, Lay CL, Gardner WS, Buchenau GW. 1973. Registration of CI 15092 and CI 15093 wheat germplasm. *Crop Science,* 13: 776-776.

Wendel JF. 2000. Genome evolution in polyploids. *Plant Mol Biol,* 42: 225-49.

Wendel JF, Schnabel A, Seelanan T. 1995. Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). *Proc Natl Acad Sci U S A,* 92: 280-4.

Wendel JF, Wessler SR. 2000. Retrotransposon-mediated genome evolution on a local ecological scale. *Proc Natl Acad Sci U S A,* 97: 6250-2.

Wessler SR. 2006. Transposable elements and the evolution of eukaryotic genomes. *Proceedings of the National Academy of Sciences of the United States of America,* 103: 17600-17601.

West JS, Bravo C, Oberti R, Lemaire D, Moshou D, McCartney HA. 2003. The Potential of Optical Canopy Measurement for Targeted Control of Field Crop Diseases.

Wicker T, Guyot R, Yahiaoui N, Keller B. 2003. CACTA transposons in *Triticeae*. A diverse family of high-copy repetitive elements. *Plant Physiology,* 132: 52-63.

Williams PC. 1993. *The world of wheat. In: Grains and Oilseeds:Handling, Marketing, Processing*, Canadian International Grains Institute, Winnipeg, Canada.

Wilson GG, Murray NE (1991) Restriction and modification systems. *Annual Review of Genetics* 25:585-627.

Wu HK, Chung MC, Wu TY, Ning CN, Wu R. 1991. Localization of specific repetitive DNA sequences in individual rice chromosomes. *Chromosoma,* 100: 330-8.

Wu J, Yamagata H, Hayashi-Tsugane M, Hijishita S, Fujisawa M, Shibata M, Ito Y, Nakamura M, Sakaguchi M, Yoshihara R, Kobayashi H, Ito K, Karasawa W, Yamamoto M, Saji S, Katagiri S, Kanamori H, Namiki N, Katayose Y, Matsumoto T, Sasaki T. 2004. Composition and structure of the centromeric region of rice chromosome 8. *Plant Cell,* 16: 967-976.

Wu T, Wang Y, Wu R. 1994. Transcribed repetitive DNA sequences in telomeric regions of rice (*Oryza sativa*). *Plant Mol Biol,* 26: 363-75.

Xu Y, Zhong L, Wu X, Fang X, Wang J. 2009. Rapid alterations of gene expression and cytosine methylation in newly synthesized *Brassica napus* allopolyploids. *Planta*, 229: 471-483.

Xue S, Zhang Z, Lin F, Kong Z, Cao Y, Li C, Yi H, Mei M, Zhu H, Wu J, Xu H, Zhao D, Tian D, Zhang C, Ma Z. 2008. A high-density intervarietal map of the wheat genome enriched with markers derived from expressed sequence tags. *Theoretical and Applied Genetics,* 117: 181-189.

Yaakov B, Kashkush K. 2010. Massive alterations of the methylation patterns around DNA transposons in the first four generations of a newly formed wheat allohexaploid. *Genome,* 54: 42-49.

Yang AS, Estécio MR, Doshi K, Kondo Y, Tajara EH, Issa JP. 2004. A simple method for estimating global DNA methylation using bisulfite PCR of repetitive DNA elements. *Nucleic Acids Research,* 32.

Yen C, Yang JL. 2009. Historical review and prospect of taxonomy of tribe *Triticeae* Dumortier (Poaceae). *Breeding Science,* 59: 513-518.

Yoder JA, Walsh CP, Bestor TH. 1997. Cytosine methylation and the ecology of intragenomic parasites. *Trends in Genetics,* 13: 335-340.

Young A. 1999. Is there really spare land? A critique of estimates of available cultivable land in developing countries. *Environment, Development and Sustainability,* 1: 3-18.

Zeder MA. 2008. Domestication and early agriculture in the Mediterranean Basin: Origins, diffusion, and impact. *Proceedings of the National Academy of Sciences of the United States of America,* 105: 11597-11604.

Zhan J, Mundt CC, Hoffer ME, McDonald BA. 2002. Local adaptation and effect of host genotype on the rate of pathogen evolution: An experimental test in a plant pathosystem. *Journal of Evolutionary Biology,* 15: 634-647.

Zhang HB, Dvorak J. 1990. Isolation of repeated DNA sequences from *Lophopyrum elongatum* for detection of *Lophopyrum* chromatin in wheat genomes. *Genome,* 33: 283-293.

Zhang X, Jiang N, Feschotte C, Wessler SR. 2004. PIF- and Pong-Like Transposable Elements: Distribution, Evolution and Relationship with Tourist-Like Miniature Inverted-Repeat Transposable Elements. *Genetics,* 166: 971-986.

Zhang Z, Shao H, Xu P, Hu M, Song W, Hu X. 2009. Focus on agricultural biotechnology: Prospective for bio-watersaving theories and their applications in the semi-arid and arid areas. *African Journal of Biotechnology*, 8: 2779-2789.

Zhang ZY, Wang LL, Xin ZY, Lin ZS. 2002. Development of new PCR markers specific to a *Thinopyrum intermedium* chromosome 2Ai-2 and cloning of the St-specific sequences. *Acta Genetica Sinica,* 29: 627-633.

Zohary D, Hopf M. 2000. *Domestication of plants in the old world*, Oxford University Press, Oxford.