DETAILED MOLECULAR CHARACTERIZATION OF THE ‘*Ph1* GENE REGION’ OF WHEAT (***Triticum aestivum* L.**)

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation/thesis of GAGANPREET K SIDHU find it satisfactory and recommend that it be accepted.

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Chair

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DETAILED MOLECULAR CHARACTERIZATION OF THE '*Ph1* GENE REGION' OF WHEAT (***Triticum aestivum* L.**)

Abstract

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Common wheat (*Triticum aestivum* L.) is an allohexaploid and consists of three similar genomes A, B and D. Wheat has a large genome size (16 million kb/haploid genome) but nearly 85% of the genome is repetitive DNA. The wheat genome thus can be divided into gene-rich and gene-poor regions. The gene-rich region present around fraction length 0.5 of the long arm of the group 5 chromosomes contains, along with many other important genes, the major chromosome pairing regulator gene, *Ph1*. The *Ph1* regulates bivalent behavior of allohexaploid wheat by allowing only homologous chromosomes to pair with each other during meiosis. To physically characterize this gene-rich region with a special focus on *Ph1*, wheat ESTs and other group 5 DNA markers were mapped on aneuploids and *Ph1* mutants to generate a high-density physical map for the region. The markers mapped to the '5L0.5 region' were compared across the Triticeae to identify agronomically important genes. *Ph1* has been localized to the '*Ph1* gene region' encompassed by the breakpoints of the terminal deletion line 5BL-1 on the proximal side and distal breakpoint of the *Ph1* gene mutant line *ph1c* on the distal side. Seven markers mapped in the '*Ph1* gene region' identified orthologous sequences on an approximately 450 KB region on rice (*Oryza sativa*) chromosome 9, revealing high synteny. Colinearity between the two

species was further confirmed by the same marker order for the '*Ph1* gene region'. Twenty-six genes were selected for their putative *Ph1* like function based on the presence of functional motifs and domains in 91 genes, present in the 450 kb region of rice including the genes involved in chromatin reorganization and microtubule attachment as key candidates for *Ph1*. The probes mapped to the '*Ph1* gene region' were used to identify positive BAC clones in barley (*Hordeum vulgare*) and tetraploid wheat (*Triticum turgidum* var *durum*), using Barley BAC library (*Hordeum vulgare* cv. Morex) and tetraploid wheat (*Triticum turgidum* var *durum* cv Langdon) BAC library. These probes identified 301 and 46 clones in the tetraploid wheat and barley BAC libraries, respectively.

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Dedication

This thesis is dedicated to my mentors,
friends and family for their guidance,
support and love

CHAPTER ONE

INTRODUCTION

1.1. Wheat Genome:

Common wheat (*Triticum aestivum* L.) belongs to grass family Poaceae, which includes other major crop plants such as oat (*Avena sativa*), barley (*Hordeum vulgare*), maize (*Zea mays*) and rice (*Oryza sativa*). The species in this family vary greatly for genome sizes, chromosome number and ploidy level: for example diploid rice (415 Mb) and hexaploid bread wheat (16,000 Mb) have approximately 40-fold difference for their genome size (Arumugunathan and Earle, 1991).

Bread wheat is an allohexaploid (2n=6x=42, AABBDD) and consists of three homoeologous genomes (Sears, 1954), originated from hybridization of three diploid progenitors. Identification of the wheat progenitors has largely come from the cytogenetic analysis of pairing studies between diploid by polyploid crosses. The donors of the A and D genome have been identified as *Triticum monococcum* (Sax, 1922; Dvorak et al., 1988) and *Triticum tauschii* (Kihara, 1944) respectively. The putative donor of B genome is *Aegilops speltoides*. The 21 chromosomes of the haploid set of *T. aestivum* have been classified into seven groups containing three homoeologous chromosomes each from the three genomes based on their ability to compensate for each other in nullisomic-tetrasomic combinations (Sears, 1966). This compensating ability of chromosomes from different ancestral origin defined their genomic relationship. The genetic conservation between these homoeologous chromosomes was further confirmed by several studies. Shephard et al in 1968 demonstrated that glutenin storage protein loci are present on chromosomes 1A, 1B and 1D. Deletion map position of a large number of wheat loci detected by EST unigenes (wheat.pw.usda.gov/NSF/progress_mapping.html) made it

possible to obtain a detailed picture of homoeologous relationships between three wheat genomes.

In spite of the high synteny between homoeologous chromosomes of bread wheat, it behaves as a strict diploid with 21 bivalents at metaphase stage in meiosis.

1.2. *Ph1* Gene:

The bivalent behavior of allohexaploid wheat is principally regulated by *Ph1*, as in its presence only homologous chromosomes can pair with each other. The gene is located on the long arm of chromosome 5B (Riley and Chapman, 1958; Sears and Okamoto, 1958). The *Ph1* gene was further mapped to a submicroscopic region (*Ph1* gene region) flanked by the breakpoints of 5BL-1 and *ph1c* (Gill et al., 1991). A marker 'xksuS1' was also identified for the '*Ph1* gene region' (Gill et al., 1991). In addition to *Ph1*, two other genes that suppress homoeologous chromosome pairing have been identified. These include *Ph2* gene present on chromosome arm 3DS (Upadhaya and Swaminathan, 1967) and another gene present on chromosome arm 3AS (Driscoll, 1972; Mello-sampayo and Canes, 1973).

Homology search during meiosis is more complicated in allopolyploids such as wheat, where three related yet distinct genomes (A, B and D) exist together in the same cell. Thus, in allopolyploid species, chromosome pairing has to overcome an additional problem, because solely, on the basis of homology, each chromosome has more than one potential partner. There have been several speculations as to how the *Ph1* gene regulates the bivalent behavior of wheat.

1.3. Mode of Action of *Ph1* gene:

Two different perhaps contradictory hypotheses have been proposed for the mode of action of *Ph1* gene: One hypothesis assumes that pairing happens in two stages. The chromosomes are premeiotically aligned during the interphase and then they pair at zygotene in the second stage. This hypothesis assumes that *Ph1* determines the premeiotic alignment of homologous and homoeologous chromosomes by separating them spatially from each other (homologous from homoeologous) and thus leads to strict homologous pairing. This is known as presynaptic hypothesis (Feldman 1966, 1993; Feldman and Avivi, 1988). The second hypothesis (Synaptic hypothesis) assumes that *Ph1* gene affects synapsis and crossing-over at meiotic prophase (Holm and Wang 1988, Dubcovsky et al. 1995, Luo et al., 1996). This hypothesis believes that *Ph1* gene affects the stringency of synapsis.

The first hypothesis believes that *Ph1* regulates arrangement of chromosomes in the cell by affecting the components like centromeres and microtubules that affect positioning of the chromosomes in the nucleus. Several studies have shown that *Ph1* affects dynamics of microtubule system (Avivi et al, 1970a, b; Avivi and Feldman, 1973; Ceoloni et al, 1984; Gualandi et al, 1984). These studies showed that the microtubule system of *Ph1* gene deficient plants is more sensitive to antimicrotubule drugs. Feldman in 1966 studied the affect of extra doses of *Ph1* gene on chromosome pairing in wheat plants carrying four (diisosomic 5BL) and six doses (triisosomic 5BL) of *Ph1* gene. In this study, extra doses of *Ph1* gene were observed to cause partial asynapsis of homologus, pairing between homoeologous and bivalent interlocking. In another study by Vega and Feldman, 1998, it was shown that *Ph1* gene affects microtubule-centromere interactions. In this study, the effect of this gene on types and rates of centromere division of univalents was observed at meiosis using monosomic lines. In the absence of *Ph1*, the

frequency of transverse breakage of the centromere of univalent chromosomes drastically reduced during meiosis as compared to plants with the *Ph1* gene.

Mikhailova et al., in 1998, demonstrated that *Ph1* gene affects chromatin organization and meiotic chromosome pairing. The behavior and morphology of two 5RL rye telosomes were studied in wheat background in the presence and absence of *Ph1* and it was observed that in plants without functional copies of *Ph1*, the rye telosomes displayed aberrant morphology, disturbed pre-meiotic associations and reduced and irregular pairing during zygotene and pachytene, In interphase nuclei of *ph1bph1b* plants, the rye (*Secale cereale* L.) telosomes showed heavily distorted morphology as well as intermingling with wheat chromosomes as compared to their distinct and parallel oriented domains in *Ph1Ph1* plants.

A study by Holm and Wang in 1988 lead to the proposal that *Ph1* affects both synapsis and crossing-over. In wheat plants lacking *Ph1*, an increased number of multivalents were observed at early zygotene in allohexaploid wheat, wheat haploids and wheat x rye hybrids. Extra doses of *Ph1* reduced the degree of synapsis. It also showed that the arrest of synapsis before completion affects the interlocking frequency at metaphase-I. This study thus supported the second hypothesis (Synaptic hypothesis) for mode of action of *Ph1* gene.

Despite the many reports on the mode of action of *Ph1* gene, how it regulates chromosome pairing is still a matter of debate.

1.4. Wheat Aneuploids:

Aneuploid stocks in wheat were largely developed in Chinese Spring (Sears 1954, 1966) to map various protein, DNA and morphological markers to different chromosomes. These included nullsomic-tetrasomic lines (in which a pair of missing chromosomes is compensated by

a pair of homoeologous chromosomes), chromosome addition lines, lines carrying isochromosomes and telocentric chromosomes etc. These stocks are highly valuable for localization of genes on different chromosomes as well as chromosomal arms. Endo in 1988 reported another powerful tool to systematically develop anueploid stocks in wheat, which will allow the sub-arm localization of different genes using a gametocidal chromosome of *Aegilops cylindrica.* When a certain chromosome from *A. cylendrica* was present in 'Chinese Spring' in monosomic condition, the gametes lacking the *A. cylendrica* chromosome underwent chromosomal breaks generating various chromosomal aberrations including deletions. Such deletions in plants were stably transmitted to next generation. Endo and Gill, 1996 successfully employed this method to generate 436 deletion lines in wheat across all 21 chromosomes, using two alien monosomic addition lines (one carrying *A. cylendrica* chromosome (Endo, 1988) homoeologous to group 2 of wheat and the other carrying *Aegilops triuncialis* chromosome) and one alien monosomic translocation line (this line had a modified 2B wheat chromosome as a small chromosomal segment from *A. speltoides* was translocated to the end of the long arm) of 'Chinese Spring'. These deletion stocks were different from each other in various morphological, physiological and biochemical traits. The deletion lines were distinguished from each other based on chromosome carrying deletion and the size of deletion identified by C-banding (Endo and Gill, 1996). The deletion lines were assigned FL (fraction length) values based on the size of deletion: for example deletion line 5BL-1 (FL-0.55) is deletion on the long arm of 5B chromosome such that 55 % of the long is present and 45 % is deleted compared to normal 5B chromosome. These aneuploid stocks have been extensively used in genetic mapping in wheat.

1.5. Genetic Mapping in Wheat:

Two types of maps have been generated in Wheat: Physical maps and Genetic linkage maps: Physical maps in Wheat have been generated by mapping 8,241 Expressed sequence tags (ESTs) on aneuploid stocks of Wheat http://wheat.pw.usda.gov/NSF) chromosomes which included a set of deletion, ditelosomic, and nullisomic-tetrasomic lines. Gill et al in 1991 generated physical map of group 5 chromosomes using *A. squarrosa* genomic library DNA clones on CS, group five nulli-tetras and *Ph1* gene mutant lines. Six probes were mapped on homoeologous group 5 chromosomes to target fine mapping of *Ph1* gene (Gill et al., 1993a).

Detailed physical maps have been generated for all seven homoeologous groups by mapping 944 expressed sequence tags (ESTs) generating 2212 EST loci on homoeologous group 1 chromosomes (Peng et al, 2004), total of 2600 loci generated from 1110 ESTs to group 2 chromosomes (Conley et al, 2004), 996 chromosome bin-mapped expressed sequence tags (ESTs) accounting for 2266 restriction fragments (loci) on the homoeologous group 3 chromosomes (Munkvold et al, 2004), 1918 loci detected by the hybridization of 938 expressed sequence tag unigenes (ESTs) on homoeologous group 4 (Miftahudin et al, 2004), 2338 loci mapped with 1052 EST probes on group 5 (Linkiewicz et al, 2004), 882 ESTs, which detected 2043 loci on homoeologous group 6 chromosomes (Randhawa et al., 2004), 919 EST clones detecting 2148 loci onto group 7 chromosomes of wheat (Hossain et al, 2004).

Another approach using gamma rays to generate chromosomal breaks has also been used to generate physical maps in Wheat. Radiation hybrid (RH) maps are developed on the basis of chromosomal breaks generated by radiation and then by analyzing the chromosome segment retention or loss using molecular markers. The use of this approach have been recently reported in wheat by Hossain et al. in 2004, to map 39 molecular marker loci to chromosome 1D in

alloplasmic (lo) durum wheat (Durum wheat (AABB) carrying 1D chromosome of *T. aestivum*, carrying the species cytoplasm-specific *scs*^{ae} gene. This line was unique as the 1D chromosome always segregated as a whole without recombination, which makes it impossible to map the gene with conventional genome mapping. Gamma rays were used to develop mapping population in hemizygous *lo-scs*^{ae} line and this population was analyzed with 39 markers and it revealed 88 breaks in the chromosome. This analysis enabled the authors to localize *scs*^{ae} gene on long arm of 1D and identify 8 linked markers.

Genetic linkage maps have been constructed in wheat and its wild relatives. RFLP (Restriction Fragment Length Polymorphism) based genetic linkage maps have been generated in the diploid progenitor species of wheat. Boyko et al. in 1999 constructed a genetic linkage map of the *A. Tauschii* (DD) composed of 546 loci. High-density genetic linkage map of *A. tauschii* was generated by mapping 249 marker loci (Boyko et al., 2002). A genetic map of diploid wheat, *Triticum monococcum* (AA), involving 335 markers, including RFLP DNA markers, isozymes, seed storage proteins, rRNA, and morphological loci, was constructed (Dubcovsky et al., 1996) Genetic linkage maps have also been constructed in tetraploid wheats. The molecular map of wild emmer wheat, *Triticum dicoccoides* (AABB) was constructed by mapping 549 marker loci on F2 population developed by using *T. dicoccoides* accession, Hermon H52 and *Triticum turgidum* cv, Langdon (Ldn) as parents (Peng et al., 2000). Detailed RFLP linkage maps for hexaploid wheat have been reported for all seven homoeologous groups linkage maps (Erayman et al., 2004). Microsatellite markers have also been used to generate a genetic linkage map in wheat (Roder et al., 1998). In this study, a total of 279 loci amplified by 230 primer sets were mapped in the reference population of the International Triticeae Mapping Initiative (ITMI) Opata 85 x W7984.

The physical maps generated in wheat have been compared with genetic linkage maps across the *Triticeae* via common markers to generate composite map for all wheat chromosomes (Werner et al. 1992; Gill et al. 1996b, Gill et al. 1996c; Kota et al. 1993; Delanay et al. 1995a, Delanay et al. 1995b; Mickelson-young et al. 1995; Weng et al. 2000), which have shown that genes are nonuniformly distributed on wheat chromosomes.

Comparisons of deletion line-based physical maps with the genetic linkage maps showed that most of recombination occurs in the gene-rich regions of the wheat genome (Gill et al., 1996b, 1996c; Sandhu et al., 2001a, Erayman et al, 2004). The gene-poor regions accounted for only ~5% of the recombination as 95% of the recombination was observed in the GRRs (Erayman et al., 2004).

1.6. Gene Distribution in Wheat:

The number of genes in all higher plants is expected to be similar although changes due to ploidy level differences may exist. The estimated number of genes in arabidopsis is 25,000 and for rice is anywhere from 32,000 to 50,000 (Barakat et al., 1997; Goff et al., 2002). The gene-containing fraction of the arabidopsis genome is 0.85 (Barakat et al., 1998). Thus, the corresponding fractions for rice, maize, barley, and wheat are expected to be 0.28, 0.05, 0.025, and <0.01, respectively (reviewed by Sandhu and Gill., 2002). The average gene size in arabidopsis and rice is 2 KB and 2.5 KB respectively and gene size tends to increase with biological complexity (reviewed by Sidhu and Gill, 2004). Thus, with the predicted size and number of wheat genes, the gene-containing fraction of the wheat genome will encompass 1.2 – 2.4% (reviewed by Sidhu and Gill, 2004).

DNA reassociation kinetics studies showed that non-transcribed repetitive DNA is an integral part of most plant genomes and its amount is proportional to the genome size (Flavell et al., 1974). The gene-containing fraction of the wheat genome has been localized to 29% by physically mapping 3025 loci including 252 phenotypically characterized genes and 17 quantitative trait loci (QTLs) relative to 334 deletion breakpoints, present as 18 major and 30 minor gene-rich regions (GRRs) (Erayman et al, 2004). The gene-rich regions are interspersed by blocks of repetitive DNA sequences visualized as regions of low gene density. GRRs differed from each other with respect to marker number, marker density and the size (3- 71 Mb). Of the 48 GRRs, 17 major GRRs contained nearly 60% of the wheat genes but covered only 11% of the genome and were present on distal 35% of the chromosomes (Erayman et al, 2004). The localization of the gene-rich regions was based on deletion line breakpoints. The actual physical size of the gene-rich regions is probably much smaller as imprecise bracketing due to fewer number of deletion lines will result in an overestimate of the spanning region (Sandhu and Gill, 2002).

GRRs were named based on their location on a particular homoeologous group, chromosomal arm and GRR location on the chromosomal arm based on the fraction length (FL). For example '1S0.8' represents a GRR present on short arm of homoeologous group 1 chromosomes around fraction length of 0.8. Two major GRRs were identified on long arm of wheat homoeologous group 5 chromosomes. Physically, these regions are present around FL 0.5 and 0.7 and thus are called '5L0.5 region' and '5L0.7 region', respectively.

1.7. '5L0.5 Region':

The '5L0.5 region' is best localized on chromosome arm 5BL of wheat, where most of this gene-rich region is bracketed by the breakpoints of deletions 5BL-1 (FL-0.55) on the proximal side and 5BL-11 (FL-0.59) on the distal side (Gill et al., 1993a; Gill et al., 1996a). The region physically spans ~2.6% of chromosome 5B. Comparisons of genetic linkage maps revealed that about 20% of chromosome 5B recombination events occur in this region (Erayman et al., 2004). The '5L0.5' region contains many agronomically important genes but is of particular importance because the *Ph1* gene has been localized to a submicroscopic region contained within '5L0.5 region' and is known as '*Ph1* gene region' (Gill et al., 1993a).

1.8. '*Ph1* gene Region':

Fine mapping relative to the breakpoints of the deletion and mutant lines has localized the *Ph1* gene to a submicroscopic sub-region of the '5L0.5 region' (Gill and Gill, 1991; Gill et al., 1993a). The gene maps distal to the breakpoint of deletion 5BL-1 but proximal to that of 5BL-11. The breakpoint of deletion 5BL-1 maps between C-band 5BL1.7 and 5BL2.1 (Endo and Gill., 1996) (Fig. 1). The distal breakpoints of the interstitial deletions, both in the *ph1b* and *ph1c* mutants, also map in the same region. Since *Ph1* is absent in deletion 5BL-1, *ph1b* and *ph1c*, it must map distal to the breakpoint of 5BL-1 but proximal to the distal breakpoints of both *ph1b* and *ph1c*. The distal breakpoint of the *ph1b* is distal to that of *ph1c* because a marker was identified that is absent in 5BL-1 and *ph1b* but present in *ph1c* (Gill et al, 1993a). The *Ph1* gene, therefore, maps in the chromosomal region between the breakpoints of 5BL-1 and *ph1c*. This chromosomal region is very small and cannot be resolved by light microscopy. This region is referred to as the '*Ph1* gene region'. A DNA marker was identified for the '*Ph1* gene region' by

screening 602 random markers present on the *T. tauschii* map (Gill et al., 1993b). Three additional markers were identified for the region by RNA fingerprinting-deletion mapping analysis of the *ph1b* and its wild type parent Chinese Spring (CS) (Sandhu and Gill., 2001b).

1.9. Genome Relations in Plants:

Different crop plants are related to each other at the genome level and the extent of synteny and colinear gene order depends upon evolutionary distance between them. The genomes of various plant species have been compared both at the gross level and micro level using various strategies. It has been shown that major genes and also quantitative trait loci for important traits, such as flowering time, plant height, dwarfism, vernalization and shattering show orthologous loci in barley, wheat, maize and rice (Devos & Gale, 1997; Sarma et al., 1998; Bailey et al; 1999). Gene orders were found to be highly conserved across various Poaceae species (Hart, 1987; Ahn and Tanksley, 1993; Ahn et al., 1993; Devos et al, 1994).

1.9a. Genome Comparison at Gross level:

Genome comparison based on RFLP maps was reported in tomato (*Lycopersicon esculentum)* and potato (*Solanum tuberosum*). Few differences were observed between tomato and potato indicating high synteny (Bonierbale et al., 1988).

. The first report on genome comparisons in cereals perhaps comes from the work performed by Hulbert et al, 1990. This study showed the comparison between maize and sorghum by mapping maize RFLP clones on sorghum. In this study, most of the probes (104/105) from maize hybridized with sorghum deciphering high synteny. Ahn and Tanksley in 1993 performed rice and maize genome comparisons. RFLP map, based on cDNA and genomic clones in rice and maize was compared. 85% of the cDNA clones from rice hybridized to maize

recombinant inbred population. Many conserved linkage groups were identified between maize and rice. In the follow up study, the authors compared rice and wheat genomes by mapping previously mapped wheat cDNA clones on rice. The results of the two studies were combined and three species were compared and authors suggested that rice, wheat and maize share extensive homoeologies in a number of regions in their genomes (Ahn et al., 1993). It has been shown that colinearity between six grass species: rice, maize, foxtail millet, Sorghum, Sugarcane and wheat is such that a single consensus genetic map for each species could be defined in terms of nineteen rice linkage groups assembled in different ways for each species (Moore et al., 1995).

The whole genome comparison of rice and wheat has been performed by using 5,780 mapped wheat EST sequences in NCBI-BLASTN for comparison against rice genome (La rota and Sorrells., 2004). It was suggested in this study that for most rice chromosomes, there is prepondence of wheat genes from one or two wheat chromosomes. The chromosome 5BL was mainly associated with rice chromosome 9 (R9) from centromere to FL-0.75 and with R3 from FL-0.75 to telomere. It was shown in two other studies that the long arm of homeologous group 5B is significantly similar to R12, R9 and R3 (Foote et. al., 1997, Linkiewicz et. al., 2004). All of the earlier studies on rice-wheat comparisons were reported on the basis of nucleotide sequence comparisons, utilizing markers for the whole wheat genome and at that time the complete sequence of rice genome with ordered BAC/PAC clones was not available.

Apart from the reports on synteny, reports on lack of synteny are also well documented for example a lack of colinearity was observed between wheat and rice for wheat leaf rust resistance gene locus *Lr1* on chromosome 5DL (Gallego et al., 1998).

1.9b. Comparison at Micro level:

Most of the earlier genome comparison data was based on linkage analysis. The construction of the large insert libraries of grass genomes has allowed isolation and sequencing of large fragments (upto 500 KB). This provides insight about conservation of gene order between different grasses at sub-megabase level.

Good conservation of the gene content and order was found between three grass species: rice, maize and sorghum for the *Sh2/a1* locus (Chen et al, 1997). In this study, the genomic fragments in three species were analyzed and sequenced for orthologous loci.

A lack of colinearity at the microlevel has been reported in a detailed study of the barley resistance locus *Rpg1* and orthologous region in rice (Kilian et al., 1995). In this study, rice BAC and YAC libraries were successfully used to drive probes to saturate barley *Rpg1* region but three probes originating from the end of a BAC clone sequence containing markers flanking the *Rpg1* locus, mapped 2.5 cM proximal to the *Rpg1* region in barley, which was previously shown to be non-orthologous in rice. This study suggests that even in regions showing high colinearity may have rearrangements at the micro-level. Recently, maize and sorghum orthologous *adh* regions were compared by genome sequencing. Nine candidate genes, including adh1, were found in a 225-kilobase (kb) maize sequence. In a 78-kb space of sorghum, the nine homologues of the maize genes were identified in a colinear order, plus five additional genes. The question of missing genes in maize was addressed by comparing sorghum with rice and for two tightly linked genes in sorghum, authors identified tightly linked orthologs in arabidopsis, suggesting that genes in maize were deleted after the two species diverged from each other (Tikhonov et al., 1999).

1.9c Conservation of Genes at Functional Level:

There have been several reports on conservation of genes across species. An interesting example is provided by recent work on the *GAI* gene in arabidopsis, *Rht*-1 gene in cereals, d8 in maize and the rice *GAI* homolog *SLR1* (Peng et al., 1997; Peng et al., 1999; Ikeda et al., 2001). Cereal homologs of *GAI* were cloned by utilizing sequence information from a rice EST, which was shown to correspond to wheat *Rht*-1 and maize *d8* genes. Similarly, Arabidopsis *CONSTANS* (CO) gene (Putter ill et al., 1995), which regulates flowering has homologues in rice (Hd-1 gene) (Song et al., 1998; Yano et al., 2000) and gymnosperms. The *UNIFOLIATA* (*UNI*) gene of pea (Hoefer et al., 1997) and *FLORICACULA* gene in Antirrhinum are homologs of *LEAFY* gene (Bla'zquez and Weigel, 2000) of arabidopsis. *LEAFY* gene homologs have been identified in several species. There are many other examples such as conservation of roles of MADS-box genes (Ambrose et al., 2000; Kyozuka et al., 2000).

It has been demonstrated that grasses form a single genetic system (Bennetzen and Freeling, 1993) of which rice provides the model genome (Havukkala, 1996) but there are surprising levels of rearrangements including deletions even in closely related species, which makes it difficult to use rice to isolate genes in other cereal crop plants (reviewed by Keller and Feuillet, 2000). A modified approach was proposed in which rice mapping and sequence data could be highly useful for characterization in larger genome cereals when the gene order between wheat and rice is conserved for the region of interest (Keller and Feuillet, 2000).

The study reported here is focused on '5L0.5' region, which is a major gene-rich region located on the long arm of chromosome 5B. The '5L0.5' region is encompassed by the breakpoints of the deletion lines 5BL-1 and 5BL-11. This GRR is very important as it contains

an array of agronomically important genes but is of particular interest as the *Ph1* gene has been localized to a submicroscopic region present in this GRR.

The specific objectives of this study are to:

1) Enrich the '5L0.5 region' with markers using aneuploid stocks of wheat with an aim to identify additional markers for the region with special focus on '*Ph1* gene region'.
2) Compare the region across different Triticeae species with an aim to identify additional genes and markers for the region.
3) Compare the markers mapped on the chromosome 5BL of wheat with rice and arabidopsis (*Arabidopsis thaliana*) and study the evolutionary relationship between the three species.
4) Study the syntenic region for '*Ph1* gene region' in rice and arabidopsis in detail.
5) Study 'rice *Ph1* gene region' in detail to identify *Ph1* gene candidates.
6) Identify homologs of the candidate genes in wheat.
7) Identify BAC clones for the '*Ph1* gene region' probes in both tetraploid wheat and barley by using Barley BAC library (*Hordeum vulgare* var. Morex) and tetraploid wheat (*Triticum turgidum* var. *durum*) BAC library.

CHAPTER TWO

MATERIALS AND METHODS

2.1. Plant Material:

Various chromosome arm and sub-arm aneuploid stocks were used to physically map DNA markers to their respective chromosomal regions. Wheat homoeologous group *5* nullisomic-tetrasomic lines (missing a pair of chromosomes, the deficiency of which is compensated by a pair of homoeologous chromosomes) (Sears 1966) were used for the chromosomal location of DNA restriction fragments. The *Ph1* gene mutant lines: *ph1b*, *ph1c*, and *dupPh1* (carries four doses of *Ph1* gene), and single-break deletion lines 5AL-3(FL-0.56), 5Al-4(FL-0.55), 5AL-10(FL-0.57), 5AL-12(FL-0.35), 5BL-5(0.54), 5BL-1(FL-0.55), 5BL-11(FL-0.59), 5DL-1(FL-0.60) and 5DL-11(FL-0.52) (Endo and Gill, 1996), bracketing the 5L0.5 gene-rich region, were used for the precise localization of marker loci. The following single break deletion lines: 5AL-3, 5AL-4, 5AL-10, 5AL-12, 5BL-5, 5DL-1 and 5DL-11 were not used for all the probes. The '5L0.5' region is bracketed by the breakpoints of 5BL-1 and 5BL-11 deletion lines on proximal and distal sides respectively. The '*Ph1* gene region' is the smaller region present in the '5L0.5' region encompassed by the breakpoints of terminal deletion line 5BL-1 on the proximal side and the distal breakpoint of interstitial *Ph1* gene mutant line *ph1c* on the distal side. The mutant *ph1b* was developed in wheat cultivar 'Chinese Spring' by X-ray irradiation of seeds (Sears 1977). The mutants *ph1c* and *dupPh1* were developed in tetraploid wheat cultivar 'Cappelli' (Giorgi and Barbera, 1981). The mutants *ph1c* and *ph1b* carry interstitial deletion of 0.89 and 1.05μm respectively, encompassing the *Ph1* gene ((Jampates and Dvorak, 1986; Gill and Gill, 1991; Gill et al., 1993b). The *dupPh1* line carries a duplication of an interstitial region carrying the *Ph1* gene and is suspected to be the same region, which is missing

in the *ph1c* mutant (Jampates and Dvorak 1986; Gill and Gill, 1991; Gill et al., 1993a). The plant material was obtained from Wheat Genetic Resource Center (WGRC), Manhattan, KS.

2.2. Marker Identification:

To identify additional markers and useful genes for the target region, eight markers, previously mapped in the '5L0.5 region' (Gill et al., 1996b) were used as anchors to compare various Poaceae maps. A total of more than 36 maps generated in wheat, barley, rye, *T. tauschii, T. monococcum*, maize *(Zea mays),* oat *(Avena sativa),* and rice *(Oryza sativa)* were used (Graingene database: http://wheat.pw.usda.gov/ ggpages/maps.html); (Bezant et al., 1996; Korzun et al., 1998; Laurie et al. 1995; Ward et al. 1998; Foote et al. 1997; Causse et al. 1994; O' Donoughue et al. 1992; Rayapati et al., 1994). Initially, markers flanked by two anchor markers were selected. Markers tightly linked to any of the anchor markers were also selected. In the second round of comparative mapping, the markers selected in the first cycle were used as anchors. The same strategy was followed to map morphological and other useful genes controlling important agronomic traits to the gene-rich region. In addition, 10 cDNA markers for the region identified by RNA fingerprinting/differential display (Sandhu t al., 2001b), were also used.

The cDNA and genomic DNA probes that were used in the construction of the physical map of the '5L0.5 region' were derived from wheat (PSR, UNL, WG, TAM, TAG, FBA, FBB), barley (ABC, ABG, BCD, cMWG, KSU, MWG), oat (CDO), and *T. tauschii* (KSU). The RFLP probes were described by the following authors: BCD, CDO, WG, Heun *et al*. (1991); KSU, Gill *et al*. (1991b); MWG and cMWG, Graner *et al*. (1991); ABC, ABG, Kleinhofs *et al*. (1993);

PSR, Sharp *et al*., 1989. UNL probes were derived from wheat cultivar 'CS' by Sandhu and Gill in 2001, using RNA fingerprinting/differential display method.

The EST probes used in the physical mapping were identified by screening the Graingene database (http://wheat.pw.usda.gov/wEST/binmaps). The ESTs corresponding to different bins on all chromosomes of wheat are listed in the database. For the intent of targeting the 5L0.5 region, we selected ESTs from following bins of the three group five homeologous chromosomes, 5AL-12-0.35-0.57, 5AL10-0.57-0.78, 5BL6-0.29-0.55, 5BL-1-0.55-0.75, C-5DL-1-0.60, 5DL-1-0.60-0.74. The '5BL6-0.29-0.55' bin was also included because it is immediately adjacent to the 5L0.5 region and the EST assignment on the Graingene database was not fully validated at that time (09/03), for different bin locations. Based on these criteria, approximately 200 ESTs were selected for physical mapping on the chromosome 5BL of wheat.

2.3. Gel-Blot Analysis:

Genomic DNA from various plant materials was isolated following Anderson et al., 1992. Two forms of leaf tissue (Fresh leaf tissue and lyophilized) were used to extract DNA depending upon the availability: For Lyophilization, the tissue was kept in the lyophilizer for approximately 7 days and after drying the tissue was ground in the grinding machine. Fresh Leaf tissue was ground in liquid nitrogen. The tissue in ground form was incubated in DNA extraction buffer (0.1M Tris pH 8.0, 0.5M NaCl, 50mM EDTA, 1.25% SDS, 0.0038% sodium bisulfite) at 65°C for 2 hours. The slurry was extracted with 0.8 volume of chloroform-isoamyl alcohol (24:1 v/v). The DNA was precipitated from the supernatant by adding two volumes of cold ethanol. The DNA pellet was washed with 70% ethanol. Dried pellet was dissolved in TE containing RNase (1µg/ml). For purification, the DNA was extracted with phenol:chloroform and recovered

by 0.3M sodium acetate and ethanol precipitation. For each sample, 5 µg of genomic DNA was digested with 40 units of restriction enzymes in the presence of spermidine (5mM) and Bovine Serum Albumin (BSA). Three restriction enzymes (*Eco*RI, *Dra*I and *Hin*dIII) were used for physical mapping. Digested DNA was electrophoretically separated on 0.8% agarose gel in 1x TBE (Tris-borate-EDTA) buffer at a constant voltage of 35V for 16 hours (Gill et al., 1993a). 'Southern' blotting onto nylon membrane (Micron Separations Inc. MA), DNA immobilization, and hybridization were performed following manufacturer's recommendations. Gels were stained with ethidium bromide (2ul/100 ml of water) and treated with 0.25M HCl for 12 minutes with gentle shaking. Gels were treated with denaturing solution (0.4N NaOH, 0.6M NaCl) for 30 minutes followed by neutralizing solution (0.5M Tris, 1.5M NaCl, pH 7.5) for 30 minutes. DNA was transferred to nylon membrane by capillary method for 16 hours, using 20x SSC (0.3M sodium citrate, 3M sodium chloride). After transfer, the membranes were soaked in 5x SSPE (3M sodium chloride, 0.2M sodium phosphate monobasic, 0.02M EDTA) at 65°C for 5 minutes, air-dried and baked at 80°C for 1 hour. DNA was cross-linked to membrane by treating the membrane with 20,000 µJoules of energy in UV stratalinker (Stratagene). Prehybridization was performed in 35 x 300 mm glass bottles containing 50 ml of prehybridization solution (6x SSPE, 5% Denhardt's solution, 0.5% SDS, 50µg/ml Salmon sperm DNA), incubated at 65°C for 16-18 hours in a hybridization rotisserie oven (Hybaid). For hybridization, about 30ng of probe was labeled with 30 µCi of 32PdCTP following random primer labeling technique (Feinberg and Vogelstein, 1983) in a 15µl reaction containing 4 units of klenow enzyme and 0.5mM cold nucleotides (ATG). Unincorporated nucleotides were removed with Sephadex G-50 spin columns. Hybridization was performed in 10 ml of hybridization solution (5% Dextran sulfate, 6x SSPE, 5% Denhardt's solution, 0.5% SDS, 50µg/ml Salmon sperm DNA) and the blots were

washed once, at 65°C in 2x SSPE, 0.5% SDS for 30 minutes. After rinsing with 2x SSC, solution the blots were wrapped in plastic and exposed to X-ray film with two intensifying screens at –80°C for three to seven days depending upon the radioactive signal.

2.4. DNA sequence analysis and motif search

In order to identify rice and Arabidopsis BAC/PAC clones exhibiting significant similarity to the mapped ESTs and DNA markers on 5BL arm, the probe sequences were used in tBLASTx searches against GenBank non-redundant database and the BAC/PAC clones which exhibited significant similarity (i.e. E-value significance $<1e^{-25}$) were selected as true homologs. The chromosomal location of the identified orthologous sequences was identified by screening Gramene database (www.gramene.org) for rice and TAIR database for Arabidopsis (www.arabidopsis.org). For the ortholog sequences of '*Ph1* gene region' and '*ph1c-ph1b* region' markers, the precise base-pair location was also identified utilizing the information available in the Gramene and TAIR databases.

The genes present in the orthologous '*Ph1* gene region' on rice were analyzed in detail for their putative function. For most of them, the putative function was listed in the Gramene database but we compared the protein sequence of all 91 genes, on NCBI conserved domain database (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) for detailed analysis of these genes. Putative function was assigned after analyzing all the results.

To identify wheat ESTs exhibiting significant similarity (cut-off value $<1e^{-25}$) to the selected rice genes, protein sequence of these genes was used in tBLASTn (Atschul et al., 1997) searches of the GenBank wheat EST database. ESTs were selected as representative of the rice genes by analyzing the position and significance of similarity and the length of the EST.

The putative function of the selected wheat ESTs was assigned by using them in BLASTN (Atschul et al., 1997) searches against GenBank non-redundant databases, and BLASTx (Atschul et al., 1997) searches against GenBank non-redundant and SwissProt databases. Putative functions were assigned after analyzing all the results

2.6. Wheat and Barley BAC Library Screening:

To generate detailed contig for '*Ph1* gene region', tetraploid wheat *(Triticum turgidum* var. *durum)* Bacterial artificial chromosome (BAC) library (Cenci et al. 2003), consisting of 28 high-density filters and Barley BAC library (*Hordeum vulagre* cv. Morex) (Yu et al., 2000) were screened with '*Ph1* gene region' specific probes. *Triticum turgidum* var. *durum* consists of A and B genomes. This library contains approximately half million clones with an average insert size of 130 kbp, providing a 5.1x coverage for each genome (Cenci et al., 2003) The Barley BAC library contains 3,13,344 clones with an average insert size of 106 kbp. Batch of two probes were used at a time for screening the libraries. The southern hybridization technique used for screening was performed as previously described in this chapter.

2.7. BAC Clone Identification:

Positive BAC clones were scored based on their pattern in each individual well located on the filters. Positive BAC clones identified by screening tetraploid wheat library were ordered from ucdavis. Individual BAC clones were inoculated into 96-deep well plates containing 2 ml LB medium plus 12.5ug/ml chloramphenicol, and grown in levitation machine at 37°C, for 12-16 hours. BAC DNA was isolated and purified using alkaline lysis method, and dissolved in 20ul TE. For identifying real positives and to see if they are present as multiple copies, approximately

7ul DNA for each individual BAC was digested with *Hin*dIII, was used for blotting onto nitrocellulose membranes. Each '*Ph1* gene region' probe was used to screen these BAC blots using Southern hybridization. Positive BACs were scored for individual probes, defining them into different loci based on their position on the film.

2.8. Meiosis Analysis:

Wheat spikes were harvested at meiosis stage between 9 AM to 10 AM. Different spikes were analyzed for their position in the plant. The spikes are taken when they are still covered by leaf sheath and their position is estimated by pressing the plant stem. The spikes, which are about 1 to 2 cm lower in position than the last node on that particular shoot, are supposed to be at meiosis between 9AM to 10 AM. The spikes can be kept in water or fixed in Carnoy fixative solution (60 ml ethanol: 30 ml chloroform: 10 ml acetic acid) for several hours (4-5 hr. is enough) at 4^0C depending upon preference. For the spikes kept in Carnoy's solution, after the meiotic cells were fixed, the plant buds were taken out of the solution and put in a Petri-dish containing water to avoid drying out of the cells. For the spikes kept in water, meiosis should be studied immediately as the cells are live. One anther from central floret was taken and placed on a glass slide and was squashed with a forcep after putting a drop of acetocarmine solution on it. The debris such as anther walls was removed by using a fine tipped forcep. Anther remainders were covered on the slide with cover slip. The slide was then heated on flame briefly. After heating, the slide was placed between a layer of paper towels and pressed straight down firmly. This squashing process flattens the cell's nuclei and spreads out the chromosomes. The slides were first observed under the 10x lens. Once meiotic cells were identified, put emersion oil on the slide and observe it under 100x lens to identify the meiotic stage. The slide could be stored in

-80^0C for future use. After finding a meiotic stage, remaining anthers from that particular bud were frozen in liquid nitrogen and kept at –80 C for RNA isolation.

CHAPTER THREE

RESULTS AND DISCUSSION

3.1. Identification of Markers for Physical Mapping:

Comparative analysis of 36 Poaceae maps using previously available 24 '5L0.5 region' probes(Faris et al., 2000) identified 72 RFLP additional markers (see page-17 for additional details). These 96 DNA markers were comprised of 90 RFLPs and six SSR markers. Nineteen of the RFLP markers were wheat cDNA (PSR, UNL), 15 wheat genomic (WG, PSR, TAM, TAG, FBA, FBB), 19 barley cDNA (ABC, BCD, KSU, cMWG), 16 barley genomic (ABG, MWG), eight oat cDNA (CDO), seven durum wheat cDNA (MGB, UBP), one rice cDNA (RZ), and six *T. tauschii* genomic clones (KSU A, D, E, F, G, H, S, M). Ten markers were cDNA clones isolated by RNA fingerprinting-deletion mapping method where the target was the *Ph1* gene-containing region (Sandhu and Gill, 2001b). The 157 EST markers were identified by screening wheat physical mapping database available at Graingene (http://wheat.pw.usda.gov/wEST/binmaps). These wheat ESTs were physically mapped using only six 5BL deletion lines and the 5BL-11 deletion line flanking the 5L0.5 region was not represented. Although the 5BL-1 deletion line was used but for about 80% of the data, the line was actually nullisomic for chromosome 5B thus was non-informative. For the present study, therefore, the markers mapping in regions 5AL-12-0.35-0.57, 5AL10-0.57-0.78, 5BL6-0.29-0.55, 5BL-1-0.55-0.75, C-5DL-1-0.60, and 5DL-1-0.60-0.74 were selected for mapping.

3.2. Marker Enrichment of '5L0.5 Region':

The 223 DNA markers were analyzed by gel-blot analysis of group 5 specific nulli-tetra lines along with deletion lines 5BL-1(FL-0.55) and 5BL-11(FL-0.59) and the results are given in figure 1. Breakpoints of these two deletion lines bracket the '5L0.5 region' present on chromosome arm 5BL. Additional deletion lines: 5AL-4 (FL-0.55), 5AL-10 (FL-0.57), 5AL-12 (FL-0.35), 5BL-5 (0.54) 5DL-1 (FL-0.60) and 5DL-11 (FL-0.52) were used for physical mapping of 157 of the 223 markers. Two of the three restriction enzymes (*Dra*I, *Hin*dIII and *Eco*RI) were used for each probe. Three enzymes were only used for the probes for which the band pattern did not get resolved with two enzymes. Out of these 223 probes, 172 probes detected 192 loci for chromosome 5BL (Fig. 1). The band pattern in nullisomic-tetrasomic lines was used to assign bands to the respective homoeologous chromosomes. A band missing in N5BT5A but present in N5AT5B and N5DT5A, was assigned to chromosome 5B. Sub-arm location of the probes was revealed by the deletion lines. A 5B specific band missing in 5BL-1 but present in 5BL-11 was assigned to the '5L0.5 region'. An example of such a pattern is given in Fig. 2 for the probe *mwg522* that maps in the '5L0.5 region'.

Including the previously mapped 24 marker loci, we identified 85 marker loci for the '5L0.5 region'.

Many of the probes detected more than one locus on 5BL. Seventeen of the '5L0.5 region' probes (*abc168, abg387, bcd307, bcd351, BE426080, BE443430, BE445248, BE494426, BE636942, BF484440, cdo412, fba127, fbb121, mwg52, mwg956, unl3, wg564*) detected a second locus on 5BL. Two probes (*cdo786, ksu8*) detected three loci on 5BL (Fig. 1). All the three loci for *ksu8* were present in the '5L0.5' region. Two loci for *cdo786* were mapped in the '5L0.5 region', while the third locus mapped proximal to the '5L0.5' region. The second locus

for *abg387, BE445248, bcd307, fba127* mapped distal to the breakpoint of 5BL-11. The second locus for the probes *abc168, BE426080, fbb121* and *unl3* was present proximal to the proximal breakpoint of *ph1b,* while for probes *BE443430, BE484440, BE494426, BE636942, bcd351, mwg52, mwg956,* the second locus localized in the '5L0.5 region'. The second locus for *wg564* and *cdo412* mapped proximal 5BL-1 and distal to the proximal breakpoint of *ph1c.*

For seventy marker loci, the corresponding B band was missing in both 5BL-1 as well as 5BL-11 and were therefore localized distal to the breakpoint of 5BL-11 (Fig. 1). Similarly, 42 marker loci were localized proximal to the breakpoint of dup*Ph1* as the corresponding bands were present in 5BL-1, 5BL-11 as well as *Ph1* gene mutant lines.

Bands corresponding to nine loci: *abc302, BE483335, BE495790, BE590997a, BE590997b, cdo412a, psr128, psr574* and *wg564* were present in 5BL-1 and 5BL-11 but missing in both mutants, therefore, mapped proximal to the breakpoint of 5BL-1 but distal to the proximal breakpoints of both *ph1b* and *ph1c* mutants (Fig. 3).

For three marker loci, *BE405060, BE442918* and *BE443187,* the *ph1c* band could not be scored, so they were placed proximal to the breakpoint of 5BL-1 and distal to the proximal breakpoint of *ph1b,* based on the banding pattern in these two lines.

3.3. Marker Enrichment of '*Ph1* Gene Region':

For detailed physical mapping for the '5L0.5 region' with a target to identify additional markers for the '*Ph1* gene region', the 5L0.5 region-specific probes were further mapped on three *Ph1* gene mutant lines: *ph1b, ph1c* and *d*up*Ph1*. The distal breakpoints of *ph1b, ph1c* and the breakpoint of the region duplicated in the dup*Ph1* line, all map in the 5L0.5 region along with the C band 5BL2.1(Fig. 1). These provide three additional breakpoints for the region,

dividing the gene-rich region into four smaller gene-rich regions. The *Ph1* gene containing region is bracketed by the breakpoints of 5BL-1 on the proximal side and the distal breakpoint of the interstitial deletion of the *ph1c* mutant on the distal side. Ten marker loci along with the *Ph1* gene mapped to the '*Ph1* gene region' as the corresponding bands were missing in both 5BL-1 and *ph1c* mutant line. The corresponding band were also missing in *ph1b* and present in double intensity in dup*Ph1* line. Markers *BE404454, Be443430a, BE443430b, BE405358, BE636942b, ksu8a, ksu8b, ksu8c, mwg592, wg564b* mapped to the '*ph1c-ph1b* region' as the corresponding band were missing in *5BL-1* and *ph1b* but present in *ph1c*. Fig. 4 shows the banding pattern for locus *BE636942b* in group 5 nulli-tetras, *Ph1* gene mutant lines and terminal deletion lines for all group 5 homoeologous chromosomes.

The fragment band for all the '*Ph1* gene region' markers was present in double intensity in *dupPh1,* suggesting the '*Ph1* gene region' is duplicated in this line (data not shown). As expected the band corresponding to the nine loci mapped proximal to 5BL-1 and distal to the proximal breakpoint of *ph1b* were also present in double intensity. Fifty-three loci in the '5L0.5 region' were mapped distal to the distal breakpoint of *ph1b* and proximal to the distal breakpoint of *dupPh1* as the corresponding bands were absent in 5BL-1 but present in *ph1b, ph1c, dupPh1* and 5BL-11. These were mapped proximal to *dupPh1* as the bands were present in double intensity in this line, while eleven marking loci showing similar band pattern for all other lines except *dupPh1* were mapped distal to the distal breakpoint of *dupPh1* as the corresponding bands were not present in double intensity in *dupPh1*.

For the probes mapped in the '*Ph1* gene region' *cdo412* identified a major locus proximal to 5BL-1 and a minor locus in the '*Ph1* gene region' (based on the intensity of the bands), while for probe *BE636942*,the band pattern was especially interesting as it identified the second locus

in the ‘*ph1c-ph1b region’*, which is a smaller gene-rich region bracketed by the breakpoints of *ph1c* on the distal side and *ph1b* on the proximal side, present in the ‘5L0.5 region’ and is immediately adjacent to the ‘*Ph1* gene region’ (Fig.1).

Ten marker loci *BE404454, BE443430a, BE443430b*, *BE405358, BE636942b, ksu8a, ksu8b, ksu8c, mwg592 and wg564b* were localized to the region bracketed by the breakpoints of *ph1c* on the distal side and *ph1b* on the proximal side.

The remaining sixty-four marker loci mapped to the ‘5L0.5 region’ were localized to region bracketed by *ph1b* and dup*Ph1*, if the bands were present as double intensity in dup*Ph1* and were localized to the region bracketed by dup*Ph1* and 5BL-11, if the corresponding band was present as normal intensity in dup*Ph1.*

3.4. Identification of Useful Genes/ Morphological Markers:

The ‘5L0.5’ region specific markers were used for comparative mapping in order to physically map useful Triticeae genes to ‘5L0.5’ region. The markers mapped to ‘5L0.5’ region were used to compare different Poaceae maps. The results are summarized in Table 1. The corresponding linked or flanking markers are also given in the table. In addition to *Ph1,* we identified 33 useful genes controlling an array of important agronomic traits that map to the ‘5L0.5’ region. These include genes controlling disease resistance, yield and its component traits, plant stature, abiotic environmental stress, heading time and several quality traits (Table 1). All of these genes showed linkage with one or more markers mapped in the ‘5L0.5’ region. Out of these 34 genes, 23 genes are on 5H chromosome of Barley, seven on 5A of wheat, one on 5B of wheat and three on 5R chromosome of rye. The actual number of the mapped genes for the region may be smaller than 34 as alleles of a gene or even the same gene in different

background, may have been counted as different genes. For example, there are two genes for yield, two for grain weight. These genes may be allelic or even the same gene mapped with different markers in the same population. Therefore, the actual number of genes may range from 28-34.

These 34 genes were further mapped to the smaller sub-regions in the '5L0.5 region' based on their linkage relationships with the markers mapping to different regions (Fig. 1). Seven genes *Egw.2, Egw.3, Ht, S5, Spn.2, Spn.3* and *Yld* mapped to '*Ph1* gene region'. Two genes *Mlj* and *RL* showed linkage to the loci mapped to the '*Ph1c-ph1b* region', while eleven genes (*FHB, HD, HD, HT, KP, mSrh, mt2, SR, SRR, var1* and *YLD*) were localized to the '*ph1b-dupPh1* region' and 13 of the 33 genes (*AadhNADP, CT, CT, GMBase, HD, KMBase, LT50, nar2, nar5, S5, SD, ST(s)* and *WS*)mapped to the region bracketed by dupPh1 and 5BL-11 on the proximal and distal side respectively. The genes controlling grain weight, spikelet number, yield as well as plant-height were mapped to the '*Ph1* gene region' (Fig. 1, Table 1).

Using comparative and physical mapping approach, 118 markers and useful genes were identified for the '5L0.5 region' including 33 genes. For the submicroscopic '*Ph1* gene region', 17 useful genes and markers were identified.

3.5. Identification of Positive BAC Clones in Tetraploid Wheat and Barley BAC libraries:

3.5a. Tetraploid Wheat BAC library Screening

The Tetraploid wheat library (*Triticum turgidum* var *durum* cultivar Langdon) was screened with probes mapped to the '*Ph1* gene region'. The tetraploid wheat (A and B genomes) library consists of 516,096 clones, which were organized in 1344 384-well plates and blotted

onto 28 high-density filters (Cenci et al., 2003). The average insert size of BAC clones is 131-kb, resulting in coverage of 5.1 X genome equivalents for each of the two genomes (Cenci et al., 2003). To screen this library, a batch of two probes was used at a time to perform southern hybridization. Fig. 10 shows the positive BAC clones on filter # 12, identified by southern hybridization with probes *unl4* and *BF474342*. Each filter is divided into six fields, which has 384 squares (wells) and each well has 16 spots, where eight clones are positioned in duplicate pattern (Cenci et al, 2003). The unique pattern in each well, together with the field number and filter number generates plate and well address for a particular clone. The nine '*Ph1* gene region' probes identified 384 positive clones in this library. The addresses for these BAC clones were identified by decoding the plate and well location for each clone based on their filter number (1-28), field number (1-6), hybridizing pattern (1-8) and well location (A1-P24).

3.5b. BAC Clone Identification:

Positive clones were ordered from UC-DAVIS (Dr. Jorge Dubcovsky) in the form of stab cultures and the DNA was isolated. The DNA of the positive clones was digested with *Hin*dIII and blotted onto nylon membranes. These blots were screened using '*Ph1* gene region' probes to check their copy number and to exclude false positives. Fig. 12 shows the hybridization results obtained by screening of a blot containing *Hin*dIII digested BAC clones with probe cdo1090. The BAC clones identified using above method were categorized into different categories based on their banding pattern (number of bands, relative distance of the bands from comb). This screening method narrowed down the list of identified BAC clones to 301 from 384.

3.5c. Barley BAC library Screening

In a similar way, barley BAC library (*Hordeum vulgare* cultivar Morex) was screened with probes mapping in the '*Ph1* gene region'. This library contains 313344 clones with an average insert size of 106 kbp (Yu et al., 2000). A batch of two probes was used at a time for southern hybridization. '*Ph1* gene region' probes hybridized to a total of 46 BAC clones, using a set of four filters (A, B, C and D). Fig. 11 shows the hybridization pattern BAC clones identified by screening of filter 'B' with probe *cdo1090.*

By screening tetraploid wheat and barley BAC libraries, 301 and 46 BAC clones were identified by screening the libraries with '*Ph1* gene probes'

3.6. Comparison of Wheat Chromosome 5BL with Rice and Arabidopsis:

The marker loci mapped to chromosome 5BL were used in comparison studies to identify corresponding loci/ regions on the two model species rice (*Oryza sativa*) and Arabidopsis (*arabidopsis thaliana*). 157 marker loci out of 192 loci mapped to chromosome 5BL were used for analysis of genome comparisons with rice and Arabidopsis by BLAST searches.

3.6a. Rice-Wheat Comparisons:

Of the 157 wheat sequences mapped to various regions on chromosome 5BL, 125 marker loci identified corresponding orthologous sequences on various rice chromosomes (Fig. 5 and Table 2). Out of these 125, 40 identified orthologous sequences on rice chromosome 9 (R9), 19 on R3, 17 on R1, fourteen on R7, ten on R5, six on R8, five on R11, four on R2, three on both R4 and R12, while two on each R6 and R10 (Fig. 5, Table 3).

3.6b. Wheat-Arabidopsis Comparisons:

Of the same 157 mapped wheat sequences, 114 corresponded to orthologous sequences on various Arabidopsis chromosomes (Fig.5 and Table 4), with the highest number corresponding to chromosome A1 and A5 (36 each). Twenty markers identified orthologous sequences on chromosome A3, thirteen on A4 and nine on A2 (Fig. 5). The analysis of wheat-Arabidopsis comparisons for different wheat regions is given in Table 3b. This comparison study revealed that similar to wheat-rice, it is hard to establish the relation between these two genomes in terms of one or two Arabidopsis chromosome for each wheat region (Table 4), but it could be interpreted that chromosome 5BL is largely similar to A1 and A5 (36/114 each) and least similar to A2 (9/114).

3.6c. Rice-Arabidopsis Homology for Wheat Chromosome 5BL:

To determine relationship between orthologous rice and Arabidopsis regions for the corresponding regions on wheat, the comparison data was analyzed in terms of these two model species. The results for this comparison are summarized in Table 5. These results indicate that R1 shows significant similarity to A1, R3 with A1, R7 with A1 and A5, and R9 with A5. The greatest similarity between these two model species was found for rice chromosome 9 as 19 wheat sequences, which identify corresponding sequences on R9 also identify corresponding sequences on A5. Significant relation was also found for rice 3 and Arabidopsis 1 as 9 (50%) wheat sequences, which identify their corresponding sequence on R3 also identify their homolog on A1.

3.6d. Wheat-Rice Homology for Different Regions on Chromosome 5BL:

To determine if different regions on 5BL could be defined in terms of one or two rice chromosomes, we analyzed the corresponding orthologous rice sequences were analyzed for their chromosomal location on rice (Table 3). Different wheat regions in table 3 are defined by

the proximal and distal breakpoints of the deletions, which bracket a particular region. The whole 5B long arm is divided into four regions: centromere-*d*up.Ph1*, ph1b*-5BL1, '5L0.5 region' and 5BL11-telomere. Out of 30 orthologous rice sequences corresponding to the C-dup.*Ph1* region, 12 correspond to R9, six to R1, three each to R5 and R11, two each to R7 and R12 and one to both R2 and R10 (Fig. 5, Table 3). Of the eight orthologous rice sequences for the *ph1b*-5BL-1 region, three correspond to R9, two each to R8 and R1 and one to R12. For the '5L0.5 region', 21 sequences identified orthologous sequences on R9, 10 on R7, Four each on R8 and R5, two each on R4 and R2 and one on R3. None were identified for R1, R10, R11, R12. For the 5BL11- telomere region, 18 correspond to R3, nine to R1, four to R9, three to R5, two each on R7 and R11, one on R2, R4 and R10.

Based on these results, wheat and rice relation in terms of these regions and corresponding rice chromosomes is shown in Fig. 6. These four regions could not be defined in terms of one or two rice chromosomes, however, it is possible that there is conservation of homology between wheat and rice for smaller regions. Based on these results and to focus more on the '*Ph1* gene region', we analyzed the comparison data for the '5L0.5 region' was analyzed in detail by studying the two mini gene-rich regions (*Ph1* gene region, *ph1c-ph1b* region) present in the '5L0.5 region'. The detailed results are given in Table 3a and Fig. 7. Eight markers mapped to the '*Ph1* gene region' identified a highly syntenous region on rice chromosome 9, while one marker identified a corresponding sequence on rice chromosome 7. In the '*ph1c-ph1b* region', six out of eight marker loci identified corresponding sequences on rice chromosome 7 while two identified orthologous sequences on rice 9.

In order to see if the regions corresponding to these two mini gene regions are present in the form of continuous blocks on the corresponding rice chromosomes or the orthologous

sequences are scattered on the two rice chromosomes, we studied the precise base pair location of the rice sequences using Gramene database. The results for this comparison are summarized in Fig. 8. The markers in '*Ph1* gene region' identified approximately 450 KB region on rice chromosome 9. These results revealed a very high synteny in wheat and rice for the '*Ph1* gene region' as the marker order in wheat (Fig. 1) and rice is same for this region (Fig. 8). Six markers in the '*ph1c-ph1b* region' identified an approximately 2.7 MB region on rice 7, while two markers mapped to this region identified sequences on rice 9 (Fig. 1, Fig. 8), immediately adjacent to the orthologous rice '*Ph1* gene region'. The only marker for '*Ph1* gene region', which identified corresponding sequence on rice 7 maps in the region corresponding to orthologous '*ph1c-ph1b* region' on rice 7. These results indicate that for these mini-gene rich regions, which are present adjacent to each other in wheat have their corresponding orthologous regions on two rice chromosomes R9 and R7**.** This 'adjacent arrangement' seems to hold in rice too as the orthologous '*ph1c-ph1b* region' is present on rice chromosome 9 and is immediately adjacent to the orthologous '*Ph1* gene region' and is towards the distal side of the chromosome (same as wheat) (Fig. 7, Fig. 8). This colinearity seems to break as the homoeologous '*ph1c-ph1b* region' is present on two chromosomes on rice.

3.6e. Detailed Analysis of '*Ph1* gene region':

The rice region orthologous to the wheat '*Ph1* gene region' is approximately 450 KB in size. We analyzed the genes present in this region for their putative *Ph1* gene-like function, based on their predicted function on the Gramene database and by screening the NCBI conserved domain database. Since, there have been many speculations regarding the mode and action of *Ph1* gene, the criteria's for selection of *Ph1* gene candidates were highly flexible. All the possible domains and motifs, which could be responsible for the speculated functions of *Ph1*

gene, were included. For example, domains and motifs involved in DNA binding, protein-protein interactions, chromatin reorganizations, unknown functions, methyltransferase activity, acetyltransferase activity etc. were selected. Twenty-six genes were selected based on their functional domains and motifs, out of the 91 genes present in rice *Ph1* gene region ranging from 181,623,98 bp to approximately 187,368,67 bp. The details of these genes along with their predicted function are given in Table 6. These include genes encoding proteins which mediate/regulate microtubule attachment and chromosome segregation, chromatin reorganization, DNA binding, DNA repair, heat shock protein activity, chaperon activity, acetyltransferase activity, Protein kinase and methyltransferase activity as well as proteins, which were specifically encoded at meiosis and anthers. Wheat EST homologs for these rice *Ph1* gene candidates were identified performing tblastn (Altschul et al., 1997) searches against wheat EST database on GENBANK. The homologs for all rice Ph1 gene candidates except rice 'chromatin reorganization' gene were identified in wheat. The wheat EST homologs along with their putative functions are listed in Table 6.

The orthologous rice locus for probe *cdo412,* which also maps to the '*Ph1* gene region' in wheat, is present also present on chromosome 9 of rice, proximal to the '*Ph1* gene region' but it was not included, as the major locus for *cdo412* is present proximal to the breakpoint of 5BL-1 in wheat (Fig. 1). If this locus was included, the total rice region spans about 1.6 MB and contains 337 genes.

To further test for the true homology of rice with wheat, the wheat ESTs that are homologs of rice sequences (listed in the Gramene database) were analyzed to search for their map location on the Graingene database. Surprisingly, mapping data for only 21 loci out of 1090 is available and 19 of them map between the deletion breakpoints of 5BL-1 and 5BL-11, which

corresponds to the ‘5L0.5 region’, containing the *Ph1* gene region (Fig.1). These results further confirmed that the region on rice chromosome 9 is truly homologous to the ‘*Ph1* gene region’ of wheat.

Results reported herein were compared with previously published results for rice-wheat homology for chromosome arm 5BL (Fig. 6). Results from this and previous studies indicate sequences on a single wheat chromosome or part there of correspond to orthologous sequences on wide range of rice chromosomes, although on the basis of number of sequences for different chromosomes, relationship between wheat and rice has been established for different wheat regions, in previous studies (Fig. 6). It was shown that 5BL holds syntenic relationship to R6, R12, R9 and R3. The two regions from the centromere to the proximal breakpoint of *ph1b* and *ph1b*- 5BL-1(FL-0.55) have been shown to be similar to regions on R6 and R12 based on four sequences. Our results indicate C-*ph1b* region has maximum homology with R9 (12 sequences) and R1 (6 sequences). While the region *ph1b*-5BL-1 (FL-0.55) is syntenic to R9, R1 and R8 (Fig. 6). We confirm the synteny of ‘5L0.5’ region to R9, but it also identifies corresponding sequences on other chromosomes, including 10 on R7, four on each R8 and R5. Similarly, although the region from 5BL11- telomere is largely similar to R3, but the there are significant sequences identified on R1, R9 and R5 as well.

Discussion

Gene synteny is a measure of conserved DNA sequences across species and the extent of conservation is proportional to their evolutionary distance. Wheat belongs to the grass family, Poaceae that includes more than 15 genera and 300 species, which includes other major cereal crops such as the small grains barley, rye, oat, maize and rice. Conventionally, species such as rice, maize, wheat etc. are studied separately. Comparative mapping is a useful approach to combine genetic information available in different species. The two prerequisites of this approach are the availability of genetic linkage maps in different species and a set of probes (anchor probes) that can be used cross-species to evaluate homoeology and conservation. RFLP maps are available in wide range of grasses such as barley (Heun et al. 1991; Kleinhofs et al. 1993; Graner et al. 1991; 1994), oat (O'Donoughue et al. 1992; 1995; Rayapati et al. 1995; Van Deynze et al. 1995b), maize (Burr and Burr 1991; Gardiner et al. 1993), sorghum (Chittenden et al. 1994), and sugarcane (da Silva et al. 1993**).** In this study, the already available markers for the '5L0.5 region' were used as anchor probes for comparison across Poaceae to identify additional probes for the region. The probes thus identified were mapped onto wheat aneuploid stocks and *Ph1* gene mutant lines. Physical mapping revealed that approximately 68% of the putative gene-rich region probes identified by comparative mapping, map to the target region. The reason for approximately 32% of the probes not mapping in the target region is that the probes identified in the first round of comparative analysis were used as anchor probes in the second round of analysis. Thus, this study demonstrates a successful application of comparative mapping to identify probes and markers for the target region.

It has been reported that conserved linkage blocks exist among different grasses (Moore et al., 1995). It has been proposed that within a distance of < 3 cM, there should be nearly 50%

colinearity between monocots and dicots (Keller and Feuillet, 2000). It is of immense importance to decipher the extent of synteny between wheat, rice and arabidopsis in order to utilize the vast amount of genetic information available these model species. We need to address the question of the use of these model species to carry out research in large genome species such as wheat. The wheat-rice genome relationships have been well documented at the gross-level based on data involving small-scale comparisons using a few markers. The study reported herein targets both gross level (whole chromosome arm, 5BL) as well as detailed micro-level ('*Ph1* gene region' and '*ph1c-ph1b* gene region') comparisons using a large number of probes (157).

Data reported herein on the whole long arm comparisons indicates high synteny between the wheat, rice and arabidopsis as approximately 80 and 71% of the markers used in this study identified corresponding sequences in rice and arabidopsis respectively. However, continuous syntenous region/regions did not correspond to either species, indicating a vast number of rearrangements during evolution. Results reported in this study were compared with previously published results for rice-wheat homology for chromosome 5BL (Fig. 6). Results reported herein and previous studies indicate sequences on a single wheat chromosome or part there of correspond to orthologous sequences on different rice chromosomes, although on the basis of the number of sequences for different chromosomes, relationship between wheat and rice has been established for different wheat regions, in previous studies (Fig. 6). It was shown that 5BL holds syntenic relationship to R6, R12, R9 and R3. The two regions from the centromere to the proximal breakpoint of *ph1b* and *ph1b*- 5BL-1(FL-0.55) have been shown to be similar to regions on R6 and R12 based on four sequences. The results reported here indicate that the C-*ph1b* region has maximum homology with R9 (12 sequences) and R1 (6 sequences). While the region *ph1b*-5BL-1 (FL-0.55) is syntenic to R9, R1 and R8 (Fig. 6). The data confirms the

synteny of '5L0.5' region to R9, but it also identifies corresponding sequences on other chromosomes, including 10 sequences on R7, four each on R8 and R5. Similarly, the region from 5BL11- telomere is largely similar to R3, but the there are significant sequences identified on R1, R9 and R5 as well**.**

After comparisons at the gross-level, the next question that arises is: Is there any colinearity if the comparisons are shifted to smaller regions on the chromosome? To address this question, the two small regions on chromosome 5BL, '*Ph1* gene region' and '*ph1c-ph1b* region', were compared with rice. These regions identified highly syntenous regions on rice. Eight of the nine probes mapped to the '*Ph1* gene region' identified a 450 KB homeologous region on rice chromosome 9. The marker order for the region was also the same between the two species. Probes corresponding to the '*ph1c-ph1b* region' identified homeologous regions on chromosome 9 and 7 of rice. In wheat, the two regions are adjacent to each other, with the '*ph1c-ph1b* region' towards the distal side of the long arm of chromosome (Fig. 1). This 'adjacent arrangement' seems to hold in rice too, as the orthologous '*ph1c-ph1b* region' on rice chromosome 9 and is immediately adjacent to the orthologous '*Ph1* gene region' and is towards the distal side of the chromosome (Fig. 8). This colinearity seems to break, as the orthologous '*ph1c-ph1b* region' is present on two chromosomes on rice. The orthologous region on rice chromosome 7 is approximately 2.5 MB. The data reported herein thus suggests that colinearity exists between the two species for some small regions.

The '*Ph1* gene region' holds immense importance as it contains the *Ph1* gene, the principal gene that regulates chromosome pairing in wheat. The *Ph1* gene was discovered about five decades back (Riley and Chapman 1958) and its role in chromosome pairing is well documented but it still has not been isolated because of lack of genetic information available in wheat. Perhaps the

sequence of rice might be helpful in the isolation of this very important gene, whose cloning may answer the most interesting question in allopolyploids, that is, how they behave as perfect diploids during meiosis inspite of the presence of more than one potential partner during pairing in the form of homoeologous chromosomes Abnormalities in pairing lead to meiotic defects, which may result in aneuploidy.

There have been several reports on conservation of genes across species. An interesting example is provided by recent work on *GAI* gene in arabidopsis, *Rht*-1 gene in cereals, *d8* in maize and rice *GAI* homolog (Peng et al., 1997; Peng et al., 1999; Ikada et al., 2001). Cereal homologs of *GAI* were cloned by utilizing sequence information from a rice EST, which was shown to correspond to wheat *Rht*-1 and maize *d8* genes. Similarly, arabidopsis *CONSTANS* (CO) gene (Putterill et al., 1995), which regulates flowering has homologues in rice (*Hd-1* gene) (Song et al., 1998; Yano et al., 2000) and gymnosperms. The *UNIFOLIATA* (*UNI*) gene of pea (Hoefer et al., 1997) and *FLORICACULA* gene in Antirrhinum are homologs of *LEAFY* gene (Bla'zquez and Weigel, 2000) of arabidopsis. *LEAFY* gene homologs have been identified in several species. There are many other examples such as conservation of roles of MADS-box genes (Ambrose et al., 2000; Kyozuka et al., 2000). Most of these examples account for the genes that participate in fundamental cellular functions, which are conserved in general, in different organisms, reflecting their importance in cell viability. Since, the process of meiotic chromosome pairing is well conserved among different organisms, it would be advantageous to identify homologs of *Ph1* gene(s) in related organisms such as rice. The homoeologous '*Ph1* gene region' on rice chromosome 9 was analyzed in detail to identify rice *Ph1* gene homologs. Twenty-six genes were selected based on their functional domains and motifs, out of the 91 genes present in rice *Ph1* gene region. Previous reports on the function of *Ph1* have documented its role in chromatin

reorganization (Mikhailova et al., 1998) and the microtubule attachment process (Vega and Feldman, 1998). The rice *Ph1* candidates selected in this study include genes encoding proteins which have domains mediating microtubule attachment and chromosome segregation, chromatin reorganization, meiosis and anther specific proteins, DNA binding domains, DNA repair proteins, heat shock proteins and chaperons, acetyltransferases, Protein kinases and methyltransferases.

This study will help meet the following long-term objectives (1) isolation of the *Ph1* gene; (2) understanding the mechanism of homology search; (3) conducting experiments where *Ph1* induced chromosome pairing would enable the transfer of desirable traits from alien genera into wheat; (4) It would address the issue of the importance of model species to carry out research in large genome species.

Table 1. List of useful genes present in the '5L0.5 region' on the long arm of group 5 chromosomes in the Triticeae identified by comparative mapping analysis.

Gene symbol	Gene	Chromosome Location	linked/flanked markers	Reference
nar2	Nitrate reductase-deficient (MoCo)	7 (5H)L	wg530	http://barleygenomics.wsu.edu/arnis/linkage_maps/maps-svg.html
nar5	Nitrate reductase-deficient (MoCo)	7 (5H)L	wg530	http://barleygenomics.wsu.edu/arnis/linkage_maps/maps-svg.html
var1	variegated	7 (5H)L	mwg923-mwg956	http://barleygenomics.wsu.edu/arnis/linkage_maps/maps-svg.html
mSrh	long vs. short rachilla hairs	7 (5H)L	mwg522	http://barleygenomics.wsu.edu/arnis/linkage_maps/maps-svg.html
Mlj	Reaction to Erysiphe graminis f. sp. hordei	7 (5H)L	wg564	http://barleygenomics.wsu.edu/arnis/linkage_maps/maps-svg.html
mt2	Mottled leaf	7(5H)L	Abc717[e]	[e]Kleinhoff's . 1996. BGN 27
S5	fertility factor	5RL	cdo786-psr574[g]	[g]Borner and Korzun, 1998
AadhNADP	Aromatic alcohol dehydrogenase-NADP	5RL	cdo786-psr575[g]	[g]Borner Korzun, 1998
Chl	Hybrid chlorosis	5RL	Ksu8-psr911[g]	[g]Borner Korzun, 1998, Bednarek et. Al, 2003
Ph1	pairing homeologous	5BL	cdo412-ksu8[h]	[h]Wall and law, 1971, Gill et al.1993
QCl.ocs-5A.1	Culm length	5AL	bcd1088	Kato et al,1999
QHt.ocs-5A.1	Plant height	5AL	bcd1088	Kato et al,1999

QYld.ocs-5A.2	Grain yield	5AL	psr326 /cdo412[i]	Kato et al., 2000
QEgw.ocs-5A.2	Ear grain weight	5AL	bcd1088	Kato et al., 2000
QEgw.ocs-5A.3	Era grain weight	5AL	psr326 /cdo412	Kato et al., 2000
QSpn.ocs-5A.2	Spikelet number per ear	5AL	bcd1088 /bcd9	Kato et al., 2000
QSpn.ocs-5A.3	Spikelet number per ear	5AL	psr326 /cdo412	Kato et al., 2000
YLD	Grain yield	7 (5H)L	abc302-mSrh	Zhu et al, 1999
KP	kernel plumpness	7 (5H)L	mSrh /abc717	Marquez-Cedillo et al. 2000
HD	heading date	7 (5H)L	mwg530	Laurie et al. 1995
HD	heading date	7 (5H)L	abc168-abc717	de la Pena et al.1999
HT	Plant Height	7 (5H)L	cdo57 /mSrh	Hays et al.1993
RL	Root length	7 (5H)L	wg564	Jefferies et al. 1999
SD	Straw diameter	7 (5H)L	mwg583	Kjaer et al. 1995
SRR	Stripe rust resistance	7 (5H)L	abc168	Chen et al. 1994
FHB	Fusarium head blight	7 (5H)L	abc168 /abc717	de la Pena et al.1999
ST(s)	salt tolerance in seedlings	7 (5H)L	wg364 /abc324	Mano and Takeda 1997
CT	cold tolerance	7 (5H)L	wg364b /wg1026	Hayes et al. 1993(a)
CT	cold tolerance	7 (5H)L	Wg364b /mR	Hayes et al. 1993(a)
LT50	Low Temperature Tolerance	7 (5H)L	Wg364b /mR	Hayes et al. 1993(a).

WS	winter survival	7 (5H)L	wg364b /Mr	Pan et al. 1994.
SR	Shoot regeneration	7 (5H)L	cdo573-mSrh	Mano and Takeda. 1996
GMBGase	green malt β-Glucanase activity	7 (5H)L	wg530	Han et al. 1995; Zwickert-Menteur et al. 1996; Zale et al. 2000. BGNL. Vol 30
KMBGase	Kilned malt β-Glucanase activity	7 (5H)L	wg530	Han et al. 1995; Zwickert-Menteur et al. 1996; Zale et al. 2000. BGNL. Vol 30

Table 2: The chromosome location of orthologous sequences of wheat genes mapped to 5BL in arabidopsis as well as rice along with the score and e-values

S. No.	Marker Accession No.	Arabidopsis Chromosome number	Score	e-value	Rice Chromosome number	Score	e-value
1	AA231753	5	85.9	3.00e-16	7	88.6	5.00E-17
2	abc168	-	-	-	8	52.8	3.00E-06
3	abc717	4	34.7	8.9e-01	4	34.7	1.5
4	abc302	1	163	1e-39	9	194	1e-48
5	abg314	-	-	-	-	-	-
6	abg387	1	69.7	9e-11	6	80.5	5e-14
7	AF302673	3	1337	0	5	368	6e-100
8	bcd351	3	33.9	1.8	6	90.9	1e-17
9	bcd508	5	110	4e-23	10	113	5e-24
10	BE352611	3	47.4	3.00e-04	-	-	-
11	BE403221	2	258	7.00e-68	1	281	6.00E-75
12	BE403518	5	229	2e-59	2	142	5e-33
13	BE403761	5	334	1e-90	-	-	-
14	BE404437	3	253	3.00E-66	3	270	2.00E-71
15	BE404454	5	130	1e-29	9	201	6e-51
16	BE405060	5	199	5.00E-50	12	224	1.00E-57
17	BE405358	3	132	5.00E-30	9	223	3.00E-57
18	BE406545	2	119	4.00E-26	10	120	3.00E-11
19	BE406569	4	109	2.00E-23	7	110	1.00E-23
20	BE406592	-	-	-	-	-	-
21	BE406612	1	57	2.00E-07	5	69.3	3.00E-11
22	BE406996	2	69.7	2.00E-11	1	69.7	2.00E-11
23	BE425296	5	33.5	2	12	47.8	1.00E-04
24	BE425968	2	166	6.00E-40	3	185	1.00E-45
25	BE426080	5	266	3.00E-70	9	302	4.00E-81
26	BE426712	4	53.5	2e-6	2	78.2	8e-14
27	BE442600	4	261	6.00E-69	2	261	4.00E-69
28	BE442676	3	324	1.00E-87	9	371	1.00E-01
29	BE442763	4	288	6.00E-77	2	301	9.00E-81
30	BE442814	-	-	-	-	-	-
31	BE442892	1	55.5	6.00E-07	9	78.6	6.00E-14
32	BE442893	-	-	-	4	68.2	2.00E-10
33	BE442918	1	219	4.00E-56	9	310	1.00E-83

34	BE443021	5	59.3	3.00E-08	-	-	-
35	BE443187	1	120	3.00E-26	9	213	3.00E-54
36	BE443423	-	-	-	-	-	-
37	BE443430	3	188	6.00E-47	7	226	2.00E-58
38	BE443610	5	259	4.00E-68	3	315	4.00E-85
39	BE443692	1	94	1.00E-18	2	139	2.00E-32
40	BE443937	5	244	8.00E-64	11	307	1.00E-82
41	BE444353	1	181	1.00E-44	-	-	-
42	BE445208	-	-	-	9	42.7	0.003
43	BE445248	5	95.1	6.00E-19	9	120	1.00E-26
44	BE445260	5	359	2.00E-98	7	359	2.00E-98
45	BE445282	1	229	4.00E-59	9	281	6.00E-75
46	BE445424	5	95.1	6.00E-19	9	120	1.00E-26
47	BE445873	2	93.2	2.00E-18	9	113	1.00E-24
48	BE471089	3	233	1.00E-60	3	283	1.00E-75
49	BE471214	-	-	-	2	32	70.5
50	BE488364	-	-	-	-	-	-
51	BE488679	2	117	1.00E-25	1	78.2	7.00E-14
52	BE488792	4	74.7	8.00E-13	9	95.1	6.00E-19
53	BE489227	-	-	-	-	-	-
54	BE493886	3	74.7	2.00E-12	4	171	2.00E-41
55	BE494426	2	175	5.00E-43	9	242	4.00E-63
56	BE495277	5	196	2.00E-49	9	240	1.00E-62
57	BE495790	4	146	2.00E-34	8	191	6.00E-48
58	BE495846	1	125	3.00E-28	1	102	3.00E-21
59	BE496112	5	191	7.00E-48	2	187	1.00E-46
60	BE496891	1	35	1.1	3	39.3	0.036
61	BE496925	5	52	7.00E-06	1	76.3	3.00E-13
62	BE497111	5	118	8.00E-26	5	44.3	0.002
63	BE497595	1	171	7.00E-42	3	192	3.00E-48
64	BE499135	5	118	6.00E-26			
65	BE499599	2	285	5.00E-06	9	401	7.00E-11
66	BE499659	-	-	-	-	-	-
67	BE499738	1	284	5.00E-75	11	281	6.00E-75
68	BE499843	1	270	2.00E-71	5	307	1.00E-82
69	BE500170	1	143	4.00E-33	3	211	1.00E-53
70	BE500452	-	-	-	3	64.3	1.00E-09
71	BE500513	1	108	4.00E-23	11	97.1	1.00E-19
72	BE500727	1	68.2	7.00E-11	8	107	8.00E-23
73	BE500811	1	233	3.00E-60	9	334	1.00E-90
74	BE500827	1	197	8.00E-50	2	211	5.00E-54

75	BE517902	1	172	3.00E-42	3	33.9	2.1
76	BE590622	1	90.9	1.00E-17	3	156	2.00E-37
77	BE590997	3	349	3e-95	8	363	1e-99
78	BE591416	-	-	-	3	88.2	2.00E-16
79	BE591423	3	61.6	7.00E-09	9	111	6.00E-24
80	BE591784	1	48.1	9e-05	1	45.4	6e-04
81	BE604829	1	31.6	7.4	1	32	5.7
82	BE604883	1	89.4	2.00E-16	1	89.4	3.00E-17
83	BE605032	5	57.4	2.00E-07	-	-	-
84	BE605135	4	113	2.00E-24	5	222	3.00E-57
85	BE636942	5	84	1.00E-15	9	142	3.00E-33
86	BE637838	5	333	1.00E-90	7	364	7.00E-100
87	BE637885	5	147	1.00E-34	7	199	3.00E-50
88	BE637923	4	75.9	4.00E-13	7	158	6.00E-38
89	BE638037	1	192	3.00E-48	3	249	2.00E-65
90	BF200863	-	-	-	-	-	-
91	BF201857	3	198	7.00E-50	11	272	3.00E-72
92	BF291672	1	121	8.00E-27	3	178	6.00E-44
93	BF293157	1	145	7.00E-34	3	226	3.00E-58
94	BF428826	4	66.2	6.00E-10	-	-	-
95	BF473933	4	187	3.00E-46	9	189	4.00E-47
96	BF474090	1	177	9.00E-44	1	141	7.00E-33
97	BF474342	3	307	1.00E-82	7	367	2.00E-103
98	BF474840	5	257	8.00E-68	9	285	2.00E-76
99	BF478520	3	109	3.00E-23	9	206	2.00E-52
100	BF478849	-	-	-	-	-	-
101	BF478950	4	75.1	1.00E-12	9	79.7	4.00E-14
102	BF483038	-	-	-	-	-	-
103	BF483731	1	71.6	6.00E-12	7	98.2	6.00E-20
104	BF483771	3	226	2.00E-58	3	239	1.00E-62
105	BF484437	-	-	-	5	277	1.00E-73
106	BF484440	5	60.5	1.00E-08	9	95.5	4.00E-19
107	BF484813	3	83.6	3.00E-15	3	181	1.00E-44
108	BG274124	3	31.6	7.5	9	63.9	1.00E-09
109	BG274279	3	237	1.00E-61	9	243	2.00E-63
110	BG313323	3	51.6	7.00E-06	3	211	5.00E-54
111	BG606780	5	37	0.35	1	35.8	0.78
112	BM137728	-	-	-	3	31.2	9.9
113	BM138069	3	41.6	0.007	1	76.3	4.33E-13
114	BM140591	5	184	1.00E-45	1	97.4	2.00E-19
115	cdo348	3	62	6e-29	1	118	7e-26
116	cdo388	3	43.1	0.0003	5	48.1	9e-05

117	cdo412	4	116	3e-25	9	176	2e-43
118	cdo786	5	173	2e-42	9	175	5e-43
119	ksu8	1	80	2e-17	7	103	4e-21
120	ksuS1	-	-	-	-	-	-
121	mwg522	5	40.8	0.008	8	50.4	2e-05
122	mwg923	-	-	-	-	-	-
123	mwg956	-	-	-	-	-	-
124	mwg624	-	-	-	5	94.4	1e-18
126	psr128	3	101	9e-21	8	103	2e-12
127	psr574	-	-	-	-	-	-
128	unl4	5	254	2e-66	9	272	7e-72
129	wg564	-	-	-	-	-	-
130	wg530	-	-	-	4	100	5e-20
131	cdo57	5	98.2	7e-20	9	114	1e-24

Table 3 : Rice - wheat relationship for chromosome arm 5BL.

Wheat Region/ Rice Chr.	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12
C- dup.Ph1	6	1	-	-	3	-	2	-	12	1	3	2
Ph1b-5BL-1	2	-	-	-	-	-	-	2	3	-	-	1
5L0.5	-	2	1	2	4	2	10	4	21	-	-	-
Ph1 gene region	-	-	-	-	-	-	1	-	7	-	-	-
Ph1c- ph1b region	-	-	-	-	-	-	5	-	2	-	-	-
Ph1b- 5BL-11	-	2	1	2	4	2	4	4	12	-	-	-
5BL-11 -T	9	1	18	1	3	-	2	-	4	1	2	-

Table 4: Wheat- arbidopsis relationship for chromosome arm 5BL.

Wheat Region/ Arabidopsis Chr.	A1	A2	A3	A4	A5
C- dup.Ph1	7	5	3	4	5
Ph1b-5BL-1	2	-	1	2	1
5L0.5	9	1	7	6	17
Ph1 gene region	-	-	1	1	6
Ph1c- ph1b region	3	-	3	-	2
Ph1b- 5BL-11	6	1	3	5	9
5BL-11 -T	18	2	9	1	6

Table 5: Rice –Arabidopsis relationship for chromosome arm 5BL of wheat.

Rice/ arabidopsis	A1	A2	A3	A4	A5	X	Total
R1	6	3	2	-	4	2	17
R2	1	-	-	-	1	-	4
R3	9	1	5	1	1	2	19
R4	2	-	-	-	-	1	3
R5	4	-	2	2	1	1	10
R6	-	-	-	-	-	2	2
R7	5	-	3	1	5	-	14
R8	1	-	2	1	1	1	6
R9	6	4	5	6	19	1	40
R10	-	1	-	-	1	-	2
R11	2	-	1	-	2	-	5
R12	-	-	-	-	2	1	3
X	-	-	-	2	-	-	
Total	36	9	20	13	36	11	

Table 6: List of *Ph1* gene candidates with putative function and their wheat EST homologs alongwith their putative function.

Rice BAC	Rice Candidate	Putative Function	Wheat EST Homolog	Blastx Function
AP006169	LOC_Os9G29930	Helix-loop helix (bHLH) protein	CV779959	bHLH protein family like
AP005759	LOC_Os9G29960	DNA binding protein(well conserved)	CN106070	Unknown protein
AP005759	LOC_Os9G29970	Microtubule attachment, chromosome segregation	CK161428	Holocentric chromosome binding protein, myosin heavy chain like protein
AP005759	LOC_Os9G30070	Basic-leucine zipper (bZIP) transcription factor	CD931016	Hypothetical protein
AP005759	LOC_Os9G30010	Methyltransferase, DNA dependent transcription regulation	BE415257	SunL protein like, Methyltransferase
AP005759	LOC_Os9G29950	WD40 (multi-protein complex assembly)	BE400277	Putative oligopeptidase, protease
AP005759	LOC_Os9G30090	WD40 like	CD888527, BE492313	WD40 reapeat family like protein, hypothetical protein
AP005759	LOC_Os9G30150	Protein Kinase	BQ238899, CN012003	Hypothetical protein, Putative protein kinase
AP005759	LOC_Os9G30160	Zinc finger, C3HC4 type (RING finger): mediates protein-protein interactions	CA594470	Putative RING Zinc finger protein
AP005633	LOC_Os9G30170	F-box protein interaction domain	CF133455	Hypothetical protein
AP005633	LOC_Os9G30180	F-box protein interaction domain	CD865309, BQ841696, CK207845	Hypothetical, F-box family protein, Hypothetical protein, splicing coactivator subunit SRm300
AP005633	LOC_Os9G30190	Protein Kinase	CA624450	Putative protein kinase
AP005633	LOC_Os9G30200	Expressed hypothetical protein	BQ842302	Hypothetical protein
AP005392	LOC_Os9G30270	Zinc finger protein	CX601356	Unknown, transposon

				like protein
AP005392	LOC_Os9G30310	Histone like transcription factor	CK162331	Putative transcription factor
AP005392	LOC_Os9G30320	Anther-specific protein	BE498862	Anther specific protein
AP005392	LOC_Os9G30380	Meiosis specific protein	CD920567, BJ299326	DNA repair protein, unknown protein Nuclear protein, unknown protein
AP005392	LOC_Os9G30400	WRKY DNA binding protein	CD902089, CA714784	WRKY DNA binding protein
AP005392	LOC_Os9G30420	Conserved domain of unknown function	CK167050	Unknown protein, UBX domain, predicted protein of unkwon function
AC108756	LOC_Os9G31050	Chromatin reorganization	-	-
AC108756	LOC_Os9G31140	Zinc finger protein	CK209862*	Putative Zinc finger protein
AC108756	LOC_Os9G31150	RING Zinc Finger	BF293596	Putative Zinc finger protein, WIP1 protein
AC108756	LOC_Os9G31210	Protein Kinase	BF484681, CD907358	Putative protein kinase like
AC108758	LOC_Os9G31300	Helix-loop-helix DNA-binding domain	CK196088, BQ752762	Putative transcription factor, unknown protein Putative helix-loop-helix protein, unknown protein
AC108758	LOC_Os9G31390	bZIP transcription factor-like protein	CD921605, BQ240568*	bZIP transcription factor like transcription factor like
AC108758	LOC_Os9G31310	Acetyltransferase-like protein	CV764450	Ubiquitin conjugating enzyme

* : not a good match

Centromere

PB, dupPh1
PB, *ph1b*
PB *ph1c*
5BL-1
DB, *ph1c*
DB, *ph1b*
Ph1 gene region
DB, dupPh1
5BL-11

5BL1.7
5L0.5 Region
5BL2.5

abc168a, abc706, BE403221, BE403518, BE403761, BE404462a, BE404462b, BE406545, BE406612, BE406996, BE425296, BE426080a, BE442814, BE442892, BE443021, BE443692, BE443937a, BE443937b, BE445260, BE488679, BE496925a, BE496925b, BE499135, BE499599, BE500811, BE591423, BF201857, BF473933, BF474840, BF478520, BF478950, BG274279, cdo348a, cdo388a, cdo786b, fbb121a, ksud42, mwg624, unl1, unl3a, unl5, unl6

fbb156, ksuA1

BE405060, BE442918, BE443187

abc302, BE483335, BE495790, BE590997a, BE590997b, cdo412a, psr128, psr574, wg564a

Ph1, bcd1088, bcd1949, BE636942a, BF474342, cdo412b, cdo786a, cdo1090, ksus1, unl4, Egw2, Egw3, Ht, S5, Spn2, Spn3, Yld

BE404454, BE443430a, BE443430b, BE405358, BE636942b, ksu8a, ksu8b, ksu8c, mwg592, wg564b, Mlj RL

AA231753, abc168a, abc717, abg314, abg387a, AF302673, bcd307a, BE426080b, BE442600, BE442676, BE442763, BE442893, BE444353, BE445208, BE445248a, BE445424, BE484133, BE493886, BE494426a, BE494426b, BE497111, BE499848, BE500727, BE605135, BE637838, BE637923, BF484440a, BF484440b, cdo385, fba127a, gwm371, gwm499, glk614, ksu60, ksug44b, ksuq10, ksuq35, mbg63, mgb10, mgb174, mgb191, mgb301, mgb341, mwg52a, mwg52b, mwg522, mwg805, mwg923, mwg956a, mwg956b, mwg2237, tag614, ubp25, FHB, HD, HT, KP, mSrh, nr2, SR, SRR, var1, YLD

abg069, bcd351a, bcd351b, cdo786c, cdo1192, mwg583, mwg768, mwg923, wg364, wg530, psb85, AldhNADPCT, CT, GMBase, HD, KMBase, LT50, nar2, nar5, SS, SD, ST(s), WS

abg055, abg314, abg387b, BE202009, BE203136, BE352611, BE404437, BE406592, BE425968, BE426712, BE443423, BE443610, BE445248b, BE445282, BE445873, BE471089, BE471214, BF474090, BE478964, BE482522, BE484028, BE485285, BE4?364, BE488792, BE489227, BE495277, BE495846, BE496112, BE496891, BE497595, BE49659, BE499738, BE500170, BE500452, BE500513, BE500827, BE517902, BE590622, BE591416, BE604829, BE604883, BE605032, BE637885, BE638037, BF200863, BF293157, BF?1672, BF428826, BF478849, BF483038, BF483731, BF483771, BF484437, BF484813, BG?124, BG313323, BG606780, BM137728, BM138069, BM140591, bcd307, bcd508, cdo348b, cdo388b, fbb121b, fba127b, mwg914, mwg2121, unl2, unl3b

abc706, cdo348, psr128, psr574, bcd157, cdo412, cdo57, bcd1949, ksus1, bcd1088, cdo1090, ksu8, fbb156, ksuA1, fba127, Xbcd1140, Xmwg52, Xcdo1192, Xmwg522, Xbcd351, Xabg055, Xwg530, Xmwg624, Xpsr150, Xpsb85, Xab473, Xbcd508, Xmwg914

C bands
Minor C bands
'DB'- Distal Breakpoint
'PB'- Proximal Breakpoint

(a) (b) (c) (d)

Fig. 1: (a) :C- banding karyotype of chromosome 5B of Wheat, (b): 5BL arm of wheat, (c): Physical map of '5L0.50' region, (d): Wheat genetic linkage map.

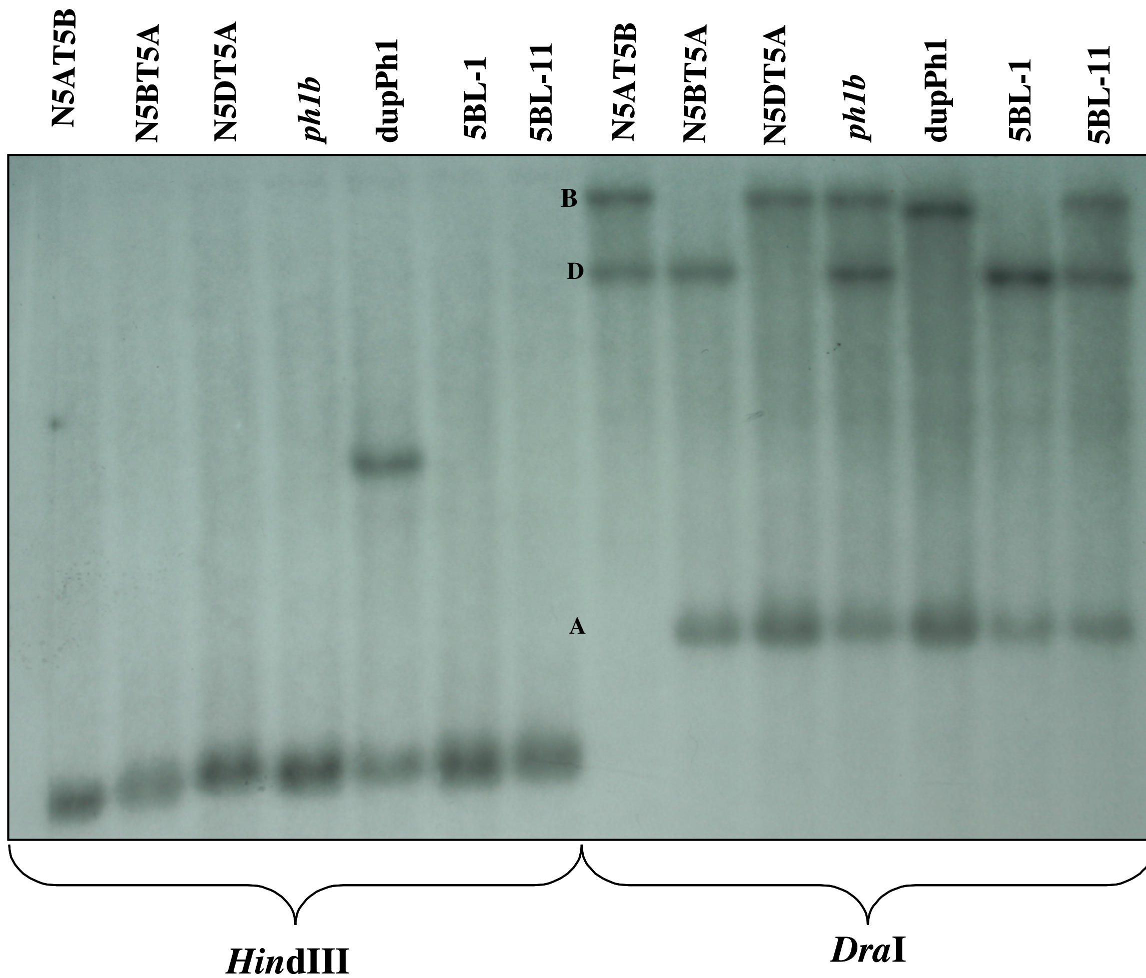

Fig. 2: Band pattern of probe mwg522 mapped to the '5L0.5 region', in aneuploid stock of Wheat, digested with two enzymes *Hin*dIII and *Dra*I.

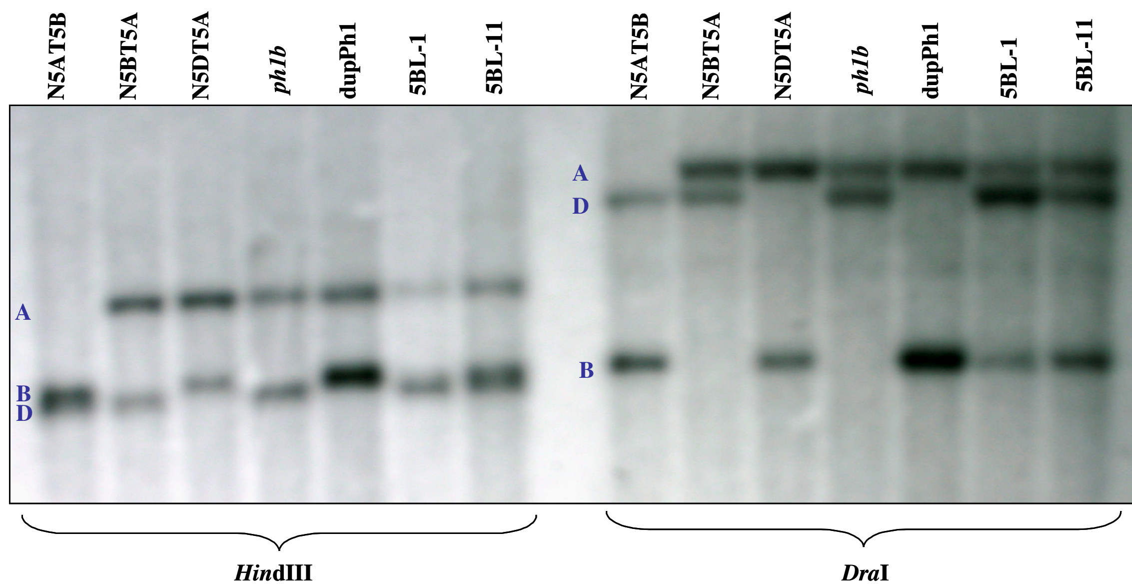

Fig. 3: Banding pattern of probe abc302 in aneuploid stock of Wheat, digested with two enzymes *Hin*dIII and *Dra*I

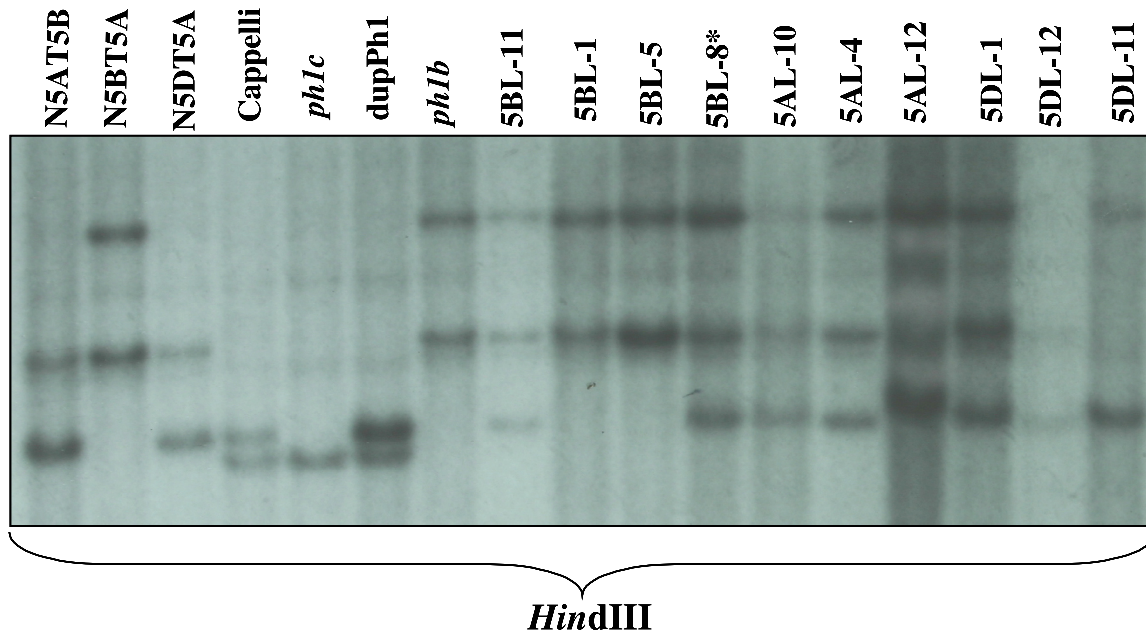

Fig. 4: Band pattern of probe *BE636942* in aneuploid stock of Wheat, digested with enzyme *Hin*dIII. (5BL-8*: The band pattern for this deletion line was not scored as the fraction length of this deletion is 0.52, but it showed presence of B-band for all the markers which were missing in both 5BL-1 and 5BL-5).

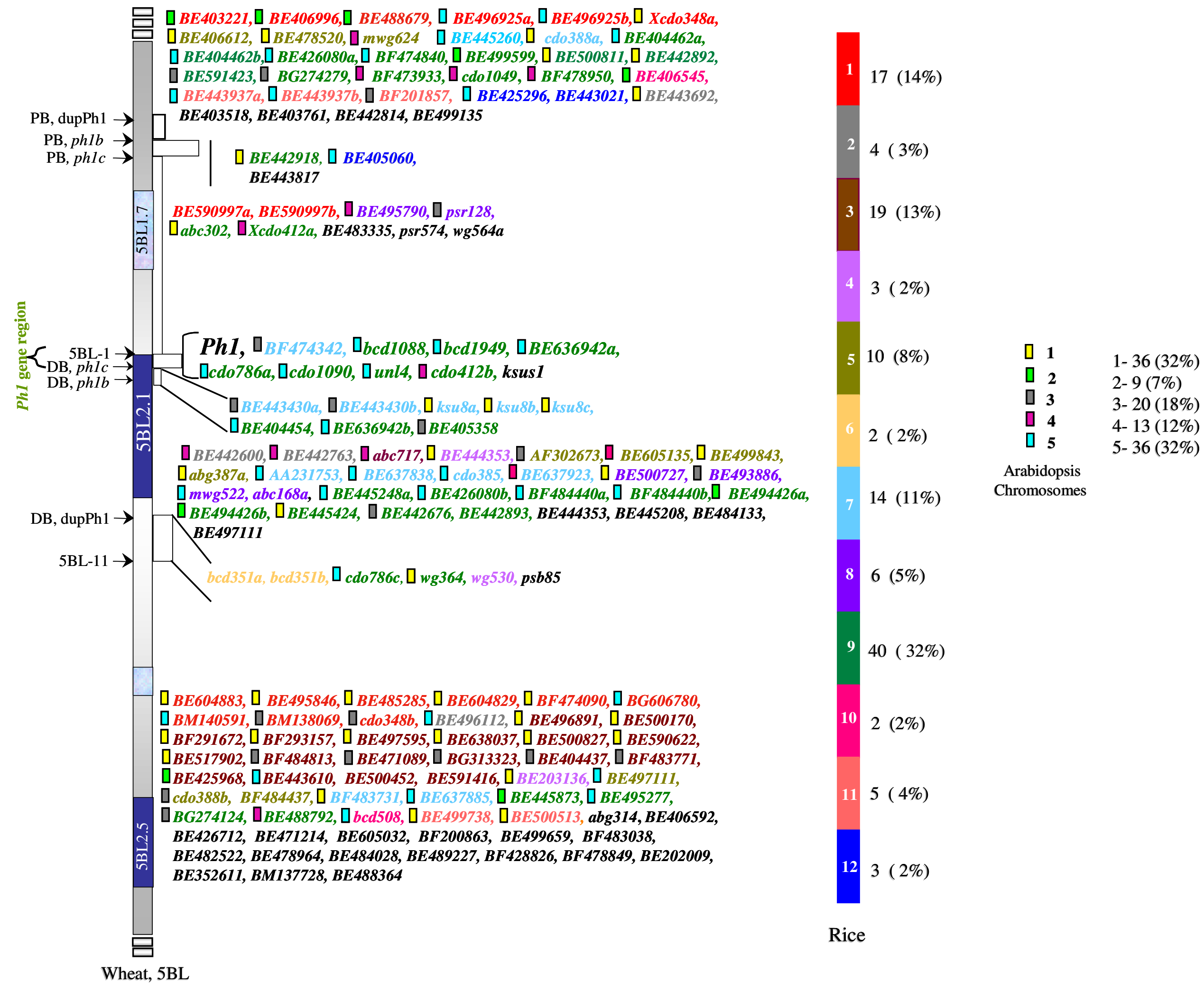

Fig. 5: Wheat-Rice-Arabidopsis Synteny on chromosome arm 5BL. All 12 rice chromosomes and five arabidopsis chromosomes are represented by blocks of different colors. The probes for which no homologs were found are written in black. *BE404454:* This probe has its homolog on rice chromosome 9(represented by same font color as chromosome 9 block) and arabidopsis chromosome 5 (represented by small block in front of it). The number and percentage of the markers giving hit on different chromosomes are indicated on the side of each color coded block.. The number of orthologous sequences identified for different rice and arabidopsis chromosomes are indicated in both numbers and percentages in front of each color coded chromosome block.

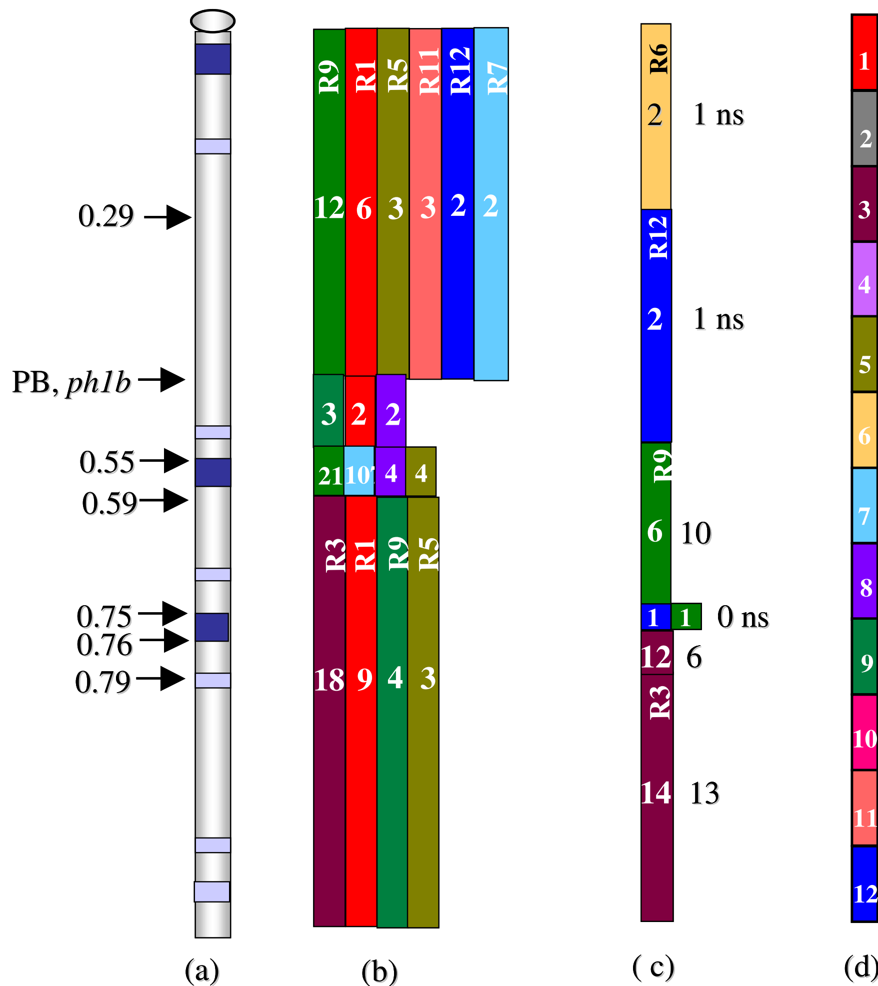

Fig. 6: (a)Wheat 5BL arm showing different regions delimited by breakpoints of different deletion lines, (b) rice-wheat synteny view: Each region on 5BL arm is color coded according to rice chromosome matching a significant number of wheat ESTs mapped to that region, the number of matches is indicated inside the colored block (c) older view;The rice-wheat synteny view for 5BL arm as published in earlier studies, (d) Color coded rice chromosomes: All twelve rice chromosomes are represented by different colors. Chromosome number for each color is indicated inside the colored block

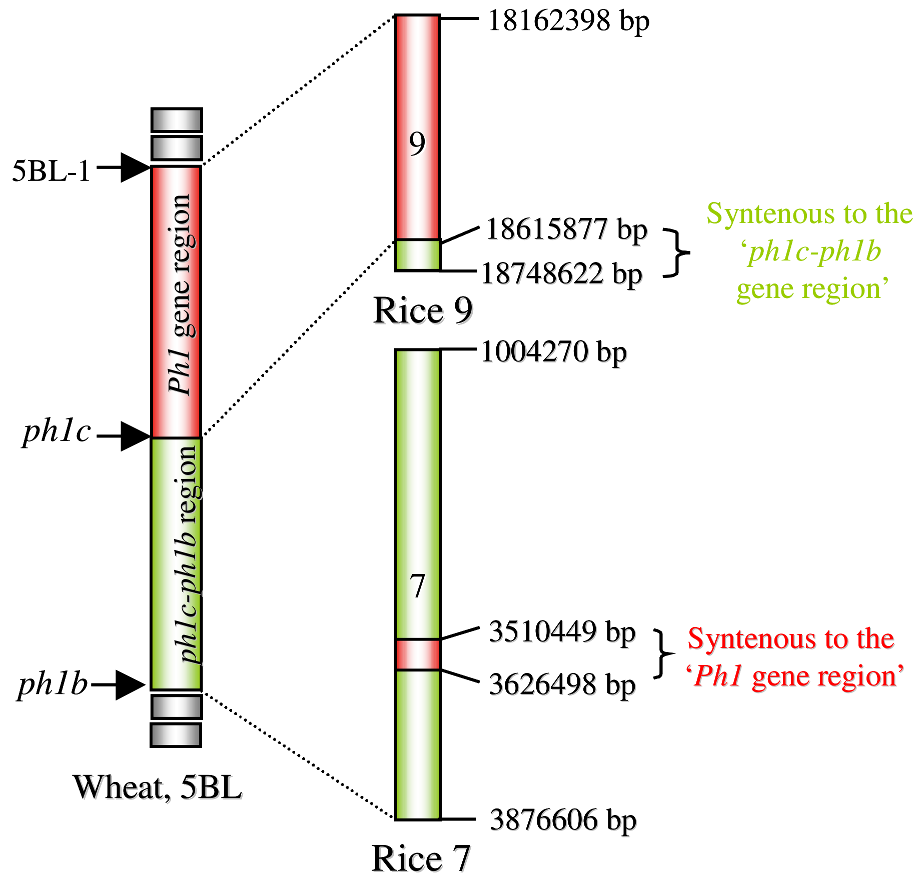

Fig. 7: Wheat-Rice Synteny for two mini gene-rich regions present in '5L0.5 region' on 5BL. Regions on chromosome 9 and 7 of Rice, syntenous to '*Ph1* gene region' and '*ph1c-ph1b* region' are color coded in same colors as the two regions on wheat chromosome.

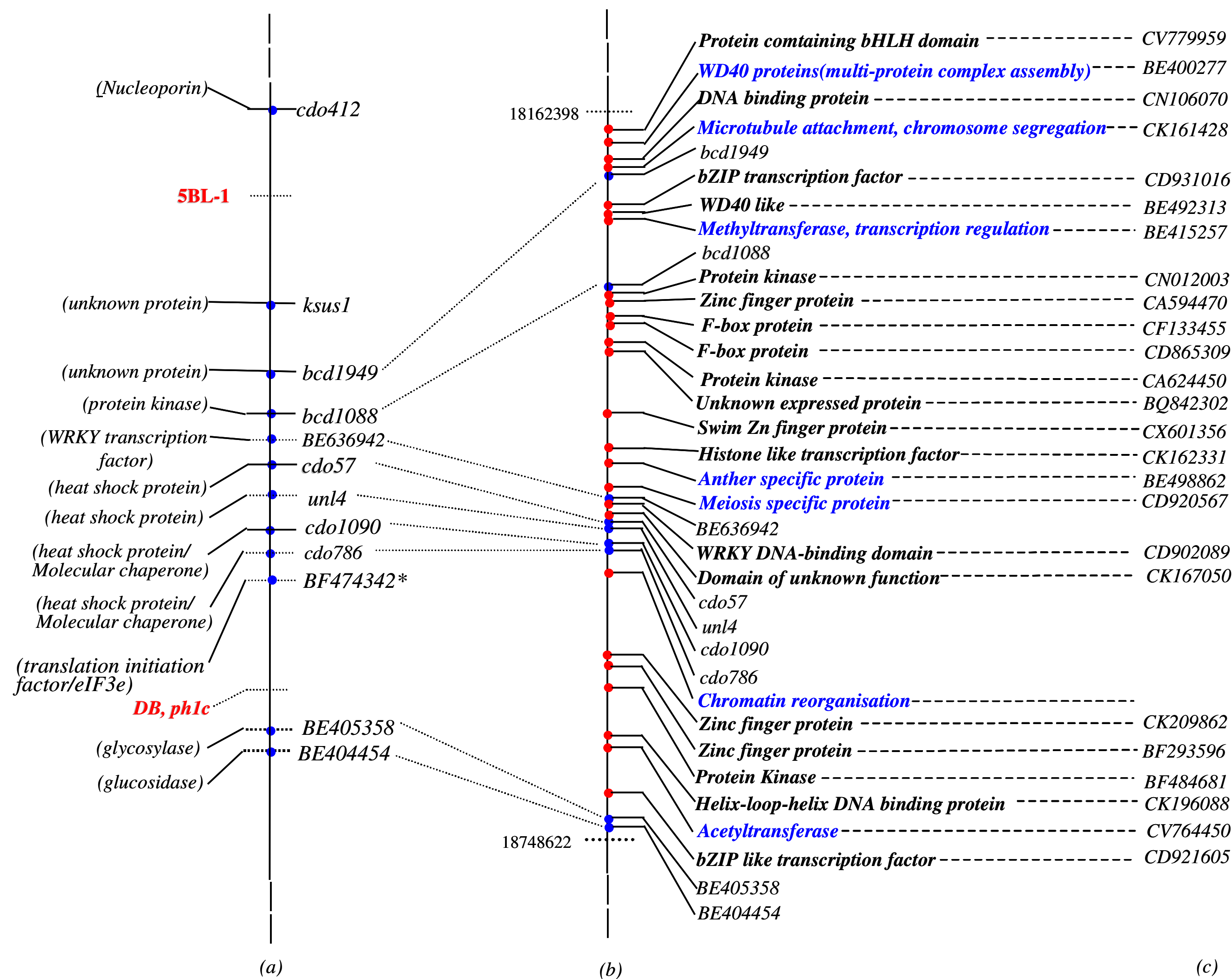

Fig. 8: (a): Genetic Map of '*Ph1* gene region', The probes for which genetic mapping data was not available are indicated by dashed lines for their location. They were placed to the location shown in the map based on their physical map position as well as their orthologous sequence on rice, *: map position not confirmed (b): 450 kb region on rice '*Ph1* gene region' of wheat.: blue dots indicate the location of mapped wheat markers on the rice '*Ph1* gene region' , while red dots indicate the rice genes present in the 450 Kb region, The genes shown in blue letters are the highest priority candidates based on their function for *Ph1* gene(c): Wheat EST homologs identified for Rice genes

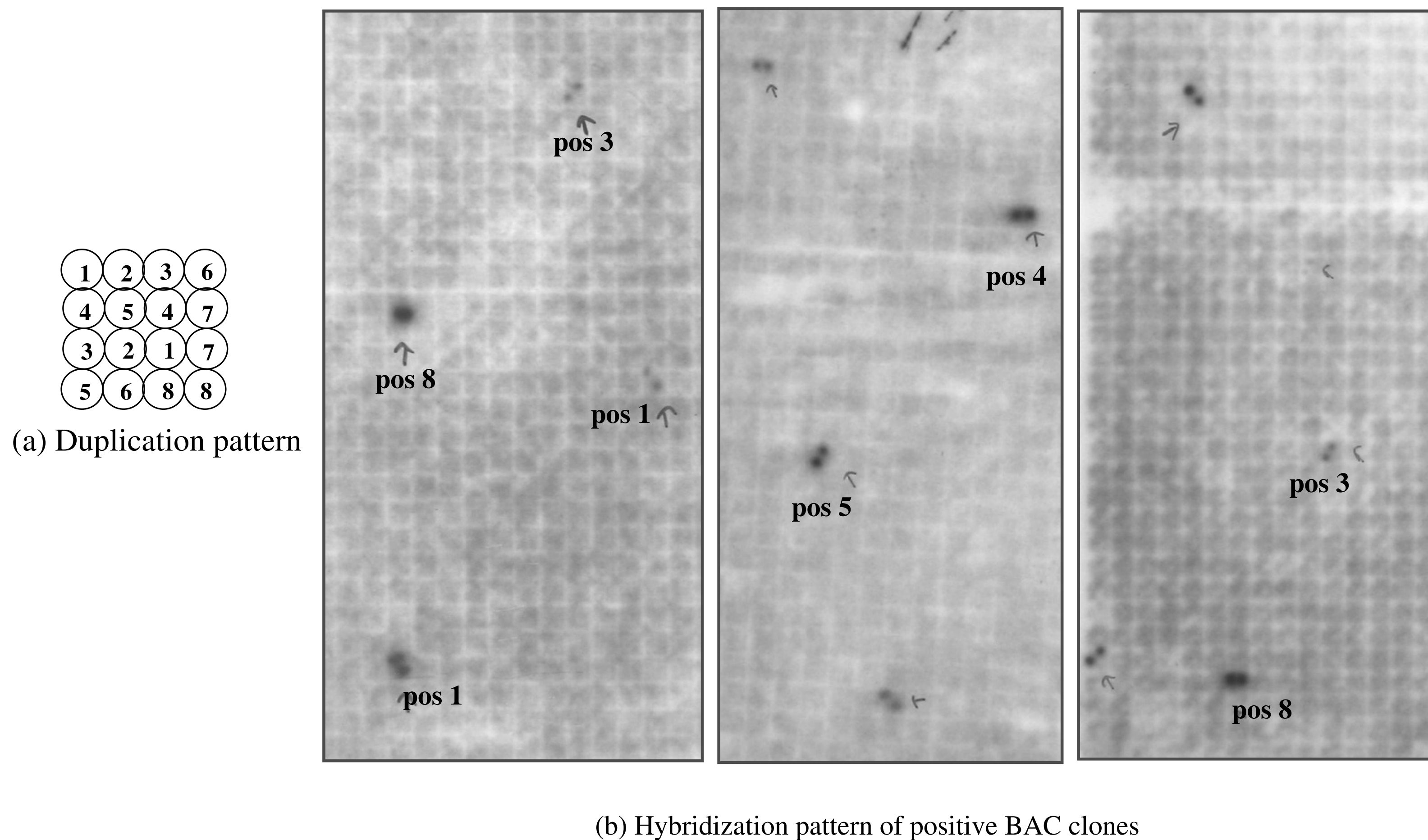

Fig. 9: (a) Duplication pattern of eight BAC clones in each square on the high density BAC library filters; (b) The different hyberidization patterns of the BAC clones identified by southern analysis of the wheat and barley BAC libraries using '*Ph1* gene probes'. Based on the pattern of the 'dots' in each square and comparing it with duplication pattern of the BAC clones as shown in (a), the hyberidizing BAC clones are assigned 'pos' numbers, which are used to decode addresses of the BAC clones in a library.

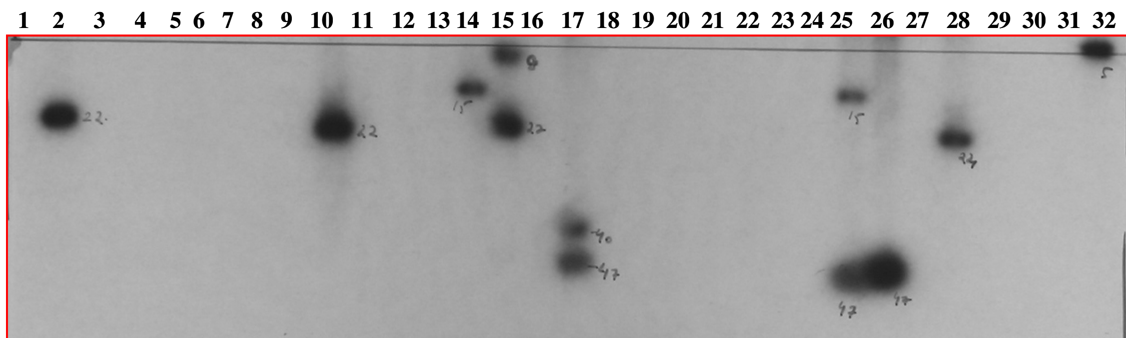

Fig. 10: Autoradiogram showing banding pattern of positive clones with probe cdo1090 in membrane containing 32 clones. Positive clones are categorized into different pattern sets based on the relative distance from comb and number of bands. Clones showing same pattern are put together in one category.

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