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Characterization of Quantitative Loci for Morphological and Anatomical Root Traits on the Short Arm of Chromosome 1 of Rye in Bread Wheat

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Sundrish Sharma
Riverside
August 28, 2009

DEDICATIONS

To my beloved grandmother

Late Smt. Sheela Devi

&

My parents

Mr. Sominder Sharma & Mrs. Sudesh Sharma,

For all the hardship they faced in raising their children.

ABSTRACT OF THE DISSERTATION

Characterization of Quantitative Loci for Morphological and Anatomical Root Traits on the Short Arm of Chromosome 1 of Rye in Bread Wheat

by

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Bread wheat (*Triticum aestivum* L.) is the second most cultivated cereal crop after rice. Many present day bread wheats carry a centric rye-wheat translocation 1RS.1BL in place of chromosome 1B. The increased grain yield of translocation lines is positively associated with root biomass. To map loci controlling root characteristics, homoeologous recombinants of 1RS with 1BS were used to generate an integrated genetic map comprised of 20 phenotypic and molecular markers, with an average spacing of 2.5 cM. To identify the chromosomal region associated with rooting ability, root phenotypic data were subjected to Quade analysis to compare genotypes. The distal 15% of the rye 1RS arm may carry QTL for greater rooting ability in bread wheat.

To identify QTL for individual root traits, a phenotyping experiment was conducted involving recombinants from each marker interval of the 1RS-1BS genetic

map. An empirical Bayes method was applied to estimate additive and epistatic effects for all possible marker pairs simultaneously in a single model. This method has an advantage for QTL analysis in minimizing the error variance and detecting interaction effects between loci with no main effect. Four common regions were identified to involve a total of 15 QTL effects, six additive and nine epistatic, for different root traits in 1RS wheat. Three of four regions were localized in the distal 15% of the 1RS arm.

The effect of different dosages (0 to 4) of the 1RS translocation on root morphology and anatomy in bread wheat was determined. The F_1 hybrid with single dose of 1RS and 1AS arms showed heterosis for root and shoot biomass. Root biomass was positively associated with increase in dosage of 1RS. This study also provided evidence of the presence of gene(s) influencing root anatomical traits. The central metaxylem vessel diameter was negatively associated with increasing 1RS dosage. Wheat genotypes with higher number of 1RS translocation arms may have inherent morphological and anatomical advantage over normal bread wheat to survive under stress conditions.

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CHAPTER 1

General Introduction

Wheat

Bread wheat is the world's second most cultivated cereal crop after rice. It is a staple food crop of the world's human population. Its consumable products range from raw grains to processed foods such as bread, macaroni, pasta, noodles, and wheat flour etc. It is cultivated under a wide range of climatic conditions ranging from within the Arctic Circle to higher elevations near the equator (Curtis 2002). The International Grains Council (IGC) forecasts world wheat production to reach 683 m tonnes in the 2008/09 season, up from 610 m tonnes in 2007/08 (http://www.igc.org.uk/en/grainsupdate/igcsd.aspx). In the United States (2008-2009) wheat is grown on an area of 22.54 million hectares with an estimated production of 68.03 million metric tonnes per year (Source: USDA).

Wheat is a member of the grass family, *Poaceae*, tribe *Tritiaceae*, and genus *Triticum*. There are different species in this genus which also differ in their ploidy level from diploid, tetraploid to hexaploid. Tetraploid durum (*Triticum turgidum* L.) and hexaploid bread wheat are the main wheats of commerce. Hexaploid wheat (*Triticum aestivum* L., 2n = 6x = 42, genome formula BBAADD), has the basic chromosome number x = 7, which is a characteristic feature of the tribe Triticeae. Bread wheat has a genome size of about 16,000 Mbp (Rota and Sorrells 2003) as compared to ~5000 Mbp of the human genome (McPherson et al. 2001), ~ 400 Mbp of rice (*Oryza sativa* L., Goff

et al. 2002), and ~100 Mbp of the *Arabidopsis* genome. The DNA of wheat contains almost 80% repetitive sequences (Smith and Flavell 1975).

The green revolution in the 1960s increased grain yield of bread wheat and rice (*Oryza.sativum*) many fold. This was attributed in part to use of stem-dwarfing genes transferred to wheat and rice cultivars. The wheat genotypes that Borlaug (1968) used in his breeding program contained dominant stem dwarfing genes (*Rht*) located on homeologous group 4 chromosomes. Dwarfing genes in those cultivars provided the high responsiveness to water and fertilizer uptake and use-efficiency, and tolerance to lodging that resulted in higher grain yield.

In contemporary studies, the aneuploid stocks of bread wheat were developed in cultivar 'Chinese Spring' and were a powerful tool for wheat genetic studies (Sears 1954, 1966, Sears and Sears 1978), and for localization of genes on to chromosomes and chromosomes arms (Mclntosh 1988). Further, Endo (1988) developed even more powerful novel aneuploid stocks, called deletion stocks. He reported a unique genetic system for the production of deletion stocks with various sized terminal deletions in individual chromosome arms. This proved very useful for subarm localization of genes (Endo et al. 1991; Mukai et al. 1990; 1991; Hohmann et al. 1994; Kota et al. 1993; Werner et al. 1992). Gill et al. (1993) described a rapid region-specific chromosome mapping strategy using deletion stocks and identified telomeric gene rich regions. Later, they studied recombination and gene distribution in chromosome 1 in wheat by

comparing physical and genetic maps. Five gene rich clusters were identified containing all of the 14 agronomic traits present on group 1 chromosomes (Gill et al. 1996).

Although wheat molecular genetic research has been retarded by polyploidy and the lack of full DNA sequence information, wheat has been extensively used for genetic mapping using molecular markers. Expressed sequence tags (EST) have been a powerful tool for designing polymorphic primers in genetic mapping experiments. Until now, a total of about 1 million EST have been submitted to the NCBI database of EST (dbEST) (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html). There is a vast information database in GrainGenes, for the *Triticeae* and *Avena* tribes (http://wheat.pw.usda.gov), which is a great resource for mapping and sequence information. Various genetic maps of wheat, from genome wide to arm specific, have been developed using EST based simple sequence repeat (eSSR) markers (Peng and Lapitan 2005), microsatellite markers (Rödor et al 1998), deletion bin mapping (Qi et al. 2003), comparative DNA sequence analysis (Rota and Sorrells 2004; Sorrells et al. 2003), wheat microarray (Bhat et al. 2007), and diversity array technology (DArT) markers (Akbari et al. 2006). Somers et al. (2003) identified single nucleotide polymorphisms (SNPs) and estimated the frequency and allelic diversity of SNPs in wheat ESTs. They identified 1 SNP per 540 bp of EST sequence in hexaploid wheat.

Rye

Rye (*Secale cereale* L., 2n = 2x = 14, genome formula RR) is the second most common grain after wheat used in the production of bread. It is a major crop in Eastern Europe, which is responsible for 80% of the total rye production (Bushuk 2001). In 2007, rye was grown on an area of 6.9 million hectares with 15.7 million tonnes of production worldwide (FAOSTAT 2007). In the United States, rye is cultivated only on 0.1 million ha with total production of around 0.2 million tonnes (FAOSTAT 2007). The genome size of rye is around 7800 Mbp (Bedbrook et al. 1980). It has about 35% more DNA than the largest genome (B genome) of wheat. The smallest chromosome of rye (1R) contains about 12% more DNA than the largest chromosome of wheat (Gustafson and Bennett 1976).

Rye is a very adaptable crop that can be grown in cold as well as temperate environments, on marginal lands. It has a well developed root system as compared to other cereals (Starzycki 1976; Czembor and Sowa 2001; and Nalborczyk and Sowa 2001). In fact, when grown under the same kind of soil and moisture conditions, root branching is better developed in rye than in wheat or oats (Weaver 1926). It is resistant to most fungal diseases. Chromosome 1 of rye carries popular rust resistance genes (Zeller 1973; Schlegel and Korzun 1997). The short arm of chromosome 1 (1RS) from 'Petkus' rye carries genes *Lr26, Sr31, Yr9* and *Pm8* conferring race-specific resistance to leaf rust (*Puccinia recondite* f. sp. *tritici*), stem rust (*Puccinia graminis* f. sp. *tritici*), stripe rust

(*Puccinia striiformis* f. sp. *tritici*) and powdery mildew (*Erysiphe graminis* f. sp. *tritici*), respectively (Mago et al. 2002). The 'Imperial' rye 1RS translocation carries the stem rust resistance gene *SrR*, but no known leaf rust, stripe rust or powdery mildew resistance genes (Koebner et al. 1986). The group of rust resistance genes *Lr26*, *Sr31* and *Yr9* from 'Petkus' rye and *SrR* from 'Imperial' rye were mapped separately, approximately 5 cM distal to the seed-storage protein gene (*Sec-1*) on the 1RS chromosome arm (Singh et al. 1990). In a separate study, the *Lr26* map location was confirmed to be distal to the *Sec-1* locus of rye on 1RS (Hsam et al. 2000).

Rye is an allogamous species and has a genome with a high heterozygosity level (Milczarski et al. 2007). The first molecular map of all 7 rye chromosomes was developed by Devos et al. (1993). It was further enriched with randomly amplified polymorphic DNA (RAPD) (Masojć et al. 2001) and amplified fragment length polymorphism (AFLP) (Bednarek et al. 2003) loci, and used as a reference map in other studies (Korzun et al. 1998; Börner and Korzun 1998). Few, but different genetic maps of rye, based on restriction fragment length polymorphism (RFLP), simple sequence repeats (SSRs), AFLP, RAPD, isozyme and gene loci, have been developed so far (Philipp et al. 1994; Senft and Wricke 1996; Loarce et al. 1996; Korzun et al. 2001; Ma et al. 2001; Hackauf and Wehling 2003; Khlestkina et al. 2004; Milczarski et al. 2007). Even with this information, rye still lags behind in available genomic resources as compared to other cereal crops. There is still a need to put more effort into generating a high resolution genetic map in rye.

Syntenic relationship among *Triticeae*

Ahn et al. (1993) were the first to report that gene synteny and order is conserved, among different genera of the tribe *Triticeae*, in rice (*Oryza sativa*), maize (*Zea mays*) and wheat. Kurata et al. (1994) studied the gene alignment of wheat and rice. The first consensus genetic map of seven different grass species was produced by Moore et al. (1995). Later, an extended and detailed version of the consensus genetic map of wheat, rice, maize, and other grass species including rye revealed the conservation of their gene content and gene order in *Poaceae* (Devos and Gale 1997; Gale and Devos 1998).

All wheat homoeologous group 1 markers examined, have complete co-linearity and arm correspondence between chromosomes 1A, 1B, 1D and 1R. There is no evidence that any arm of 1R pairs in meiosis with any wheat chromosome arm outside homoeologous group 1 (Devos et al. 1993). Sorrells et al. (2003) did a high resolution large scale comparative DNA analysis of ESTs mapped in wheat in relation to rice. The structural relationship between the genomes indicated the homology of chromosomes 5 and 10 of rice with the chromosome 1 homoeologous group of wheat. In other similar comparative mapping, DNA sequences of ESTs were aligned with rice chromosomal sequences (Hackauf et al. 2009) and they also indicated homology of chromosome 5 and 10 with chromosome 1 of rye.

1RS Translocation in wheat

These syntenies of wheat and rye chromosomes (excepting some known structural rearrangements that break the overall synteny of individual chromosomes) permit the formation of compensating translocations of wheat and rye chromosomes. A compensating translocation is genetically equivalent to either of the two parental chromosomes; that is, it carries all relevant genes, but not necessarily in the same order. On the other hand, homoeology between wheat group 1S and rye 1S arms permitted induction of homoeologous genetic recombination, thus the development of recombinants of much smaller segments of rye 1RS to wheat than the entire arm.

Many of the present wheat cultivars developed by breeding for disease resistance carry a spontaneous centric rye-wheat translocation 1RS.1BL (or 1RS.1AL) that has been very popular in wheat breeding programs (Lukaszewski 1990; Rajaram et. al. 1990). This translocation contains a short arm of rye (*Secale cereale* L.) chromosome 1, (1RS) and the long arm of wheat chromosome 1BL (or 1AL). It must have occurred by misdivision of centromeres of the two group 1 chromosomes, and fusion of released arms and first appeared in two cultivars from the former Soviet Union, Aurora and Kavkaz. Rye chromosome arm 1RS in the translocation contains genes for resistance to insect pest and fungal disease (Zellar and Hsam 1984) but as it spread throughout wheat breeding programs it became apparent that the translocation was also responsible for a yield boost in the absence of pests and disease (Merker 1982; Rajaram et al. 1983; Villareal 1991;

Ehdaie et. al. 2003). Besides the presence of genes for resistance and yield advantage on 1RS, there is a disadvantage of 1RS in wheat due to the presence of the rye seed storage protein secalin, controlled by the *Sec-1* locus on 1RS, and the absence of the wheat loci, *Gli-B1* and *Glu-B3*, on the 1RS arm. Lukaszewski (2000) modified the 1RS.1BL translocation by removing the *Sec-1* locus and adding *Gli-B1* and *Glu-B3* on the 1RS arm.

Lukaszewski (1993; 1997) developed a set of wheat−rye translocations, derived from 'Kavkaz' winter wheat that added 1RS to wheat arms 1AL, 1BL, and 1DL in spring bread wheat 'Pavon 76', a high yielding spring wheat from CIMMYT. Studies showed that the chromosomal position of 1RS in the wheat genome affected agronomic performance as well as bread-making quality (Kumlay et al. 2003; Ehdaie et al. 2003).

Using the 1RS translocation, Lukaszewski (2000) developed a total of 183 wheat-rye short arm recombinant lines for group 1 chromosomes in a near-isogenic background of cv. Pavon 76 bread wheat. Out of 183 recombinant chromosomes, 110 were from 1RS-1BS combinations, 26 from 1RS-1AS and 47 from1RS-1DS combinations. Mago et al. (2002) used some of these lines to link molecular markers with rust resistance genes on 1RS. These recombinant breakpoint populations provide a powerful platform to locate region specific genes.

Genetics of roots

Wheat roots have two main classes, seminal roots and nodal roots (Esau 1965). Seminal roots originate from the scutellar and epiblast nodes of the germinating embryonic hypocotyls, and nodal roots, emerge from the coleoptiler nodes at the base of the apical culm (Manske and Vlek 2002). The subsequent tillers produce their own nodal roots, two to four per node and thus contribute towards correlation of root and shoot development (Klepper et al. 1984). The seminal roots constitute from 1-14% of the entire root system and the nodal roots constitute the rest (Manske and Vlek, 2002). Genetic variation for root characteristics was reported in wheat and other crop species (Throughton and Whittington 1968; Zobel 1974). Genetic variability for seedling root number was studied among different *Triticum* species at diploid, tetraploid, and hexaploid level and it was found to be positively correlated with seed weight (Robertson et al. 1979). In a hydroponic culture study in winter wheat, Mian et al. (1993) found significant genotypic differences in root and shoot fresh weights, number of roots longer than 40 cm, longest root length and total root length. Wheat genotypes with larger root systems in hydroponic culture were higher yielding in field conditions than those with smaller root systems (Mian et al. 1994). Also, wheat yield stability across variable moisture regimes was associated with greater root biomass production under drought stress (Blum et al. 1983).

Studies in other cereal crops associated quantitative trait loci (QTL) for root traits with the QTL for grain yield under field conditions. Champoux et al. (1995) provided the first report of specific chromosomal regions in any cereal likely to contain genes affecting root morphology. They reported that QTL associated with root traits such as root thickness, root dry weight per tiller, root dry weight per tiller below 30 cm, and root to shoot ratio shared common chromosomal regions with putative QTL associated with field drought avoidance/tolerance in rice. Price and Tomos (1997) also mapped QTL for root growth using a different population than that used by Champoux et al. (1995) in rice. In a field study of maize recombinant lines, QTL for root architecture and aboveground biomass production shared the same location (Guingo et al. 1998). Tuberosa et al. (2002) reported the overlap of QTL for root characteristics in maize grown in hydroponic culture with QTL for grain yield in the field under well-watered and droughted regimes occurred in 8 different regions. They observed that QTL for weight of nodal and seminal roots were most frequently and consistently overlapped with QTL for grain yield in drought and well watered field conditions. Also, at four QTL regions, increase in weight of the nodal and seminal roots was positively associated with grain yield under both irrigation regimes in the field.

There are a few reports on QTL studies for root traits in durum wheat but none has been reported in bread wheat. Kubo et al. (2004) studied root penetration ability in durum wheat. They used discs of paraffin and Vaseline mixtures as substitute for compact soil. Later, a QTL analysis was done for the number of roots penetrating the

poly vinyl disc, total number of seminal and crown roots, root penetration index and root dry weight (Kubo et al. 2007). The QTL for number of roots penetrating the poly vinyl disc and root penetration index was located on chromosome 6A and a QTL for root dry weight was located on 1B.

Wang et al. (2006) demonstrated significant positive heterosis for root traits among wheat F_1 hybrids. They showed that 27% of the genes were differentially expressed between hybrids and their parents. They suggested the possible role of differential gene expression in root heterosis of wheat, and possible other cereal crops. In a recent molecular study of heterosis, Yao et al. (2009) speculated that up-regulation of TaARF, an open reading frame (ORF) encoding a putative wheat ARF protein, might contribute to heterosis observed in wheat root and leaf growth.

Rye, wheat and barley develop 4-6 seminal roots which show a high degree of vascular segmentation (Aloni and Griffith 1991). Feldman (1977) traced files of metaxylem to their levels of origin in maize root apex and showed their differentiation behind the root apex in three-dimensional model. In drier environments, Richards and Passioura (1989) demonstrated that genotypes, when selected for narrow root xylem vessels as against unselected controls, yielded up to 3%-11% more than the unselected controls depending upon their genetic background. This yield increase in the selections with narrow root vessel was correlated with a significantly higher harvest index, also higher biomass at maturity and kernel number. Huang et al. (1991) indicated the decrease

in diameter of metaxylem vessel and stele with increase in temperature which resulted in decreased axial water flow in wheat roots. The decrease in axial water flow is very critical in conserving water during vegetative growth and making it available during reproductive phase of the plant. In a recent study on root anatomy, QTL for metaxylem were identified on the distal end of the long arm of chromosome 10 of rice (Uga et al. 2008). In another comparative study of rye DNA sequences with rice genome, the distal end of the long arm of chromosome 10 of rice showed synteny to the 1RS chromosome arm (Hackauf et al. 2009).

The 1RS.1BL (or 1RS.1AL) chromosome is now being used in many wheat breeding programs. Rye has the most highly developed root system among the temperate cereals and it is more tolerant to abiotic stresses such as drought, heat, and cold than bread wheat (Starzycki 1976). Introgression of rye chromatin into wheat may enlarge the wheat root system. Manske and Vlek (2002) reported thinner roots and higher relative root density for 1RS.1BL translocations compared with their non-translocated bread wheat checks in an acid soil, but not under better soil conditions. Repeated studies with the 1RS translocation lines of Pavon 76 have demonstrated a consistent and reproducible association between root biomass and the presence and position, of the rye 1RS arm (Ehdaie et al. 2003). The increased grain yield of 1RS translocations under field conditions observed and reported earlier (Rajaram et al. 1983; Villareal et al. 1991; Moreno-Sevilla et al. 1995) may be due to the consistent tendency of 1RS to produce more root biomass and also to the higher transpiration rate measured (Ehdaie et al. 2003).

Those authors have shown a significant increase of root biomass in wheat lines with 1RS translocations, and a positive correlation between root biomass and grain yield. All translocations of 1RS: with 1A, 1B, and 1D chromosomes have shown increased root biomass and branching as compared to Pavon 76 and there was differential expression for root biomass among these translocation lines with ranking 1RS.1AL > 1RS.1DL > 1RS.1BL > Pavon 76. In Colorado, the 1RS.1AL translocation with 1RS from Amigo (Insave rye) showed 23% yield increase under field conditions over its winter wheat check, Karl 92 (Owuoche et al. 2003).

Research outline

Most plant studies concentrate on the above ground organs and neglect roots. The evidence of increase in root biomass in 1RS translocation wheats and the availability of a set of near-isogenic lines of Pavon 76 with the same 1RS chromosome arm provided a tool to characterize the loci for root traits in the wheat-rye translocation in bread wheat. Until now, roots have not been studied extensively for the localization of root traits in bread wheat. The present study was conducted with the following three objectives:

- Development of a high resolution genetic map for the 1BS-1RS arms by PCR-based mapping

- Identification and physical mapping of the region on 1RS arm responsible for root characters
- Study the anatomy of roots of different dosages of 1RS chromosome arms in Pavon 76 wheat background

Chapter 2 of this dissertation focuses, primarily, on creating a consensus high resolution genetic map of the 1RS-1BS chromosome arm by using 1RS-1BS recombinant breakpoints. A total of 20 physical and molecular markers were mapped on the 1RS-1BS chromosome arm with an average map distance of 2.5 cM. A preliminary phenotypic analysis identified the distal 15% of the 1RS chromosome arm carries the gene(s) for increased rooting ability in bread wheat.

As mentioned earlier, there has not been any report on mapping QTL for different root trait in wheat. Chapter 3 of this dissertation reports the successful mapping of QTL for different root traits in bread wheat. This was an extended study of chapter 2 to phenotype the 1RS-1BS recombinants that were used to generate the integrated 1RS-1BS genetic map. To map root QTL, a novel E-BAYES approach was used for the statistical analysis to calculate the additive (main) and additive × additive (epistatic) effects of loci present on 1RS in bread wheat. This study is the first report of root QTL in wheat and also the first report of using the E-BAYES method in the wheat crop. This work successfully validates the approach of using recombinant breakpoints for genetic mapping and identifying the QTL for complex agronomic traits.

The affirmative conclusions from the previous and present research encouraged the research reported in chapter 4, where Pavon 76 genotypes, differing in dosage of the 1RS arm, were used to study the response of different doses of root loci on phenotypic expression. The genotypes were subjected to phenotypic analysis and the study of dosage effect on root anatomy. A single dose of 1RS and four doses of 1RS showed higher root biomass as compared to zero and double dosages. Histological study of roots showed thinner roots with narrow stele diameter in quadruple doses of 1RS than zero and single dose. Results showed thinner roots but more root biomass in the presence of 1RS. This chapter also reports the anatomical structure of xylem vessels in the roots. It showed an increase in the number of xylem vessels from the root tip to the base of the seminal root. This is also the first report of a histological study of 1RS bread wheat roots.

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CHAPTER 2

Integrated genetic map and genetic analysis of a region associated with root traits on the short arm of rye chromosome 1 in bread wheat

ABSTRACT

A rye-wheat centric chromosome translocation 1RS.1BL has been widely used in wheat breeding programs around the world. Increased yield of translocation lines was probably a consequence of increased root biomass. In an effort to map loci controlling root characteristics, homoeologous recombinants of 1RS with 1BS were used to generate a consensus genetic map comprised of 20 phenotypic and molecular markers, with an average spacing of 2.5 cM. Physically, all recombination events were located in the distal 40% of the arms. A sample of 68 recombinants was used and recombination breakpoints were aligned and ordered over map intervals with all the markers, integrated together in a genetic map. This approach enabled dissection of genetic components of quantitative traits, such as root traits, present on 1S. To validate our hypothesis, phenotyping of 45-day old wheat roots was performed in five lines including three recombinants representative of the entire short arm along with bread wheat parents 'Pavon 76' and Pavon 1RS.1BL. Individual root characteristics were ranked and the genotypic rank sums were subjected to Quade analysis to compare the overall rooting ability of the genotypes. It appears that the terminal 15% of the rye 1RS arm carried gene(s) for greater rooting ability in wheat.

Introduction

Many present day bread wheat cultivars carry a centric rye-wheat translocation 1RS.1BL in place of chromosome 1B (Braun et al. 1998). Originally the translocation was thought to have been fixed because the 1RS arm of rye (*Secale cereale* L., 2n = 2x = 14, genome formula RR) carries genes for resistance to various leaf and stem fungal diseases and insects (Zellar and Hsam 1984). However, the translocation increased grain yield even in the absence of pathogens (Villareal et al. 1991, 1995). It has been shown recently that this yield increase may be a direct consequence of a substantially increased root biomass (Ehdaie et al. 2003).

Studies by Ehdaie et al. 2003 showed a significant increase of root biomass in wheat lines with 1RS translocations and a positive correlation between root biomass and grain yield. In sand cultures, all three 1RS translocations on 1AL, 1BL, and 1DL in 'Pavon 76' genetic background showed clear position effects with more root biomass and root branching over Pavon 76 (a high yielding spring wheat from CIMMYT). The root biomass among these translocation lines ranked as follows: Pavon 1RS.1AL > Pavon 1RS.1DL > Pavon 1RS.1BL > Pavon 76. On the other hand, in Colorado, the 'Amigo' 1RS.1AL translocation from a different rye source ('Insave') in wheat cv. 'Karl 92', showed 23% yield increase under field conditions over its winter wheat check, Karl 92 (Owouche et al. 2003). In 1RS.1BL translocation wheats grown in acid soils, roots were thinner and there was a higher root length density, and this likely enhanced the root

surface area (Manske and Vlek 2002). The yield advantage of 1RS translocation lines may be partly attributed to the increase in root biomass that increases uptake of water and nutrients from the soil (Ehdaie et al. 2003; Snape et al. 2007 and Ehdaie and Waines 2008).

To elucidate the mechanisms responsible for increase in root biomass in 1RS wheats, it is necessary to genetically map and identify loci responsible for enhanced root traits. The objective of this study was to develop a consensus genetic map of the 1RS-1BS chromosome arms using a population of induced homoeologous recombinants, and subsequently use the genetic map to tag the 1RS chromosomal region responsible for increased root traits.

Molecular linkage maps of cereals are being improved rapidly by adding new types of markers, merging different species-specific maps and comparative mapping of markers between related genomes. Efficient use of resulting dense maps requires detailed insights into the relationship between genetic and physical distances (Künzel et al. 2000). Various types of markers have been mapped on the 1S arm of wheat (Rödor et al. 1998; Peng et al. 2004) and rye (Mago et al. 2002). PCR-based markers were developed for 1RS.1AL and 1RS.1BL wheat-rye translocations in wheat (Weng et al. 2007). However, these translocations have not been used extensively to generate consensus map of wheat and rye chromosomes which would be a useful tool to study different agronomic characters influenced by the presence of rye chromatin. Recombination mapping has an

advantage over deletion-bin mapping in generating higher resolution maps. In deletion mapping, the number of available breakpoints, hence the number of bins, limits the resolution, and after the set developed by Endo (Endo and Gill 1996) there have been no further efforts to generate new breakpoints. In genetic mapping, resolution is limited primarily by the number of available markers, and these increase steadily. Another advantage of recombination mapping is the ability to study the genes on rye chromosomes, where, because of diploidy, it is difficult, if not impossible, to practice deletion bin mapping. In this study, using a set of recombinants, we generated a consensus map of 1RS-1BS that integrates physical and molecular markers and attempted to narrow the regions containing major QTL for root characteristics.

Materials and Methods

Plant material

Experimental material was provided by Dr. A.J. Lukaszewski, University of California, Riverside. It consisted of a set of rye-wheat recombinant lines in a near-isogenic background of bread wheat cv. Pavon 76. Pavon 76 is a spring hard wheat from the breeding program at Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), Mexico. The set includes centric translocation 1RS.1BL in Pavon 76, where the 1RS arm is from cv. Kavkaz and 1BL arm is from Pavon 76 (Lukaszewski 1993) and

a set of 68 recombinants of the 1RS arm with 1BS in Pavon 76 (Lukaszewski 2000). 1RS-1BS recombination was induced by the absence of the *Ph1* locus. All recombinants are single breakpoints; therefore, the short arm of each recombinant chromosome contains one segment of 1RS (either terminal or proximal to the centromere) and a complementary segment of 1BS. Each recombinant has a normal 1BL long arm. Since they were produced by crossing over, they can be used to generate a genetic map. However, since recombination in wheat is predominantly in the distal portion of each arm (Gill et al. 1996), physically, these recombination breakpoints cover only the terminal 40% of the arm's length.

Genetic mapping

The primary source of PCR-based markers were DNA sequences of cDNAs that had been used to define 763 expressed sequence tags (EST) allocated by low-resolution deletion mapping to wheat 1S chromosomes (Peng et al. 2004, http://wheat.pw.usda.gov/cgibin/westsql/map_locus.cgi). Based on posted Southern blot images, 91 EST loci allocated to 1BS were selected for primer design. The unigenes corresponding to these ESTs were identified from HarvEST: Wheat assembly WK (www.harvest-web.org) and used for primer design. Primer pairs were designed using PRIMER 3 software http://frodo.wi.mit.edu/ (Rozen and Skaletsky 2000). It was expected that a majority of thus produced markers would be polymorphic in the wheat-rye

context. Additionally, 16 1BS specific SSR/eSSR primer pairs were chosen from earlier studies (Roder et al. 1998: Peng et al. 2005). Nine eSSR primer sequences were provided by Dr. N. Lapitan of Colorado State University, Fort Collins, CO, and information on seven SSR primers was from Rödor et al. 1998. Pre-screening was done using five genotypes: Pavon 76, Pavon 1RS.1BL, Pavon Dt.1BL, T-1, and 1B+5.

DNA Extraction

DNA was extracted from young leaf tissue of rye-wheat recombinants and their parental lines using Plant DNAzol (Promega, Madison, WI) and further purified using a DNeasy plant kit (Qiagen, USA). Quality and quantity of the purified DNA were assessed on a 0.8% agarose gel by electrophoresis and using a UV spectrophotometer.

PCR, gel electrophoresis and scoring for EST-based markers

PCR was performed in a DNA Engine Dyad® PELTIER Thermal Cycler System (MJ Research, USA). The 20 µl reaction mixtures consisted of 50 ng of template DNA, 2 µl of 10X PCR Buffer with 15 mM of $MgCl_2$ (Qiagen, USA), 1 unit of HotStarTaq DNA Polymerase (Qiagen, USA), 0.2 mM dNTPs (Sigma Chemical Co., St. Louis, MO), and 2 pmole each of forward and reverse primer synthesized by Operon Biotechnologies, Inc. After 10 min of denaturation at 95°C, amplifications were performed for 35 consecutive cycles each consisting of 45 sec at 95°C, 30 sec at 60°C, 45 sec at 72°C, followed by an 8

min extension step at 72°C. About 5 µl of PCR products were run on a 1.2% agarose gel. Gels were stained with ethidium bromide and gel images captured using a gel documentation system. Gel images were scored manually for presence or absence of bands/ polymorphism.

PCR, gel electrophoresis and scoring for SSR-based markers

A tailed primer approach (Oetting et al 1995) was used for simple sequence repeat (SSR) analysis. To facilitate labeling, unlabeled M13 tail (5' CACGACGTTGTAAAACGAC 3') was added to the 5' end of forward SSR/eSSR primers (see Table 1) while reverse SSR primers did not contain a tail. An IRDye-labeled M13 forward primer (third primer) was included in the PCR which generated labeled PCR product in the subsequent cycles of PCR for easy detection. PCR was performed in a DNA Engine Dyad® PELTIER Thermal Cycler System (MJ Research, USA). The 10 µl reaction mixtures consisted of 10 ng of template DNA, 1X Thermophilic DNA polymerase buffer (50 mM KCl, 10 mM Tris-HCl (ph 9.0 at 25°C), and 0.1%Triton X-100, Promega, Madison, WI), 2.0 mM $MgCl_2$, 0.5 unit of Taq DNA polymerase (Promega, Madison, WI), 0. 2 mM dNTPs (Sigma Chemical Co., St. Louis, MO), 0.25 pmoles forward and reverse primers synthesized by Operon Biotechnologies, Inc., and 0.25 pmoles of M13F primer labeled at the 5' end with an infrared dye IRD700 (LI-COR, Lincoln, NE). After 4 min of denaturation at 94°C, amplifications were performed with

35 cycles each consisting of 1 min at 94°C, 1 min at the specific annealing temperature (see Table 1), 90 sec at 72°C, then followed by a 15 min extension step at 72°C.

The PCR products were separated on 18cm denaturing 7% Long Ranger (BMA, Rockland, ME) polyacrylamide gels using a LI-COR IR^2 4200LR Global DNA sequencer dual dye system (Lincoln, NE). Formamide loading dye was added at an appropriate ratio to get sharp bands (Caruso et al. 2008). PCR products were denatured at 94°C for 3 min and transferred to ice before loading on the gel. Approximately 0.25 µl of diluted and denatured PCR product was loaded in each lane of the gel. At least 3 wells in each gel were loaded with 50-350 bp sizing standard (LI-COR, Lincoln, NE) to facilitate scoring. Gels were scored manually for presence (1) or absence (0) of wheat alleles.

Phenotyping of shoot and root traits

A phenotyping experiment to study root characters was set up in the glasshouse in sand-tube cultures (Champoux et al. 1995) in PVC tubes, 80 cm long and 10 cm in diameter during 2006, 2007 and 2008. The study involved five lines: Pavon 76 and Pavon 1RS.1BL as the parents, and recombinants T-14, 1B+38, and 1B+2 (Ehdaie and Waines 2006). The three recombinant lines were chosen from the set of 68 lines to subdivide the recombining portion of the arms into three segments of roughly equal lengths.

Seed of these five lines were surface sterilized with 5% commercial bleach for 5 min, washed for 10 min in distilled water, soaked in water for 24 hrs and then germinated on wet filter paper in Petri dishes. Five day old seedlings were transplanted to 80 cm PVC tubes containing 1 m polythene tubing, closed at one end, with 8.5 kg of silica sand #30. Two small holes were made at the bottom of polythene tube to allow drainage of excess water. The PVC tubes were supported in metal frames and arranged in a randomized complete block design with four replicates. Plants were harvested 45 days after germination when the differences for root characters could be efficiently measured among different recombinant lines. Data for different shoot characters were recorded and the tubes containing roots were stored at 4^{o}C until processing. Roots were washed and recovered without damage using a floatation technique (Böhm 1979). The shoot characters measured were longest leaf length (LLL), maximum width of the longest leaf (LLW), leaf area (LA), plant height (PH), number of tillers (NT), and dry shoot biomass (SB). The root characters measured were number of roots greater than 30 cm (NR >30), longest root length (LRL), total length of roots greater than 30 cm (TRL), shallow root weight (SRW - root weight above 30 cm), deep root weight (DRW - root weight below 30 cm), dry root biomass (RB), and root biomass to shoot biomass ratio (R/S).

Statistical analysis

The shoot and root data were subjected to the analysis of variance (ANOVA) for each year (Steel et al. 1997). The combined ANOVA across years was performed for

each measured and calculated trait. The overall rooting ability of each genotype was calculated by ranking each genotype for individual root traits in each replication in each of the three years. Genotypes with the highest values were ranked 5 and those with the lowest values were ranked 1. Subsequently, all the ranks of root characters for each genotype were summed providing a measure of the rooting ability index for each genotype at different replications (blocks). The genotypic rank sums averaged across the years were subjected to the non-parametric Quade analysis developed for randomized complete block designs (Conover 1980; Quade 1979) to differentiate genotypes for overall rooting ability.

Results

PCR-based 1BS specific markers

To map a locus specific marker, we targeted the 3' UTR of ESTs for primer design due to its polymorphic nature. Primers were designed from the corresponding unigenes from the HarvEST assembly. Ninety one EST loci were selected from the 763 EST loci assigned to wheat 1S by Peng et al. (2004). These 91 primer pairs were screened against five genotypes: Pavon 76, Pavon 1RS.1BL, Pavon Dt.1BL, T-1, and 1B+5. In the initial screening, eight primer pairs showed polymorphism for the presence/absence of a DNA band. These eight primer pairs

were used for screening the population of 68 primary recombinant lines. Four of the primer pairs (see Table 1) showed reproducible polymorphism. The amplicon sizes ranged from 500 bp to 1.1kb. Two were the expected fragment sizes and the other two primer pairs, *Xucr_4* and *Xucr_6*, produced longer amplicons than the expected size due to amplification of introns (Table 1). The primer pair for *Xucr_4* amplified two PCR products, of 700bp and 575 bp, but only the 575 bp band was polymorphic (Figure 1 a & b).

SSR-based 1BS Specific markers

To add more markers, we selected 16 1BS specific primer sequences from the lists of Röder et al. 1998 and Peng et al. 2005, Pre-screening of these primers against the same five lines produced polymorphism for eight markers, but only four of these showed a convincing polymorphism for the entire population. Amplified PCR products were comparable to the expected size range of 150-200 bp (Table 1).

Table 2.1 Primer sequences and PCR conditions for amplification of bread wheat 1BS specific markers

Locus	Marker name	Primer sequences (5' to 3')	Genebank accession	HarvEST: Unigene #	Source	SSR Motif	Annealing temp. (^{0}C)	Expected Fragment size (bp)	Observed Fragment size (bp)
B8	*Xucr_3*	F: TGCCTCTCTTGCACTTAGCA R: TGGGCTGCTAAAAGGATCAC	BE498153	27072	EST	-	60	486	500
F6	*Xucr_4*	F: CAAGGAGGTTGGTTTCCTGA R: CGAATACAAGCCGTTCATCA	BE497177	12930	EST	-	60	440	575
C7	*Xucr_5*	F: CTTGCGTCTACGTCGAGGAT R: GGTCATTTGATCGGCTTCAT	BE490021	22324	EST	-	60	499	500
E5	*Xucr_6*	F: TCGAAGGAGAATACGCTGGT R: GCCCATAAGATTTTGCAACG	BF483035	9666	EST	-	60	442	1100
Xcwem6c	*Xucr_8*	F: CACGACGTTGTAAAACGAC CCTGCTCTGCCATTACTTGG R: TGCACCTCCATCTCCTTCTT	BF483588	10053	SSR	(AG)12	55	165	165
Xgwm18-1B	*Xucr_1*	F:CACGACGTTGTAAAACGAC TGGCGCCATGATTGCATTATCTTC R: GGTTGCTGAAGAACCTTATTTAGG	-	-	SSR	(CA)17GA(TA)4	50	188/182	180
Xgwm264-1B	*Xucr_7*	F: CACGACGTTGTAAAACGAC GAGAAACATGCCGAACAACA R: GCATGCATGAGAATAGGAACTG	-	-	SSR	(CA)9A(CA)24	60	157/165	160
Xgwm273-1B	*Xucr_2*	F: CACGACGTTGTAAAACGAC ATTGGACGGACAGATGCTTT R: AGCAGTGAGGAAGGGGATC	-	-	SSR	(GA)18	55	171/165	165

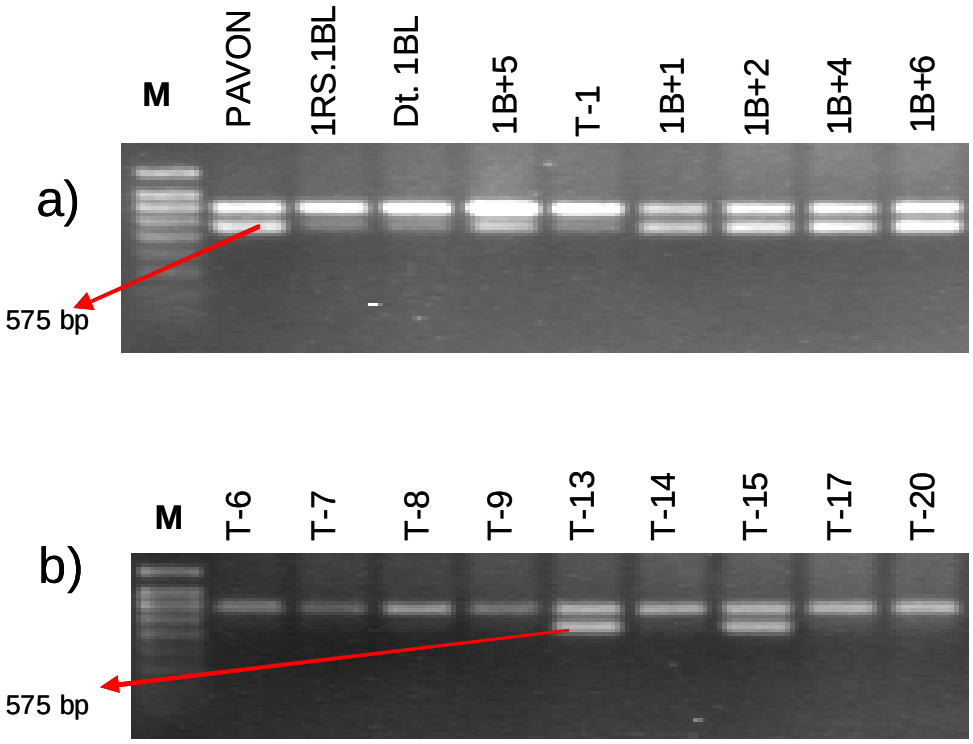


Figure 2.1: Polymerase chain reaction amplicon from 1BS-specific dominant marker *Xucr_4* (575 bp) among different wheat lines on 1.2% agarose gel elctrophoresis. a) & b), The numbers at the top of panels correspond to lines carrying different lengths of 1RS chromatin in wheat backgrounds. 1B+ lines are 1BS arm with distal 1RS chromatin and T-lines are 1RS arm with distal wheat chromatin

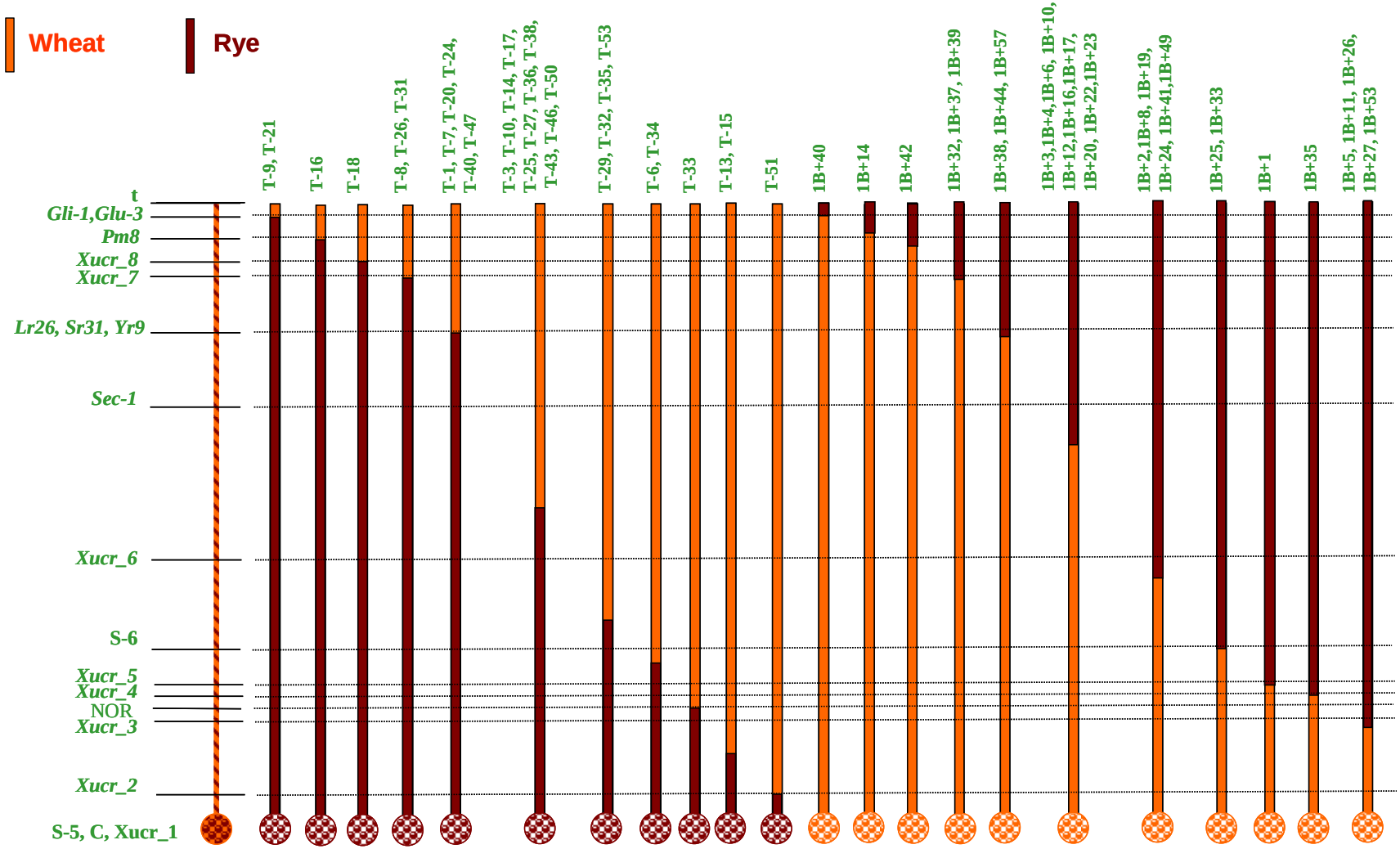
Genetic map based on the 1RS-1BS recombinant breakpoints

A calculative approach was used to generate a genetic map of the 1RS-1BS arms. The 1RS-1BS map from the previous study (Lukaszewski, 2000), based on twelve markers and 103 recombinants, was enriched by eight additional molecular markers and recalculated using 68 lines. The population of recombinants was divided into two configuration groups, 1B+ lines with distal 1RS, and T- lines with distal 1BS, comprising 34 lines each. Both groups were scored seperately for the presence/absence of each of the 20 markers, with the presence of a marker denoted as a score of 1 and the absence as 0. Each line was then ranked according to its total score for all markers and each group was further divided into 11 subgroups on the basis of its ranking. Thus, the entire mapping population was divided into 22 subgroups (Figure 2). In this fashion, the 20 markers subdivided the genetic maps of the arms into 15 intervals (Figure 2). Since each of the 68 primary recombinants used in mapping was preselected, guranteed recombining cell having single crossingover event, the total genetic length of the map is 50cM, and each breakpoint is then equal 50/68 cM while the distance between any two given markers is calculated by the formula:

Genetic distance = (50/total no. of lines) × no. of breakpoints per interval

and no mapping functions needed to be applied.

Figure 2.2: Diagrammatic representation of genetic positions of recombinant breakpoints of 1RS-1BS in Pavon 76 bread wheat. White bars are representing wheat 1BS and black bars represent rye 1RS. On the left side of the figure is the list of markers present starting from the centromere at the bottom (represented by circle; white = wheat and black = rye centromeres) to the top towards telomeres. Each black and white bar represents the 1RS-1BS arm showing the position of the markers with respect to recombinant breakpoints and these bars represent only the 40% distal end of the chromosome


Wheat
Rye
t
Gli-1,Glu-3
Pm8
Xucr_8
Xucr_7
Lr26, Sr31, Yr9
Sec-1
Xucr_6
S-6
Xucr_5
Xucr_4
NOR
Xucr_3
Xucr_2
S-5, C, Xucr_1
T-9, T-21
T-16
T-18
T-8, T-26, T-31
T-1, T-7, T-20, T-24, T-40, T-47
T-3, T-10, T-14, T-17, T-25, T-27, T-36, T-38, T-43, T-46, T-50
T-29, T-32, T-35, T-53
T-6, T-34
T-33
T-13, T-15
T-51
1B+40
1B+14
1B+42
1B+32, 1B+37, 1B+39
1B+38, 1B+44, 1B+57
1B+3,1B+4,1B+6, 1B+10, 1B+12,1B+16,1B+17, 1B+20, 1B+22,1B+23
1B+2,1B+8, 1B+19, 1B+24, 1B+41,1B+49
1B+25, 1B+33
1B+1
1B+35
1B+5, 1B+11, 1B+26, 1B+27, 1B+53

Phenotyping of shoot and root traits

Phenotyping of recombinants was necessary to test the general applicability of the consensus 1RS-1BS map in locating a 1RS region showing better rooting ability. Various shoot and root traits were studied using two parents and three recombinants covering the whole 1RS-1BS map. There were significant differences among years for all shoot characters measured, except for the maximum width of the longest leaf (Table 2). Significant differences were found among the genotypes for shoot characters, except for maximum width of the longest leaf and leaf area. Genotype × year interaction was significant only for the number of tillers per plant (Table 2). This interaction was due to changes in the magnitude of the genotypic means across different years (non-crossover interaction) rather than changes in ranking of the means. Therefore, shoot characters in Table 2 are represented by means averaged across years. Pavon 1RS.1BL was taller, had longer leaf length and a greater root to shoot ratio than Pavon 76. Since Pavon 1RS.1BL and Pavon 76 had similar shoot biomass (Table 2), greater root to shoot biomass ratio in the former genotype indicated greater root biomass in 1RS.1BL than Pavon 76 (Table 3). Leaf area in 1B+2 was the highest (30.1 cm^2) followed by Pavon 1RS.1BL (29.7 cm^2). Despite significant differences observed among the genotypes for shoot characters, the differences were relatively small, except for the shoot biomass in 1B+2 in the third year (not shown) which was due to greater number of tillers per plant and plant height (Table 2). Otherwise, the rest of the genotypes did not show large differences for combined as well as for individual years for most of the shoot traits.

There were significant differences among years for all root characters measured (Table 3). Significant differences were found among the genotypes for all the root characters measured, except for longest root length. The genotype × year interaction was significant only for the number of roots greater than 30 cm. Therefore, means for root characters were averaged across three years (Table 3). The number of roots greater than 30 cm and root biomass in Pavon 1RS.1BL were greater than those in Pavon 76 which confirmed the results reported earlier (Ehdaie et al. 2003; Ehdaie and Waines 2006). Number of roots greater than 30 cm in Pavon 1RS.1BL, 1B+2 and 1B+38 were similar, but greater than those in Pavon 76 and T-14 (Table 3). A similar trend was observed for the total length of roots greater than 30 cm. Shallow root weight was highest in Pavon 1RS.1BL (267 mg plant^{-1}) followed by 1B+2 (255 mg plant^{-1}), and 1B+38 (227 mg plant^{-1}). The lowest shallow root weight belonged to T-14 (193 mg plant^{-1}). Deep root weight in Pavon 1RS.1BL, 1B+2, and 1B+38 were similar, but greater than those in Pavon 76 and T-14. Greater dry root biomass observed in Pavon 1RS.1BL compared to Pavon 76 was due to a combination of greater shallow and deep root weight in the former than the later genotype (Table 3).

Quade analysis was used to compare the rooting ability index (RAI) of the examined genotypes based on the mean rank sums of root characters. The Quade statistic (Sj) ranged from -11.0 for T-14 to 11.5 for Pavon 1RS.1BL (Table 3). Differences among Sj were statistically significant at $P< 0.10$ (Quade 1979). Pavon 1RS.1BL had the highest

RAI (26.3) followed by 1B+2 (22.0) and 1B+38 (20.2). Pavon 76 (11.3) and T-14 (10.2) had the lowest RAI (Table 3).

Table 2.2: Summary of combined ANOVA and mean values of plant height (PH), number of tillers (NT), longest leaf length (LLL), maximum width of the longest leaf (LLW), leaf area (LA), shoot biomass (SB), and root to shoot biomass ratio (R/S) for bread wheat Pavon 76, Pavon 1RS.1BL, and three recombinant lines 1BS-1RS grown in sand tubes for 45 days (mid-tillering stage) averaged across three years.

Genotype	**PH** cm	**NT** no.	**LLL** cm	**LLW** cm	**LA** cm^2	**SB** mg	**R/S**
Pavon 76	45.8† c	4.2 b	32.2 c	1.01 a	27.3 b	725 bc	0.41 bc
1RS.1BL	48.0 b	4.1 b	35.6 a	1.00 a	29.7 ab	798 b	0.45 a
T-14	47.7 bc	4.1 b	34.6 ab	0.94 a	27.4 b	634 c	0.39 bc
1B+2	49.4 a	5.1 a	33.6 bc	1.05 a	30.1 a	862 a	0.40 c
1B+38	46.3 bc	4.1 b	33.7 bc	0.96 a	27.1 b	730 bc	0.42 ab
Year	**	**	**	NS	**	**	**
Genotype	*	**	*	NS	NS	**	**
Genotype × year	NS	**	NS	NS	NS	NS	NS
CV‡	6	14	7	9	14	18	11

† Means followed by the same small letter within a column are not significantly different at $P < 0.05$ and according to LSD test.
CV‡ = Coefficient of variation.
* = Significant (p = 0.05)
** = Significant (p = 0.01)
NS = Not Significant (P)

Table 2.3: Summary of combined ANOVA and mean number of roots greater than 30 cm (NR>30), longest root length (LRL), total root length of roots greater than 30 cm (TRL), shallow root weight (SRW), deep root weight (DRW), dry root biomass (RB), Quade statistic (Sj), and rooting ability index (RAI) for bread wheat Pavon 76, Pavon 1RS.1BL, and three recombinant lines 1BS-1RS grown in sand tubes for 45 days (mid-tillering stage) averaged across three years.

Genotype	NR>30	LRL	TRL	SRW	DRW	RB	Overall	
	no.	--------cm-------		----------mg----------			S_j [§]	RAI [Ψ]
Pavon 76	5.3† b	91 a	350 b	220 bc	69 b	289 cd	-7.0	11.3 B
Pavon 1RS.1BL	6.5 a	91 a	422 a	267 a	89 a	355 a	11.5	26.3 A
T-14	5.3 b	88 a	347 b	193 c	65 b	258 d	-11.0	10.2 B
1B+2	6.2 a	89 a	389 a	255 ab	94 a	350 ab	3.5	22.0 A
1B+38	6.1 a	94 a	390 a	227 b	83 a	311 bc	3.0	20.2 A
Year	*	**	**	**	**	**		
Genotype	**	NS	**	**	**	**		
Genotype × year	**	NS	NS	NS	NS	NS		
CV‡	15	10	14	19	24	16		

† Means followed by the same small letter and capital letters within a column are not significantly different at $P < 0.05$ and at $P < 0.10$ according to LSD test and Quade test, respectively.

CV‡ = Coefficient of variation.

[§] The critical value for a difference $|S_i - S_j|$ to be significant = 9.06

* = Significant (p = 0.05), ** = Significant (p = 0.01)

NS = Not Significant ($P > 0.05$)

Ψ Mean rank sums for root characters

Discussion

Genetic mapping in wide hybrids has been performed for many plant species, particularly in diploids including barley (Graner et al. 1991), chickpea (Winter et al. 2000), lentils (Eujayl et al. 1998), onion (van Heusden et al. 2000), *Nicotiana* species (Lin et al. 2001), and tomato (Bernatzky and Tanksley 1986; Helentjaris et al. 1986). In the early days of genetic mapping with molecular markers, wide hybrids were the approach of choice, for it guaranteed much higher levels of polymorphism than in intra-specific hybrids. Despite notable instances of non-Mendelian segregation and skewed distribution of recombination, wide hybrids produced useful genetic maps with higher marker saturation at considerably less cost and effort (van Heusden et al. 2000). Use of wide hybrids in allopolyploids is more complicated than in diploids as allopolyploids tend to have some kind of chromosome pairing control system in place that limits crossing over to homologues. Hence, homoeologous pairing may be low or even non-existent. In this study, 68 recombinants produced by crossing over were used. These recombinants were selected at random from a population of 103 such recombinants developed by Lukaszewski (2000). The entire recombinant population was selected from a population of ca. 17,000 progeny with the *Ph1* system disabled. If the assumption is made that crossing over in the *Ph1+* and *Ph1-* wheats is the same, and they appear to be, except for the absence of multiple crossovers per arm (Lukaszewski, 2000) then the sample analyzed here would be equivalent to a population of 136 backcross progeny, a sensible number giving the maximum resolution level (spacing of breakpoints)

of 0.7 cM. Physical distribution of 68 recombinant breakpoints used in the present study is shown in Figure 3.

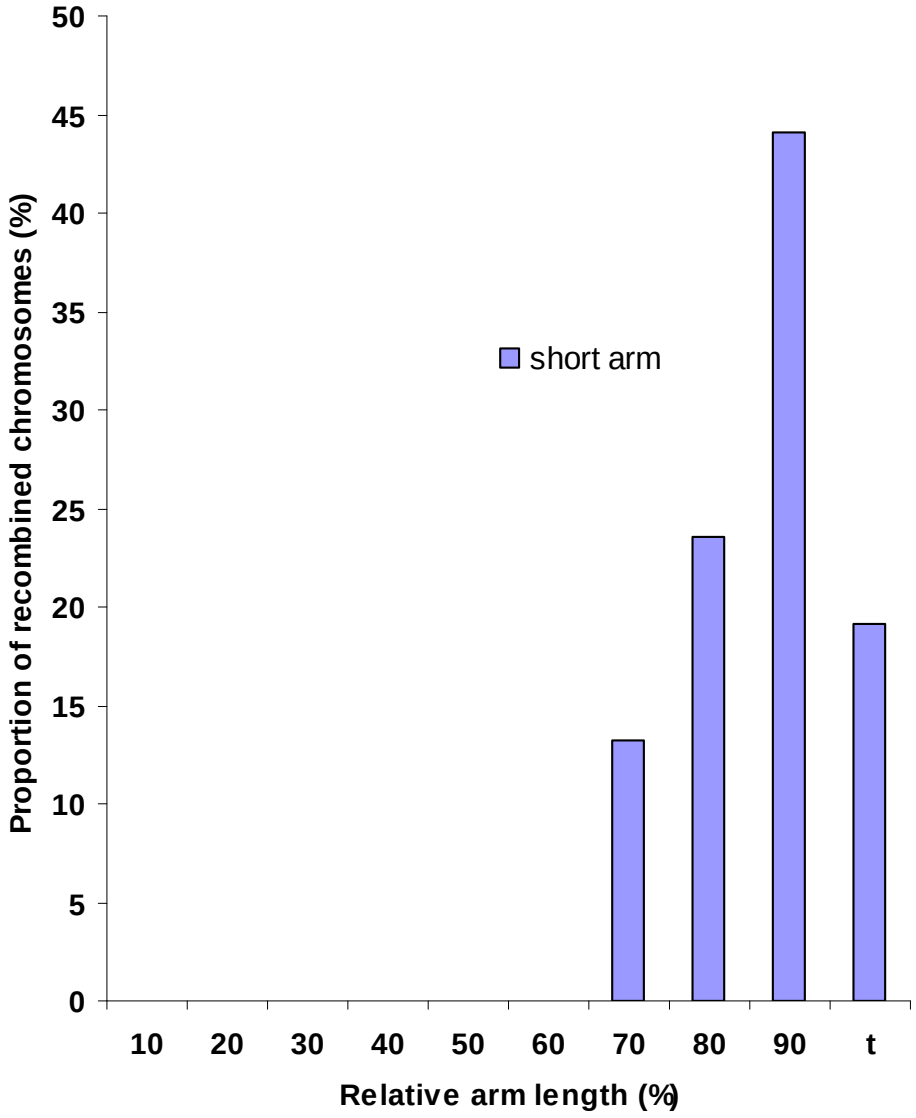


Figure 2.3: Physical distribution of recombinant breakpoints of short chromosome arm of 1R-1B along Centromere (0) – telomere (t) axis

With a total of 20 physical and molecular markers, we constructed the combined genetic map of 1RS-1BS recombinant breakpoints in Pavon 76 background. The genetic map produced here has an average density of 2.5 cM. The maps shown in Figures 2 and 4 represent only the physical 40% of the distal ends of the 1S arms as no recombination took place in the proximal 60% of the arm. Any loci in this region would show complete linkage with the centromere.

The advantage of using these recombinant breakpoints in developing a genetic map is in their physical differences from one another. Here, we used two methods to map. Firstly, we used the recombinant breakpoints to develop a genetic map of the arm, and secondly, we used the map to classify the breakpoints. Each interval or genetic distance between two markers is represented by one or more recombinant breakpoints that most often include both reciprocal configurations of the chromosomes. This physical separation of the breakpoints further refines the genetic map to 0.7 cM level. This reciprocal nature of chromosomes with breakpoints in any given interval will permit allocation of identified genetic loci to very narrow physical segments of rye chromatin. This may be a useful tool in dissecting the genetic components of a particular gene of interest or an important agronomic trait present on 1RS.

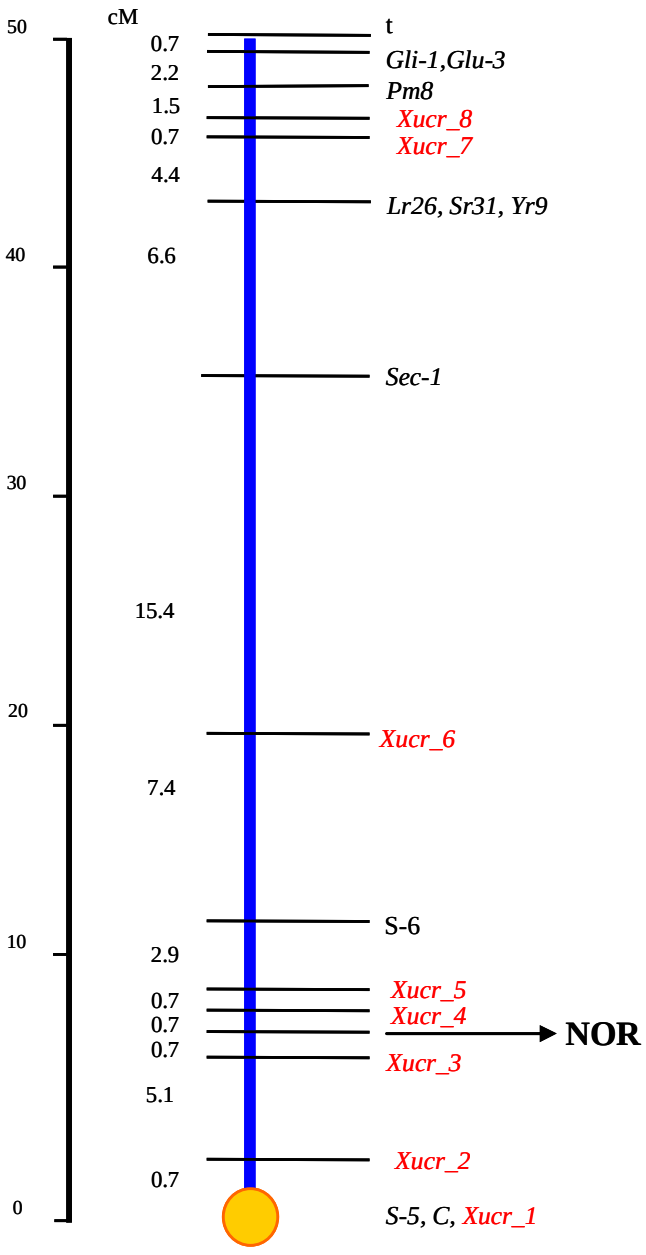


Figure 2.4: Combined Genetic map of the rye-wheat 1RS-1BS arm showing the genetic distances (in cM) between markers. Genetic map is representing 40% of the distal end of 1RS and 1BS chromosomes

Somers et al. (2003) detected an average of one single nucleotide polymorphism (SNP) per 540 bp ESTs in wheat and demonstrated the reliability of designing PCR primers for locus-specific amplicon production. In this study, we targeted a single chromosome arm, 1BS, and expected that most of the 91 EST loci allocated to this arm could be converted to 1BS locus-specific amplicon markers. Given that the recombination was intergeneric, we expected that most of these markers would be polymorphic between wheat and rye, and so would produce a highly saturated map. However, only four PCR-based markers showed reliable polymorphism, though we can not rule out sequence-based polymorphisms that we could not detect. The sequences of the 91 EST loci on wheat 1S arms were used to search rice orthologs using TIGR rice version 4.0 gene models, and 54 of these sequences had rice blastx hits of e-20 or better, comprising 52 rice gene models. Of the 52 models, 27 were clustered on chromosome 5 of rice, which is known to be syntenic to chromosomes 1S of Triticeae (Sorrells et al. 2003; Peng et al. 2004; Gale and Devos 1998). In the present study, two gene models with genebank accessions viz; BE497177 and BF483588 were found to be represented as markers, *Xucr_4* and *Xucr_8*, respectively (see Table 1). The rice annotation of *Xucr_8* is described as a drought induced 19 protein (Di19) which can be helpful in studying drought. However, a high frequency of insertions and deletions in rice and maize make these genomes more fluid at the DNA sequence level than indicated by Southern analyses (Sorrells et al. 2003).

Testing a total of 16 SSR and eSSR markers yielded four polymorphic amplified products which were comparable to the previous studies (Rödor et al. 1998; Peng et al. 2005). Rödor et al. (1998) found different band sizes for cultivar and synthetic wheat, though the differences were comparable. Only 1 out of 9 eSSR markers could be mapped on 1BS-1RS. This low success rate may be due to low polymorphism between wheat and rye 1S arms. Whether this indicates high conservation of genic regions across species we cannot tell at this point. Peng et al. (2005) showed amplification of 15 wheat eSSR markers in rye as well as barley. They detected polymorphism between wheat and barley but did not mention polymorphism between wheat and rye. A comparison of the current 1RS-1BS map with the SSR (Rödor et al. 1998) and eSSR maps (Peng et al. 2005) of the 1B chromosome shows a good agreement in marker order, location, and relative positions in its distal part, regardless of the projection mode used. A similar approach was used to generate a genetic map of *ph1b*-induced 2R-2B intergeneric recombinants, with a similar success (Lukaszewski et al. 2004), validating the wide-hybrid approach to mapping.

The integration of physical and molecular markers in the present 1RS-1BS map also provided the alignment of recombinant breakpoints over each map interval. This information offers great potential to study agronomic traits affected by the introduction of alien 1RS chromatin into wheat. Our working hypothesis was if a 1B+ line shows some specific trait then this trait should be absent in its complimentary T- line, and vice-versa. To check the applicability of this concept, we looked at different root characters in wheat.

Studies during the past few years showed an increased root biomass in wheat with the alien 1RS chromosome arm over standard spring wheat Pavon 76 and found a positive correlation of increased root biomass with grain yield (r = 0.90) (Ehdaie et al. 2003). Recent studies in rice (*O. sativa* L.) and maize (*Zea mays* L.) have also correlated the QTLs for root traits with QTLs for yield under field conditions (Yadav et al. 1997; Price et al. 1997, 2000; Tuberosa et al. 2002). A major limitation to study roots is the difficulty in making observations as the process is very laborious and time consuming. In the present study, we tested only three recombinant chromosomes with breakpoints selected to divide the recombining portion of the arm into three segments of roughly similar lengths. The experiments were conducted in three years at the same months of the year, in replicated trials, to determine the magnitude of the genotype × year interaction and the repeatability of results from the sand-tube technique. Significant variation was observed from year to year (Table 3), with considerably higher means in the third year. This might have been due to relatively lower temperatures in the third year providing better conditions for vegetative growth. The significant genotype × year interaction observed for number of roots greater than 30cm (Table 3) was due to only one genotype, T-14, producing more roots only in year 2. Otherwise, other genotypes showed similar trends for this character across the three years. The lack of significant genotype × year interaction for most of the root characters examined (Table 3) indicated high repeatability for these root traits. Quade analysis used on rank sums separated the five genotypes in two groups viz., Pavon 1RS.1BL, 1B+2 and 1B+38 containing distal rye chromatin with

higher rooting ability and Pavon 76 and T-14 lacking the distal rye segment with lower rooting ability (Table2).

Overall, the five genotypes examined showed relatively small variation for shoot characters but they differed in root characteristics including root biomass. Based on the results, we propose the presence of quantitative trait locus or loci (QTL) for root traits in the distal 15% of the physical length of 1RS arm. In cereals, most of the gene rich regions for agronomic traits are concentrated in the distal ends of the chromosomes (Gill et al. 1996). Kim et al. (2004) conducted field studies for the agronomic performance of 1R from different sources of origin. They found 1RS increase the grain yield significantly, and interestingly, all the lines with 1RS did not show significant differences for shoot biomass. They did not look at the root traits which could have also been useful. In a similar study, Waines et al. (2004) compared 1RS from different sources to study root biomass in hexaploid as well as tetraploid wheats. The translocated hexaploid wheats with $1RS_{Amigo}$ and $1RS_{Kavkaz}$ showed 9% and 31% increase in root biomass than Pavon 76, respectively. Similar results were reported for the durum wheat "Aconchi" (without 1RS) versus Aconchi with the 1RS arm. These studies point towards the definite presence of gene(s) for greater rooting ability on 1RS, and also the differential expression of alleles from different sources of 1RS in root traits. In a recent study on rice root anatomy, Uga et al. (2008) identified a QTL for metaxylem anatomy on the distal end of the long arm of chromosome 10. In another comparative study of rye DNA sequences with rice genome, the distal end of the long arm of chromosome 10 of rice was syntenic to 1RS (Hackauf et al. 2009). Both these studies provide evidence to support the general

applicability of our mapping method to locate the probable region on 1RS, carrying gene(s)/QTL for root traits.

Our present finding on root studies prepares a platform to find gene(s)/QTL for root traits on 1RS. Future work will focus on use of a larger number of recombinant lines to narrow down the QTL region of 1RS responsible for increased root traits and find the molecular markers linked to these QTL. Ultimately, this would lead to our goal of physical mapping and then positional cloning of the root QTL.

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Chapter 3

Dissection of QTL effects into additive and epistatic components for root traits on the short arm of rye chromosome 1 in bread wheat

ABSTRACT

A high resolution chromosome arm-specific mapping population was used in a first ever attempt to locate/detect gene(s)/QTL for different root traits on the short arm of rye chromosome 1 (1RS) in bread wheat. This population consisted of induced homoeologous recombinants of 1RS with 1BS, each originating from a different crossover event and therefore distinct from all other recombinants in the proportions of rye and wheat chromatin present. It provides a simple and powerful approach to detect even small QTL effects using fewer progeny. A promising empirical Bayes method was applied to estimate additive and epistatic effects for all possible marker pairs simultaneously in a single model. This method has an advantage for QTL analysis in minimizing the error variance and detecting interaction effects between loci with no main effect. A total of 15 QTL effects, six additive and 9 epistatic, were detected for different root traits in 1RS wheat. Epistatic interactions were further partitioned into inter-genomic (wheat and rye alleles) and intra-genomic (rye-rye or wheat-wheat alleles) interactions affecting various root traits. Four common regions were identified involving all the QTL for root traits. Two out of four regions carried QTL for almost all the root traits and also were responsible for all the epistatic interactions. Evidence for inter-genomic interactions is provided. Comparison of mean values of recombinants from these four regions further supported the QTL detection.

Introduction

The root, the hidden half of a plant, is important for numerous functions including water and nutrient uptake that make it difficult to over look the root's importance to plant productivity (MacMillan et al 2006). It is an irony that this organ has inspired fewer plant scientists to work on it than the number who work on above-ground plant parts. The limited research effort in improvement of roots may be because of the difficulty in observing, measuring and manipulating them (Shen et al. 2001).

Rye (*Secale cereale* L., 2n = 2x = 14, genome formula RR) is well known for its abiotic stress tolerance (Hackauf et al. 2009) and resistance against diseases and pests (Zeller and Hsam 1984). Very likely, those resistances facilitated the selection and establishment of a spontaneous centric rye-wheat translocation 1RS.1BL in place of chromosome 1B of bread wheat (*Triticum aestivum* L., 2n = 6x = 42, genome formula BBAADD) (Mettin et al. 1973). The translocation spread throughout the world even when these resistance genes were not important, and eventually made it into hundreds of released cultivars (Braun et al. 1998). It was realized that the translocation increased grain yield even in the absence of pathogens (Villareal et al. 1991), and eventually, the yield gain was attributed to a substantially increased root biomass (Ehdaie et al. 2003; Ehdaie and Waines 2008). A larger root system increases uptake of water and nutrients from the soil (Ehdaie et al. 2003).

Cereal roots have two main classes, seminal roots and nodal roots (Esau, 1965). Seminal roots originate from the germinating embryonic hypocotyls, and nodal roots emerge from the coleoptile nodes at the base of the apical culm (Manske and Vlek 2002). Weaver (1926) compared the root systems of rye and bread wheat, and reported rye had longer seminal roots. The genetic control of root characteristics is poorly understood as the growth pattern changes greatly depending on the environment and almost always it is obscured from direct observation. Root traits are believed to be complex and controlled by many genes, each with a small genetic effect. Genetic loci controlling such traits are called quantitative trait loci (QTL). With the advent of molecular markers, it has become possible to estimate the genome location and size of QTL, including those for root characters. Research has recently been undertaken to map root QTL in rice (Champoux et al. 1995; Price and Tomos 1997; Zheng et al. 2000), maize (Lebreton et al. 1995), common bean (Ochoa et al. 2006), and *Arabidopsis* (Gerald et al. 2006). In wheat, many QTL have been identified for above ground traits of agronomic importance (Spielmeyer et al. 2007; Maccaferri et al. 2008; Zhang et al. 2008) but no information on root genes or QTL has been reported. Disregarding root pathogens, the most recent wheat gene catalogue contains not a single reference to roots (McIntosh et al. 2008).

Most quantitative traits are determined by many interacting loci with small genetic effects that are modified by environmental factors (Falconer and Mackay 1996). Interaction of these alleles at different loci is called epistasis (Fisher 1918; Holland 2001). Epistasis is now considered an important source of genetic variation with some

components, especially Additive × Additive receiving more attention (Goodnight 2000; Jannink 2003) due to their heritable nature (Ehdaie and Cress 1973). Efficient methods have been developed to map QTL with additive (main) effects (Lander and Botstein 1989; Xu 2003; Zhang et al. 2005) but mapping QTL with epistatic effects is still at the juvenile stage. There were efforts to detect epistasis using Bayesian models (Yi et al. 2003; 2006) but they were unable to guarantee detection of all such effects. The Bayesian approach uses a given prior distribution (a prior estimate of an unobserved parameter) and Markov chain Monte Carlo (MCMC) sampling to infer posterior distribution conditional on the data (observable) (Wu et al 2007). Recently, Xu (2007) developed an empirical Bayes method (E-BAYES) that requires no MCMC samplings, yet still estimates the variance parameters for the priors of the regression coefficients. Simultaneous estimation of additive (main) effects of all individual markers along with epistatic effects from all combination of marker pairs made this approach for estimation of significant epistatic effects where many may go undetected.

Recent studies have shown the efficiency of chromosome specific mapping populations over traditional crosses in detecting a given effect with fewer progenies (Singer et al. 2004). The power of such a population, in a statistical sense, has been demonstrated in animal studies such as mice (Nadeau et al. 2000; Singer et al. 2004). In plants, stepped aligned recombinant inbred strains (STAIRS) were generated in *Arabidopsis*, using chromosome substitution strains (Koumproglou et al. 2002), but there is no detailed report until now on using such a mapping population for analysis of

complex traits. It is another irony that chromosome substitution lines have been incorporated in wheat breeding programs since the 1950s (Sears 1953; Law et al. 1983) but it is only now that they are being appreciated for generating a chromosome specific mapping population to study quantitative traits. This paper presents a first ever attempt to characterize QTL effects for wheat root traits, using the E-BAYES method in combination with a chromosome specific mapping population. Here, we report the detection of additive and epistatic effects and also further dissection of gene interaction effects into inter-genomic and intra-genomic epistatic effects.

Materials and Methods

Mapping population

The root study was done on a total of 29 recombinant lines, each having a different recombination breakpoint. These 29 lines were selected from a population of 68 1RS-1BS recombinants, as used to generate 1RS-1BS integrated map (Sharma et al. 2009). This map consisted of a total of 20 markers in 15 intervals spanning 35-40% of the physical length of the chromosome arm, with average spacing of ca. 2.5cM. Assuming the total map length of 50 cM (each chromosome is a single breakpoint translocation), the 68 1RS-1BS recombination breakpoints bring the average resolution of this map to 0.7cM.

Phenotyping

Plants were grown in PVC tubes, 80 cm long and 10 cm in diameter and regularly watered. After 45 days plants were harvested, roots were washed by the floatation technique (Böhm 1979) and various characters were measured. The experiment involved 32 lines including Pavon 76, Pavon 1RS.1BL and Pavon Dt. 1BL as checks, grown in a glasshouse for four seasons in a randomized complete block design with four replications. The shoot characters measured were; the longest leaf length, maximum width of the longest leaf, leaf area, plant height, number of tillers per plant, dry shoot biomass, and the root characters were; number of roots greater than 30 cm, longest root length, total length of roots greater than 30 cm, shallow root weight (depth < 30 cm), deep root weight (depth > 30 cm), total root weight (TRW), and root biomass to shoot biomass ratio.

The genotype of each marker was coded as +1 for the wheat allele and -1 for the rye allele. The overall mean phenotypic value of each line across the environments was taken as the input phenotype for that line representing the genotypic value of that line. All QTL detected would represent those showing consistent effects across environments.

Statistical analysis

The empirical Bayes method was used in the data analysis (Xu 2007). The linear model to describe the vector of phenotypic values for a trait is:

$$\mathbf{y} = \mathbf{1}\mu + \sum_{l=1}^{m} \mathbf{Z}_l \gamma_l + \sum_{l'>l}^{m} (\mathbf{Z}_l \times \mathbf{Z}_{l'}) \gamma_{ll'} + \boldsymbol{\varepsilon} \quad \text{(I)}$$

where, $\mathbf{y}$ is an $n \times 1$ vector, μ is the population mean, $\mathbf{Z}_l$ is genotype indicator variable for a given locus l, γ_l is the additive effect for locus l, $\gamma_{ll'}$ is epistatic effect between loci l and l', and ε is the residual error. The notation $\mathbf{Z}_l \times \mathbf{Z}_{l'}$ represents the direct product of vectors $\mathbf{Z}_l$ and $\mathbf{Z}_{l'}$. Excluding μ, the total number of QTL effects for m = 15 markers is $p = m(m+1)/2 = 120$, including $m = 15$ main effects and $m(m-1)/2 = 105$ pair-wise epistatic effects. We now use j to index the jth genetic effect (including additive and pair-wise epistatic effects) for $j = 1, \ldots, p$. We can rewrite model (1) as

$$\mathbf{y} = \mathbf{1}\mu + \sum_{j=1}^{p} \mathbf{X}_j \beta_j + \boldsymbol{\varepsilon} \quad \text{(II)}$$

Comparing model (II) to model (I), we can see that $\mathbf{X}_j = \mathbf{Z}_l$ and $\beta_j = \gamma_l$ if the jth effect is a main effect, and $\mathbf{X}_j = \mathbf{Z}_l \times \mathbf{Z}_{l'}$ and $\beta_j = \gamma_{ll'}$ if the jth effect is an epistatic effect. Therefore, model (2) is a general model for both the main and the epistatic effects.

As far as the method of estimating genetic effects is concerned, distinction between a main effect and an epistatic effect is unnecessary (Xu and Jia 2007).

QTL effects were estimated by the two step approach of Xu (2007). First, variance components were estimated by a typical random model variance-component analysis using maximum-likelihood method (Hartley and Rao 1967) and second, estimation of QTL effect by best linear unbiased prediction (BLUP) given the estimated variance components (Robinson 1991; Xu and Jia 2007). This analysis provided BLUP estimates of QTL effects β_j and that value was squared to calculate the genetic variance explained by the QTL. Therefore, the total genetic variance of the population was calculated as $V_{Ga} = \sum_{j=1}^{p} \beta_j^2 \times \mathbf{1}$ for *j*th additive (main) effect and $V_{Ge} = \sum_{j=1}^{p} \beta_j^2 \times \sigma^2 (m_l \times m_{l'})$ for *j*th epistatic effect, where $\sigma^2 (m_l \times m_{l'})$ is the variance of interaction between markers at *l* and *l'* loci. The total phenotypic variance for the population for each trait was calculated as $V_P = \sum_{i=1}^{n} (Y_i - \overline{Y})^2 / (n-1)$, where Y_i is the mean value of the *i*th genotype across environments, $\overline{Y}$ is the average of all the mean values of all the genotypes for a trait, and n is the number of genotypes. The proportion of the phenotypic variance that is explained by the *j*th QTL was calculated as $H = V_{Ga} / V_P$ for additive (main) effect and $H = V_{Ge} / V_P$ for epistatic effect. To declare an estimated effect as "significant", each estimated effect was converted into a *t*-test statistic, $t_j = |\beta_j| / S_{\beta_j}$, and then further

converted in a LOD score using $\mathrm{LOD}_j = t_j^2 / 4.61$. All effects with the LOD scores larger than a critical value were declared as significant. The critical value was calculated by a permutation analysis (Churchill and Doerge 1994). The (1-α) × 100th percentile of the distribution of the LOD scores of the reshuffled sample was a good approximation of the true critical value, where α is a controlled experimental type I error (Xu and Jia 2007).

Results

We used the eBayes option of PROC QTL (Hu and Xu 2009) to analyze the phenotypic data for different traits (see materials and methods). The eBayes option of PROC QTL implements the empirical Bayes method of Xu (2007). The computer program can be downloaded from http://www.stratgen.ucr.edu. The critical t-values were calculated for all additive (main) and epistatic effects and most stringent values for all the phenotypic traits fell in the 3.8 – 4.0 range at an experimental type I error of α = 0.007, but we chose a maximum critical value of 4.0 to avoid any false detection of QTL (Table 1). To check the effect of the presence of 1RS in wheat, the mean of 'Pavon 76' (spring wheat from CIMMYT, Mexico) was compared with all the recombinant lines with 1RS segments including Pavon 1RS.1BL across the environments (Table 2). Further, means of different recombinants were compared relative to the position of 1RS segments in recombinants to validate the mapping of root QTL located in the present study.

Table 3.1: Significant genetic effects of different QTL linked with different root traits, namely, number of roots greater than 30 cm (NR>30), longest root length (LRL), total root length of roots greater than 30 cm (TRL), shallow root weight (SRW), deep root weight (DRW), and total root weight (TRW)

Root Trait	Marker I	Marker II	Genetic effect	Proportion of Phenotypic variance	LOD Score	Types of Effect
NR > 30cm	9	-	0.64	0.34	4.86*	Additive
	2	13	0.79	0.26	4.69*	Epistatic
	13	-	0.83	0.57	3.90*	Additive
	10	-	0.75	0.47	3.60*	Additive
LRL	12	14	2.29	0.18	5.68*	Epistatic
	5	-	2.74	0.52	3.36§	Additive
TRL	13	-	56.01	0.56	4.92*	Additive
	2	13	52.82	0.24	4.19*	Epistatic
	10	-	47.28	0.40	3.91*	Additive
SRW	4	5	71.47	0.15	5.57*	Epistatic
	13	14	55.55	0.18	3.53*	Epistatic
DRW	13	14	32.81	0.31	7.61*	Epistatic
	4	5	26.79	0.11	4.67*	Epistatic
TRW	4	5	96.72	0.14	5.42*	Epistatic
	13	14	89.77	0.23	4.88*	Epistatic

* = significant LOD scores (p = 0.007)
§ = close to LOD threshold value

Table 3.2: Mean phenotypic values of root and shoot traits for both the parents Pavon 76 and Pavon 1RS.1BL and 1RS-1BS recombinant lines

	N	NR	LRL	TRL	SRW	DRW	TRW	NT	PH	LL	LW	LA	SB	R/S
Pavon 76	16	9.0	89.7	559.7	374.7	148.9	523.6	6.5	52.1	35.7	1.06	31.1	1623.6	0.36
Pavon 1RS.1BL	16	11.2	90.0	691.7	405.5	152.0	557.4	5.8	54.3	35.8	1.05	31.0	1604.7	0.39
Recombinant lines	464	10.2	83.0	611.9	411.2	158.0	569.2	6.8	53.2	34.7	1.05	30.2	1641.9	0.39
➢ **T – lines**	224	9.8	81.2	579.4	397.7	148.6	546.3	6.5	52.6	34.4	1.03	29.4	1559.9	0.39
♣ **1B + lines**	240	10.7	84.7	642.2	423.8	166.8	590.6	7.1	53.9	34.9	1.07	30.9	1718.5	0.38
♣ **(1B + lines) II, III, IV QTL**	128	10.8	84.5	653.7	419.3	162.2	581.5	7.1	53.4	34.8	1.06	30.5	1703.6	0.39
♣ **(1B + lines) I, II, III and IV QTL**	64	10.7	85.1	632.7	463.4	188.8	652.2	7.5	53.8	36.1	1.06	31.1	1803.1	0.39
Range of recombinants		7.5-12.0	71.9-91.9	411.2-733.2	283.4-586.3	77.0-232.7	360.2-819.0	5.1-8.4	48.4-58.4	30.8-39.7	0.96-1.17	26.6-35.4	1207.8-2119.8	0.34-0.44

N = number of plants per mean across genotypes × four seasons × four replications
NR = number of roots greater than 30 cm, LRL = longest root length, TRL = total root length of roots greater than 30 cm, SRW = shallow root weight, DRW = deep root weight, TRW = total root weight, NT = number of tillers, PH = plant height, LL = longest leaf length, LW = maximum width of the longest leaf, LA = leaf area, SB = shoot biomass, and R/S = root to shoot biomass ratio
Recombinant lines = all 29 recombinants used
T - lines = 14 recombinants with distal wheat segment
1B + lines = 15 recombinants with distal rye segment
(1B + lines) II, III, and IV QTL = 8 recombinants with distal most 1RS region containing QTL regions I, II, and III
(1B + lines) I, II, III, and IV QTL = 4 recombinants with distal most 1RS region containing QTL region I, II, III, and IV

Root traits

All root characters measured showed significant QTL effects on the short arm of chromosome 1 of rye; a total of 15 QTL effects were found. Six of these were additive and nine showed epistatic interactions. Of the nine epistatic interactions, five were inter-genomic interactions between wheat and rye alleles and the rest were intra-genomic interactions (Table 1). The highest single additive effect explained 57% of the phenotypic variation for the number of roots > 30cm long; the same effect explained 56% of the total phenotypic variation for the total root length. This QTL is tightly linked to marker *Pm8*, a powdery mildew resistance locus. The highest intra-genomic epistatic effect explained 31% of the phenotypic variance for deep root weight with a LOD score of 7.61 (Table 1, Figure 1d). It was detected between two adjacent regions marked on the map by loci *Pm8(13)* & *Gli-1,Glu-3(14)* (Figure 2). The highest inter-genomic epistatic effect was detected for the number of roots > 30 cm. This inter-genomic interaction involved *Pm8(13)* & *Xucr_2(2)* (Figure 2), and explained 26% of the phenotypic variation with LOD score of 4.69 (Table 1, Figure 1a).

Shoot traits

With the exception of shoot biomass, no significant QTL was detected for any shoot trait measured and no significant QTL effect was detected for the root to shoot

biomass ratio. Two significant intra-genomic epistatic effects were detected for shoot biomass. The first one, *Xucr_4(5)* & *Sr31(10)* loci explained 42% (LOD = 7.34) and the second one, *Xucr_8(12)* & *Gli-1,Glu-3(14)* explained 16% (LOD = 6.80) of the phenotypic variation (Data not shown).

Mean comparisons

The means for all phenotypic traits of Pavon 76, Pavon 1RS.1BL, and all of the genotypes with 1RS segments are presented in Table 2. Pavon 1RS.1BL which contains the entire 1RS arm showed higher means for root traits compared to Pavon 76. The recombinants had, in general, higher means for root traits than Pavon 76 (Table 2). The recombinants were divided into two groups on the basis of the location of the rye segment: distal (1B + lines) or proximal (T – lines). In both groups, the overall means for root traits were higher than that of Pavon 76, but means of 1B + lines were higher than those of T – lines (Table 2).

Figure 3.1: Three dimensional graphic representation of QTL effects for six different root traits of the 1RS-1BS recombinant bread wheat population. (a) number of roots greater than 30 cm (NR>30), (b) longest root length (LRL), (c) total root length of roots greater than 30 cm (TRL), (d) shallow root weight (SRW), (e) deep root weight (DRW), and (f) dry root biomass (TRW). The main (additive) effects are on the diagonals and the epistatic effects are on the left triangle of the 3D plots. (The graphical scales for LOD scores in individual graphs are different, as generated by the software program).

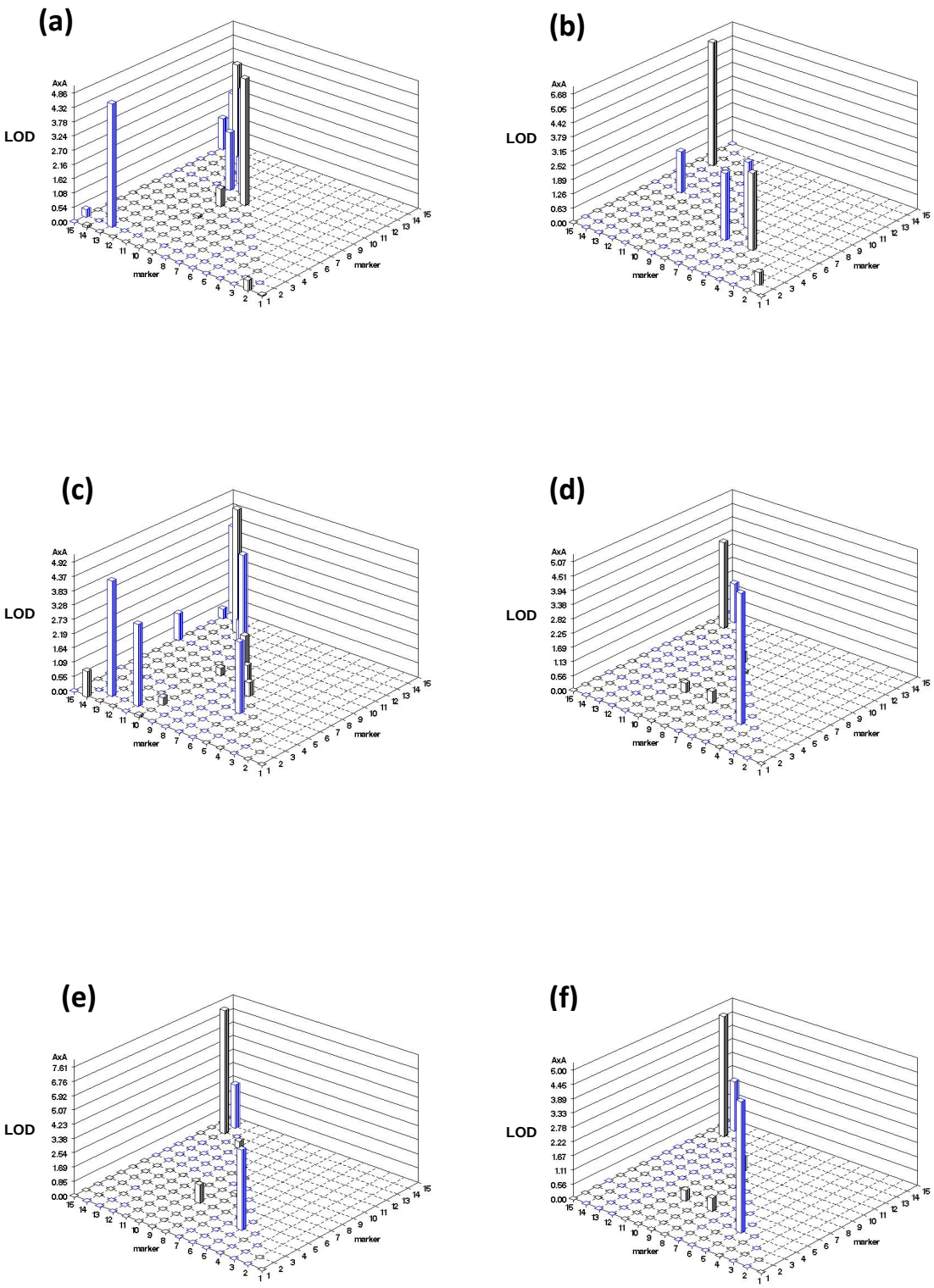
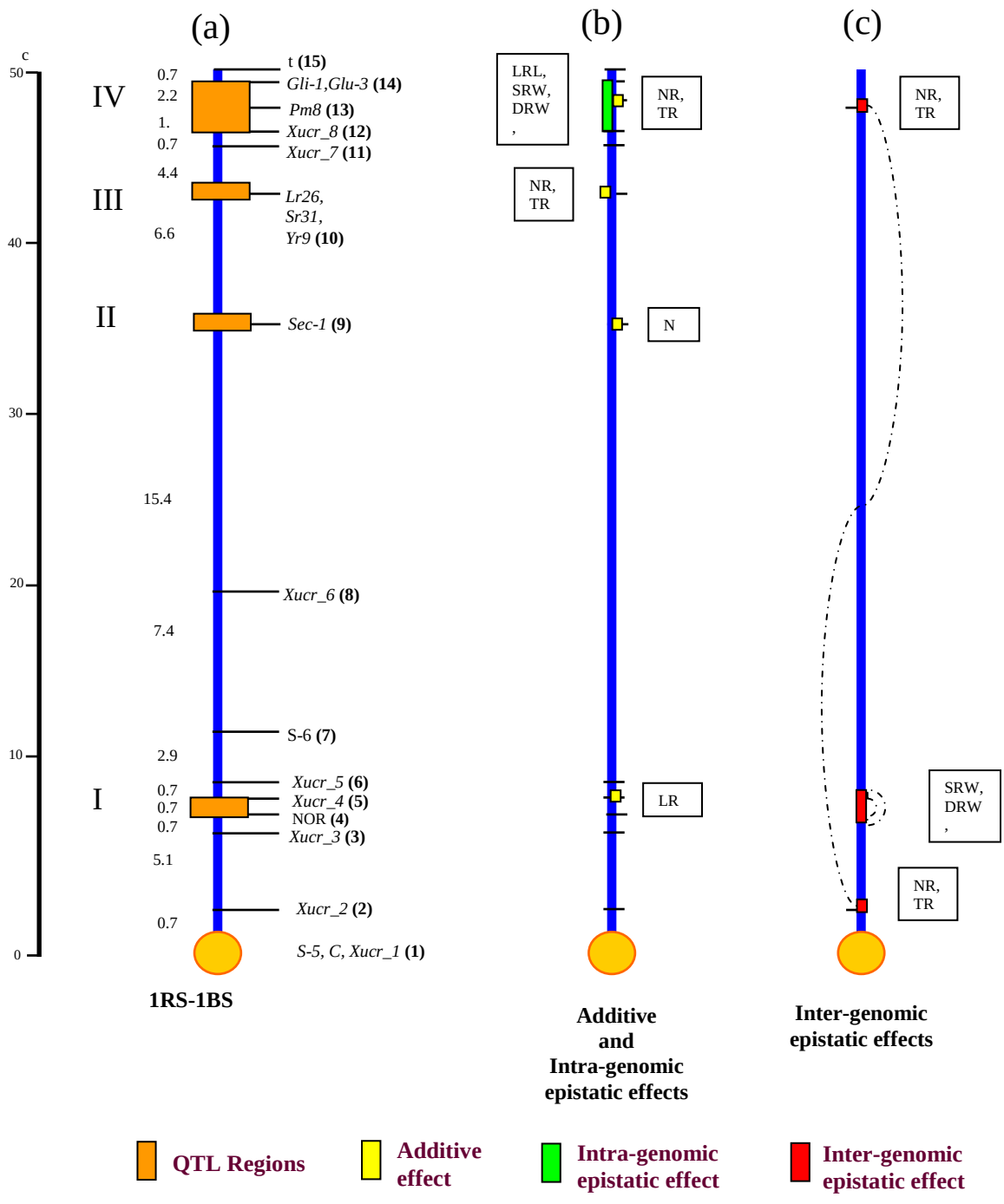

(a)
LOD
AxA
4.86
4.32
3.78
3.24
2.70
2.16
1.62
1.08
0.54
0.00
marker
(b)
LOD
AxA
5.68
5.05
4.42
3.79
3.15
2.52
1.89
1.26
0.63
0.00
marker
(c)
LOD
AxA
4.92
4.37
3.83
3.28
2.73
2.19
1.64
1.09
0.55
0.00
marker
(d)
LOD
AxA
5.07
4.51
3.94
3.38
2.82
2.25
1.69
1.13
0.56
0.00
marker
(e)
LOD
AxA
7.61
6.76
5.92
5.07
4.23
3.38
2.54
1.69
0.85
0.00
marker
(f)
LOD
AxA
5.00
4.45
3.89
3.33
2.78
2.22
1.67
1.11
0.56
0.00
marker

Figure 3.2: (a–c) Diagrammatic representations of QTL for root traits on the consensus genetic map of 1RS-1BS recombinant lines. (a) Values on the left side of the 1RS-1BS map are genetic distances and right side of the map shows different markers. Values in the parentheses are the number of markers used in this study as reference starting at 1 from the proximal side of the 1RS-1BS map to 15 at the distal end. I, II, III, and IV are the main regions comprising most of the QTL effects for root traits, (b) location of different QTL effects on 1RS-1BS map, yellow rectangles are the additive effects, green rectangles are intra-genomic epistatic effects (between rye-rye or wheat-wheat loci), and (c) location of inter-genomic epistatic interaction (between rye and wheat loci) - red rectangles connected by curved dashed line. Boxes with borders show different root traits, R- rye alleles are responsible for QTL effect, and W- wheat alleles are responsible for QTL effect.


(a)
(b)
(c)
c
50
40
30
20
10
0
IV
III
II
I
0.7
2.2
1.
0.7
4.4
6.6
15.4
7.4
2.9
0.7
0.7
0.7
5.1
0.7
t (15)
Gli-1,Glu-3 (14)
Pm8 (13)
Xucr_8 (12)
Xucr_7 (11)
Lr26,
Sr31,
Yr9 (10)
Sec-1 (9)
Xucr_6 (8)
S-6 (7)
Xucr_5 (6)
Xucr_4 (5)
NOR (4)
Xucr_3 (3)
Xucr_2 (2)
S-5, C, Xucr_1 (1)
1RS-1BS
LRL,
SRW,
DRW
,
NR,
TR
NR,
TR
N
LR
NR,
TR
NR,
TR
SRW,
DRW
,
NR,
TR
Additive
and
Intra-genomic
epistatic effects
Inter-genomic
epistatic effects
QTL Regions
Additive
effect
Intra-genomic
epistatic effect
Inter-genomic
epistatic effect

Discussion

Preliminary studies (Ehdaie and Waines 2006; Sharma et al. 2009) indicated the distal region of 1RS covering ca. 15% of its genetic map, in the genetic background of bread wheat Pavon 76, had a significant effect on root traits. Since root traits are quantitative in nature and controlled by polygenic inheritance (Ehdaie et al. 2001), it was expected to find several QTL associated with the root traits studied.

Advantage of E-Bayesian Statistics

There were many statistical approaches to identify and estimate QTL effects such as linear regression model, interval mapping, maximum likelihood method, and Bayesian statistics. Also, there were a number of software, such as MAPMAKER/QTL and Cartographer to map QTL for different phenotypic traits in plants (Price and Tomos 1997; Spielmeyer et al. 2007) as well as in animals (Carlborg et al. 2005; Yi et al. 2006). The E-BAYES method used in this study was chosen for its advantages over other commonly available software and methods. E-BAYES outperformed all other Bayesian methods, stochastic search variable selection (SSVS), penalized likelihood (PENAL), and least absolute shrinkage selection operator (LASSO), in terms of minimizing the error variance. It made shrinkage very selective by providing optimal estimates of variance components, with unshrinking of large effects while small effects are shrunk to zero (Xu 2007). There were reports of an empirical Bayes method developed by other groups

(George and Foster 2000; Yuan and Lin 2005) but they did not shrink each regression coefficient by its own prior and also they were not as easy to understand as E-BAYES developed by Xu (2007). In this study, E-Bayesian approach was applied for the first time in wheat to dissect QTL for root traits.

Chromosome arm specific mapping population

The mapping population was novel and different from conventional breeding populations. It is analogous to near-isogenic lines (NILs) where two lines differ for a genomic segment in an otherwise homogeneous background, the distinction here is cross genome differences. In a recent study (Keurentjes et al. 2007), a comparison between mapping power of the RIL (recombinant inbred line) and NIL populations revealed that population size of RIL is more important than that of replication number, where due to Beavis effect (Xu 2003), the explained variances are overestimated in smaller populations. On the other hand, mapping power of a NIL population relies more on replication number than population size (Keurentjes et al. 2007). The lower localization resolution of NILs can be increased by generating chromosome or region specific mapping populations. The population in this study was a set of single chromosome arm recombinant lines. In such sets, the two parents differ by a single chromosome arm, the remaining chromosome arm pairs being as identical as ca. ten backcrosses can achieve. This population has statistical advantage in detecting QTL with relatively small phenotypic effects in fewer progeny than by analyzing a large segregating population

(Nadaeu et al. 2000). It is due to the absence of phenotypic noise of segregating unlinked QTL in usual mapping populations with heterogeneous background. Thus, an undetectable effect in a large segregating population can be converted to a detectible effect even in fewer progeny in a chromosome specific population (Nadaeu et al. 2000; Singer 2004). Nadaeu et al. (2000) proved this concept, explaining a sample of 28 mice specific for a single chromosome, was sufficient for the strong detection of a specific trait locus which they could detect with weak evidence in an intercross progeny of 300 individuals. The present study was a step further in using a chromosome arm-specific population. Here, the parent lines differed by the presence/absence of the 1RS.1BL wheat-rye translocation. A set of 1RS-1BS recombinant chromosomes was generated (Lukaszewski 2000), each one originating from a different crossover event and therefore differing from all other recombinants in the proportions of rye and wheat chromatin present. This near-isogenic line approach in essence eliminates the question of population size as a factor in QTL detection. If a QTL is present on the studied arm, it will manifest itself in any properly conducted experiment. The number of lines/recombinants used only affects mapping resolution, that is, the size of the segment to which a trait can be assigned. Thus, 29 lines used in this study, each originating from a single crossover, produce an average resolution of ca. 1.7 cM, a feat not frequently achieved in mapping populations.

Dissection of QTL effects for root traits

We examined a total of 13 phenotypic traits including seven shoot traits and six root traits. A total of 15 QTL effects for root traits were detected using the E-BAYES method. Further partitioning of the root QTL showed six additive effects and nine epistatic effects. Two different types of epistatic effects were recognized that we termed intra-genomic and inter-geomic epistatic effects. Intra-genomic epistatic effect resulted from interaction between the alleles of two different loci of either rye or wheat. Inter-genomic epistatic effect involved interaction between different alleles of two loci of different genomes, such as one from wheat and one from rye. Pumphrey et al. (2009) reported allelic interactions between different genomes of bread wheat in a synthetic hexaploid *T. aestivum*. The differential root traits among wheat genotypes with 1RS translocation on 1AL, 1BL, and 1DL (Ehdaie et al. 2003) could likely be due to inter- and intra-genomic epistatic interactions.

Common QTL for different root traits

Most of the additive and epistatic effects detected for variation in different root traits shared common QTL regions. Markers *Sr31(10)* and *Pm8(13)* shared the QTL with additive (main) effects for the number of roots >30 cm and total root length. One of these markers, *Pm8*, showed inter-genomic epistatic interaction with *Xucr_2(2)* and this epistatic effect was again common for number of roots >30 cm and total root length.

Number of roots >30 cm was affected by four QTL involving almost all markers within the distal 15% of the 1RS-1BS genetic map. Three QTL effects were involved in the expression of total root length and all three shared the same region as for number of roots >30 cm. All the three characters for root biomass viz; shallow root weight, deep root weight, and total root weight, showed two epistatic effects each, and both epistatic effects were common. Pairs of loci involved were NOR*(4)* & *Xucr_4(5)* for inter-genomic epistatic effect and *Pm8(13)* & *Gli-1,Glu-3(14)* for the other intra-genomic epistatic effect. *Pm8(13)* and *Gli-1,Glu-3(14)* also showed significant interactions with other markers for other root traits. This repetitive detection of the same chromosome regions or the associations of the same markers with QTL effects further consolidates our approach to map QTL for root traits in wheat.

Epistasis between loci without main effect

In conventional QTL mapping, the focus is on detecting the QTL with main (additive) effects and then applying an epistatic model to examine the epistatic effect between the QTLs with main effects. In nature, there are loci with small genetic effects which sometimes go undetected in a phenotype but their interaction with other similar loci may have a significant effect. It would be a disadvantage not to include them in the genetic model. Here, we proved the superiority of our method in detecting epistatic effects between two pairs of loci and none of those two pairs of loci had main effects as against other methods where epistatic effects were estimated for loci with main effects

only (Kao and Zeng 2002; Maccaferri et al. 2008). This has clearly been shown in the intra-genomic epistatic effects between pairs of loci, *Xucr_8(12)* and *Gli-1,Glu-3(14)* for longest root length. Similar intra- and inter-genomic epistatic effects were also explained for shallow root weight, deep root weight, and total root weight. All these three characters for root biomass showed two pairs of loci involved in two different epistatic interactions and none of these loci had detectable additive effect for these characters.

We studied seven shoot characters for the estimation of QTL effects. Interestingly, we did not find any significant main or epistatic effect for any of the shoot characters except shoot biomass. Variation in shoot biomass was explained by two intra-genomic epistatic effects; *Xucr_4(5)* with the *Sr31(10)* region and *Xucr_8(12)* with *Gli-1, Glu-3(14)* (data not shown). The second epistatic effect was also detected for "longest root length". It is not unexpected that shoot biomass shares QTL with some of the root characters as they are involved in the growth and development of the same plant. Recently, a QTL study was conducted for only three shoot characters; plant height, heading date, and grain yield, in durum wheat (Maccaferri et al. 2008). They found five QTLs for plant height and one out of the five was on chromosome 1BS, and this QTL was detected only in 7 out of 16 environments. Similar to these results, we also found a very weak QTL effects for number of tillers and plant height. They fell short of our LOD threshold value, but QTL for number of tillers shared the same location with shoot biomass in our study (data not shown) which was expected.

Major QTL regions

Four regions were identified that carry almost all the QTL with both additive and epistatic effects (Figure 2). In Figure 2, they have been marked as I, II, III, and IV as we move from the centromere towards the telomere. Region I was involved in five of the six root traits and covered 0.7 cM, which is the highest resolution we could obtain. Region II involved only one marker, *Sec-1(10),* showing a single additive effect (number of roots >30 cm). Region III covered 0.7 cM and was involved in two main QTL effects for number of roots >30 cm and total root length along with one epistatic effect for shoot biomass. Region IV covered 3.7 cM with three markers. This region indicated presence of QTL effects for all the root traits and also for shoot biomass. Three out of four QTL regions were located in the satellite region of chromosome 1RS. Two of them were located in the distal most 10% of the 1RS region which is in agreement with previous studies (Ehdaie and Waines 2006; Sharma et al. 2009).

Validation with mean comparisons

In Table 2, mean comparisons of Pavon 76, Pavon 1RS.1BL, and all the recombinant lines also revealed the higher mean values of root traits in the presence of 1RS chromatin. The presence of distal 1RS segments (1B + lines) showed higher mean values for root traits than the presence of proximal 1RS (T - lines). Lower mean values of T-lines may be attributed to the missing distal segment with two markers in all T-lines,

one of which belonged to QTL IV. As discussed earlier, three out of four QTL regions identified were located in the distal 10% of the 1RS-1BS arm. For further mean comparisons, 1B + lines involving QTL regions IV, III and II were chosen due to their close proximity with each other. As expected, they showed the higher mean values for root traits compared to T – lines, and significantly higher than those of Pavon 76. The comparison of 1B + lines with distal 1RS up to region IV showed higher mean values than Pavon 76, Pavon 1RS.1BL, and all the different sets of recombinant lines. These mean comparisons confirmed the detection of QTL using the E-BAYES method. From the above discussed mean comparisons of recombinant lines, it was obvious the distal part of 1RS is important for root traits in bread wheat. Hence, we propose the rye loci as major contributors in most of the additive effects and intra-genomic epistatic interactions detected for the root traits.

Root traits in cereals were associated with drought tolerance. An earlier study (Waines et al. 1998) revealed the association of rye 1RS, 2RS, 5R, and 7R chromosomes and chromosome arms with drought tolerance. Other recent studies confirmed the involvement of these rye chromosomes in drought tolerance in the bread wheat background (Koszegi et al. 1996; Mohammadi et al. 2003). In cv. Chinese Spring, all seven rye chromosomes appeared to carry genes influencing drought tolerance and the evapo-transpiration efficiency (Waines et al. 1998). The methodology used in this paper may provide a general method to analyze these other chromosome arms for QTL analysis and study of epistatic effects.

Here, we addressed the nature of QTL effects for root traits in wheat by studying the additive allelic effects and the intra- and inter-genomic epistatic interactions contributed by rye 1RS. This study provided important information on root genetics which can be pivotal for alien introgressions of genes involved in complex traits. This may also be helpful in marker assisted selection by selecting for a desired combination of alleles for root manipulation towards better adaptability and stability to drought stressed environments. The combinatorial use of chromosome arm-specific mapping population and E-Bayesian approach makes it novel and possible to study genetic interactions with greater sensitivity and precision than can be done in natural or segregating populations. This may also be another valuable approach to understand complex traits in other organisms.

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Chapter 4

Dosage effect of the short arm of chromosome 1 of rye on root morphology and anatomy in bread wheat

ABSTRACT

The spontaneous translocation of the short arm of chromosome 1 of rye (1RS) in bread wheat is responsible for higher grain yield and root biomass. Recent studies have confirmed the presence of QTL for different root morphological traits on the 1RS arm in bread wheat. The present study was conducted to address two questions in wheat root genetics. First, does the presence of the 1RS arm in bread wheat affect its root anatomy? Second, how does root morphology and anatomy of bread wheat respond to different dosages of 1RS? Near-isogenic plants with different number, 0 to 4, of 1RS translocations were studied for the root morphology and anatomy. The F_1 hybrid, with single doses of the 1RS and 1AS arms, showed heterosis for root and shoot biomass. In other genotypes, with 0, 2 or 4 doses of 1RS, root biomass was incremental with the increase in the dosage of 1RS in bread wheat. This study also provided evidence of the presence of gene(s) influencing root xylem anatomical traits in bread wheat. It was found that root vasculature follows a specific developmental pattern along the whole length of the seminal root and 1RS dosage tends to affect the root anatomy differently in different regions. This study indicated that the inherent differences in root morphology and anatomy of different 1RS lines have advantage over normal bread wheat to survive under stress conditions.

Introduction

All higher plants have roots and the root fraction of the plant's total mass varies widely, even within the same species. Although, roots encounter many fluctuations in their external environments that affect their growth, it is their tendency to accommodate and survive these as a whole system that makes them strongly homoeostatic (Barlow 1986). Knowledge of these modifications in the root system at a morphological and anatomical level, whether due to environmental changes or genetic control, is of importance (Weaver 1926).

In response to the external environment, root morphological traits and root growth have been studied widely in a number of crop species. Drought stressed *Lolium perenne* plants have increased number and growth of lateral roots (Jupp and Newman, 1987). In barley, potassium deficiency is associated with reduced length of laterals and larger root diameter compared to phosphorus deficiency (Hackett 1968). In wheat, temperature has a profound effect on dry weight and root length (Bowen and Rovira 1971; Huang 1991). Besides root morphology, anatomical traits are also influenced greatly by the surrounding environment and have been widely studied in different crop species such as maize (Hose et al. 2001), rice (Uga et al. 2008), and other cereals (Aloni and Griffith 1991; Erland 1995). In winter and spring wheat, chilling and high temperature decreased the diameter of the central metaxylem vessel (Terizioglu and Ekmekci 1995; Huang et al. 1991).

There are reports which suggest specific morphological and anatomical root traits help stress tolerant plants survive a particular stress condition. In rice, traits such as deep root to shoot ratio and deep specific root length were found to contribute to drought avoidance in the field (Yoshida and Hasegawa 1982; Fukai and Cooper 1995). Drought tolerant wheat varieties have smaller xylem vessel diameters (Yang et al. 2007). This anatomical adaptation of the tolerant wheat genotypes proved to be an advantage for survival and higher grain yield under water stress (Richards and Passioura 1989; Huang et al. 1991, 1993; Ou et al. 2005).

The genetic control of root characteristics is poorly understood especially in bread wheat. Robertson et al. (1979) characterized genetic variability for seedling root number within the genus *Triticum* to examine its value in a breeding program and found this trait to be positively correlated with seed weight. In bread wheat, xylem vessel diameter was found to have greater genetic variation and higher heritability with significant response to selection (Richards and Passioura 1981). In a wheat backcross breeding program, lines selected for reduced xylem vessel diameter yielded 3-11% more in driest environments than unselected controls, depending on genetic background (Richards and Passioura 1989). Besides these findings, there is still no report in bread wheat of the chromosomal localization of genes that affect root anatomy.

Weaver (1926) compared the root systems of rye and bread wheat under natural conditions and reported rye had deeper seminal roots. The spontaneous translocation of

the short arm of chromosome 1 of rye (1RS) (*Secale cereale* L.) to the long arm of chromosome 1B (1BL) in bread wheat (*Triticum aestivum* L.) was first identified in the late 1930s (Kattermann 1938; Mettin et al. 1973). Over the past few decades, there have been several reports of better performance of 1RS translocated wheats for grain yield over other commonly grown wheat genotypes (Rajaram et al. 1983; Villareal et al. 1991; Kim et al. 2004; Owuoche et al. 2003). In other studies, increase in grain yield among 1RS wheats was found to be positively correlated with higher root biomass (Ehdaie et al. 2003) while there were no significant differences found for shoot traits (Kim et al. 2004). Roots of 1RS.1BL translocation wheats were thinner and there was a higher root length density when grown in acid soils and this likely enhanced the root surface area (Manske and Vlek 2002).

Lukaszewski (1993) reconstructed the complete chromosomes of 1B and 1R from 1RS.1BL translocation and later, produced three new centric translocations viz., 1RS.1Al, 1RS.1BL, and 1RS.1DL in ‘Pavon 76’ (Lukaszewski 1997). Each of three translocations had the same 1RS arm but in different location in the genome and each was mitotically stable. All three translocations performed better for grain yield under field conditions (Ehdaie et al. 2003; Kumlay et al. 2003) and had greater root biomass. These translocation lines were ranked for root biomass as Pavon 1RS.1AL > Pavon 1RS.1DL > Pavon 1RS.1BL for root biomass (Ehdaie et al. 2003). Recently, a genetic map was generated using 1RS-1BS recombinant breakpoints in wheat and their genetic analysis

indicated the distal 15% of the physical length of chromosome 1RS may carry the gene(s) for better rooting ability and root morphological traits (Sharma et al. 2009).

The present study was conducted to address the dosage effect of 1RS translocation in bread wheat. We used wheat genotypes that differ in their number of the 1RS translocations in a spring bread wheat 'Pavon 76' genetic background. For generating F_1 seeds, Pavon 1RS.1AL was the preferred choice due to its better performance for root biomass than other 1RS lines (Ehdaie et al. 2003). Here, we report the dosage effect of a 1RS chromosome arm on the morphology and anatomy of wheat roots. The results from this study validate previous results of the presence of genes for rooting ability on the 1RS chromosome arm. This study also provides evidence for presence of genes affecting root anatomy on 1RS.

Material and Methods

Plant Material

Five genotypes, Pavon 76, F_1 (Pavon 1RS.1AL × Pavon 76), Pavon 1RS.1AL, Pavon 1RS.1DL, and Pavon 1RS.1AL-Pavon 1RS.1DL were used. They were coded as R_0, RA_1, RA_2, RD_2, and RAD_4, respectively. Here, 'R' denoted the dosage of chromosome arm 1RS, the second letter A and D denoted the chromosome 1 of the

respective genome of wheat to which 1RS is translocated and numbers in the subscripts are dosage number of 1RS chromosome arms present in the genotype. Pavon 76 is a spring bread wheat from the breeding program of Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), Mexico. For a single dose of 1RS, crosses were made between Pavon 1RS.1AL and Pavon 76 and F_1 seed was used as RA_1. Seeds of RA_2, RD_2, and RAD_4 genotypes were provided by Dr. A.J. Lukaszewski, University of California, Riverside.

Experimental Set up

To study the effect of different dosages of the 1RS chromosome arm of rye in wheat background and their effect on root morphology, an experiment was set up in the glasshouse in a randomized complete block design with six replicates per genotype for four seasons. Anatomy of the primary seminal root was studied for two of these seasons. Seeds from the above mentioned five genotypes were surface sterilized with 5% commercial bleach for 5 min, washed for 10 min in distilled water, soaked in water for 24 hrs and then germinated on wet filter paper in Petri dishes. 3-5 day old seedlings were transplanted to two gallon pots, lined with a plastic bag containing 6.5 kg of silica sand #30. Plants were watered when necessary with half strength Hoagland's solution Small holes were made at the bottom of the plastic bags to allow drainage of excess water.

Phenotypic data

Plants were harvested 45 days after germination and pots containing roots were stored at 4^{o}C until processed. Roots were washed and recovered without damage using the floatation technique (Böhm 1979). Different plant characters were measured including plant height (PH), number of tillers (NT), longest leaf length (LLL), maximum width of the longest leaf (LLW), leaf area (LA), longest root length (LRL), root biomass (RB), shoot biomass (SB), and root biomass to shoot biomass ratio (R/S).

Heterosis

Mid-parent heterosis (MPH) and best-parent heterosis (HPH) of the F_1 hybrid (RA_1) over its parents (R_0 and RA_2) for root biomass and shoot biomass were calculated as:

MPH = (F_1 - mean P)/mean P in percent,

HPH = (F_1 - high P)/high P in percent

Root Anatomy

Microscopy

After washing, root samples were collected in two different seasons from the seminal roots of up to six plants of each genotype at three different locations viz; root tip, middle of the root and 1 cm from the base of the root adjacent to seed. Root samples were immersed in half-strength Karnovosky's fixative (2.5% gluteraldehyde and 4% formaldehyde in 50 mM phosphate buffer, pH 7.2). The roots were left in the fixative at 4^{o}C for 24 hours. The roots were dehydrated by passing through a graded ethanol series of 10%, 35%, 50%, and 70% and then left overnight in 70% ethanol followed by another 2 hour treatment in 85%, 90%, and 100% and then subsequently overnight in 100% with 1% Erythrosine B (C.I. 11A). Then the samples were transferred through an ethanol/xylene series of 3/1, 1/1, and 1/3 for 30 min to 1 hour each. Then, tissues were placed in pure xylene for 2 hours followed by overnight treatment. Infiltration and embedding was done in paraffin.

Paraffin blocks containing tissues were sectioned 10 µm thick using an A/O rotary Microtome and stainless steel knives, sections were mounted on slides and stained with Safranin O (C.I. 50240) and Fast Green FCF (C.I.19) using Sass's procedure (Ruzin, 1999). Samples were observed on a Zeiss standard compound bright field microscope. Digital images were taken using an Infinity 1 digital camera (Luminere Inc, Canada).

Root tips were serially sectioned in the transverse plane starting at the tip of the root cap. Data were recorded for the different root anatomical features such as root diameter, stele diameter, central metaxylem (CMX) vessel diameter, CMX vessel area, CMX vessel number, and number of xylem poles from different regions of the roots of all the above mentioned five genotypes. Data on CMX vessel diameter and area were made on transverse sections of root tips at a distance of 1370 µm from the tip for all genotypes. Calibrations and measurements were carried out by camera compliant software 'Analyze Infinity' (Luminere Inc, Canada). Correlation coefficients were calculated and regression analysis was performed.

Statistical analysis

The morphological data were subjected to analysis of variance (ANOVA) for each year (Steel et al. 1997). Data of all the genotypes with different dosages of 1RS were analyzed using a split plot design and the combined ANOVA across seasons was performed for each measured and calculated trait. The anatomical data were also subjected to ANOVA to determine the genetic differences among genotypes. Simple correlation coefficients were calculated to determine the relationship among different anatomical traits. Linear regression analysis was carried out for different anatomical root traits to assess the relationship between dosage of 1RS chromosome arm and the root anatomical traits in bread wheat. Statistix 8 program (Analytical Software, Tallahassee FL) was used to carry out correlation and regression analysis.

Results

Phenotypic study

There was lesser total root biomass in Pavon 76 (R_0) than any of its 1RS sister lines. Among the 1RS lines, F_1 (RA_1) and RAD_4 had higher root biomass (Figure 4.1). There were significant differences among genotypes for plant height, number of tillers, length of longest leaf, maximum width of longest leaf, root biomass, shoot biomass, and root to shoot ratio (Table 4.1). For leaf area, differences among genotypes were not significant. All the genotypes showed significant difference for all the phenotypic traits across the seasons. Genotype × season interactions were not significant for leaf area and root to shoot ratio. The RAD_4 genotype performed better for no. of tillers, longest leaf length, and root to shoot ratio but performed least for plant height, maximum width of longest leaf, and leaf area. The RA_1 hybrid performed significantly better for root biomass and shoot biomass than other genotypes. Mean values of RA_2 were higher for maximum width of the longest leaf and leaf area. For root biomass, RA_1 and RAD_4 were significantly better performers followed by double dosage genotypes (RA_2 and RD_2) and then Pavon 76 (R_0) (Figure 4.1, Table 4.1). All the genotypes were similar for shoot biomass except RA_1 which outperformed them.

Mid-parent and Best-parent heterosis

Mean values of the F_1 hybrid (RA_1) were significantly higher than both of its parents (R_0 and RA_2) for root biomass and shoot biomass (Table 4.1). MPH and HPH for root biomass were calculated to be 34.4% and 25.7%, respectively (Table 4.2). For shoot biomass, heterotic gain of F_1 over mid-parent and high parent was 15.3% and 13.6%, respectively (Table 4.2).

Table 4.1: Summary of combined ANOVA and mean values of plant height (PH), number of tillers (NT), longest leaf length (LLL), maximum width of the longest leaf (LLW), leaf area (LA), root biomass (RB), shoot biomass (SB), and root to shoot biomass ratio (R/S) for bread wheat Pavon 76 (R_0), F_1 - Pavon 1RS.1AL × Pavon 76 (RA_1), Pavon 1RS.1AL (RA_2),Pavon 1RS.1DL (RD_2), and Pavon 1RS.1AL-Pavon 1RS.1DL (RAD_4) grown in sand pots for 45 days (mid-tillering stage) averaged across four seasons

Genotype	**PH** cm	**NT** no.	**LLL** cm	**LLW** cm	**LA** cm^2	**RB** mg	**SB** mg	**R/S**
R_0	54† b	11 b	35 c	1.38 ab	38.1 ab	1073 bc	3692 b	0.33 bc
RA_1	55 b	11 b	37 b	1.31 b	37.7 ab	1549 a	4319 a	0.36 ab
RA_2	58 a	11 b	37 b	1.42 a	40.9 a	1232 b	3802 b	0.36 ab
RD_2	58 a	9 c	36 bc	1.35 ab	38.3 ab	1087 b	3637 b	0.31 c
RAD_4	52 c	13 a	39 a	1.20 c	37.1 b	1461 a	3743 b	0.38 a
Season	*	*	*	*	*	*	*	*
Genotype	*	*	*	*	NS	*	*	*
Genotype × Season	*	*	*	*	NS	*	*	NS
CV (%)‡	7	15	7	10	14	24	17	16

† Means followed by the same small letter within a column are not significantly different at $P < 0.05$ and according to LSD test.
CV‡ = Coefficient of variation.
* = Significant ($p = 0.05$)
NS = Not Significant ($p = 0.05$)

Table 4.2: Mid-parent heterosis (MPH) and best-parent heterosis (BPH) for root and shoot biomass of bread wheat hybrid F_1 - Pavon 1RS.1AL × Pavon 76 (RA_1) over parents Pavon 1RS.1AL (RA_2) and Pavon 76 (R_0) grown in sand pots for 45 days (mid-tillering stage). Mean values are averaged across four seasons

Trait	**Mean**			**MPH** (%)	**BPH** (%)
	Hybrid (RA_1)	**Mid-parent**	**Best-parent (RA_2)**		
	----------	(mg)	----------		
Root biomass	1549	1153	1232	34.4	25.7
Shoot biomass	4319	3747	3802	15.3	13.6

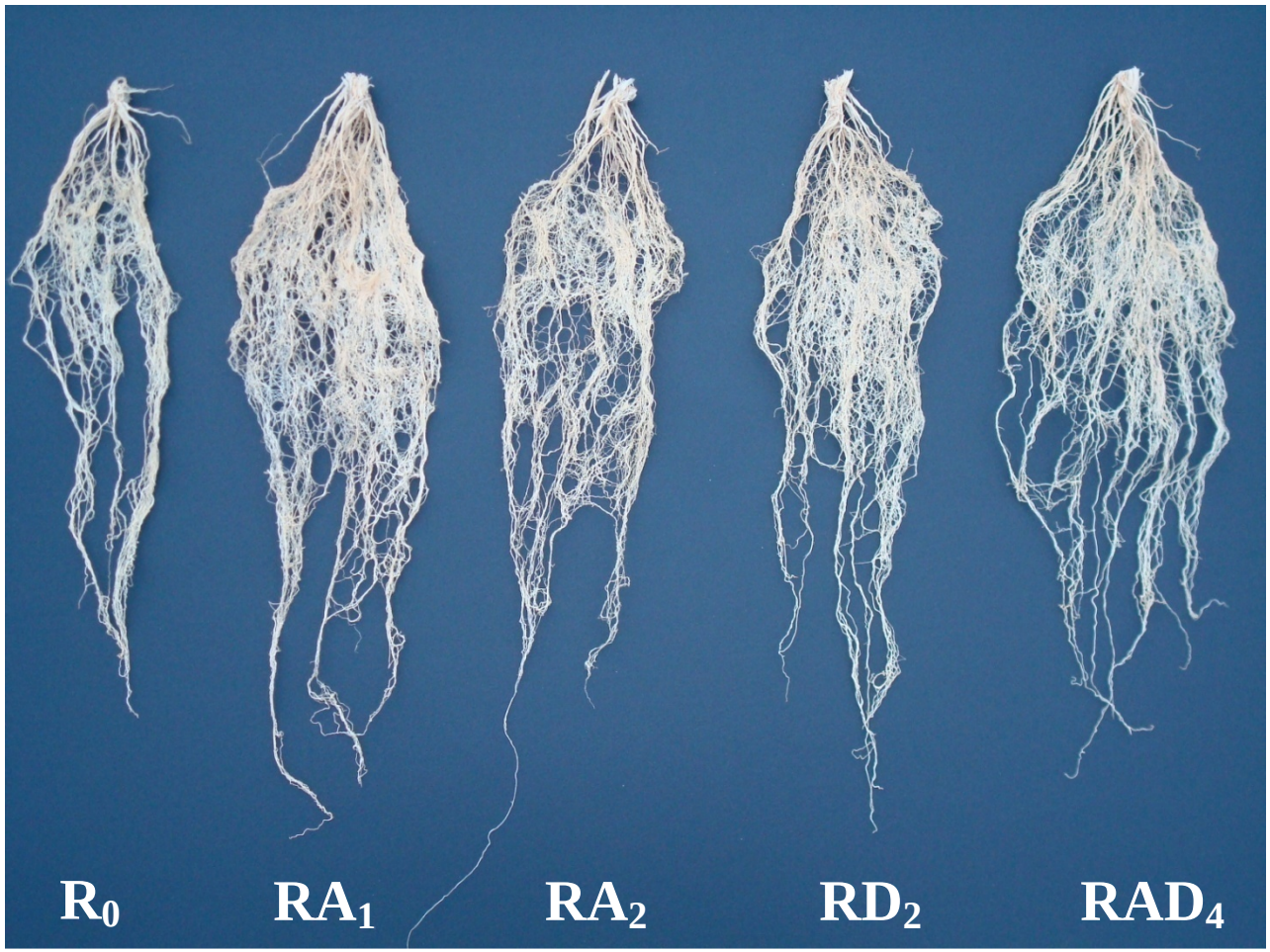

Figure 4.1: Roots of different wheat genotypes with different number of 1RS translocations in spring bread wheat 'Pavon 76' background harvested 45 days after germination (grown in pots). Pavon 76 (R_0) = 0 dose of 1RS, Pavon 1RS.1AL × Pavon 76 (RA_1) = 1 dose of 1RS, Pavon 1RS.1AL (RA_2) = 2 dose of 1RS on 1AL, Pavon 1RS.1DL (RD_2) = 2 dose of 1RS on 1DL, and Pavon 1RS.1AL 1RS.1DL (RAD_4) = 4 dose of 1RS on 1AL and 1DL

Root anatomy

The size, number and arrangement of metaxylem vessels varied along the length of the root from top to tip region for each genotype. In the top region there were 3-6 metaxylem vessels that were not necessarily central in position. More of these were present in the transverse sections of R_0, RA_1 and RA_2 than in RD_2 and RAD_4 (Figures 4.2a-e). The diameter of the stele was larger in R_0 and RA_1. In the middle region of the root, there were two centrally located metaxylem vessels in the stele of R_0 and RA_1 and a single CMX vessel in RD_2 and RAD_4 (Figures 4.2 f-j). The diameter of the stele was smaller than in the top region of the root for all genotypes. In the root tip region, there was a single differentiating central metaxylem vessel in all the genotypes which was narrower in RA_2, RD_2, and RAD_4 genotypes (Figure 4.2 m, n, o). There was not much variation for other anatomical traits in the root tip region between the genotypes (Figure 4.2).

Top-region of the root (TOP)

There were significant differences among genotypes for stele diameter, total metaxylem vessel area, number of central metaxylem vessels, and number of xylem poles in the top region of the seminal root. Differences among genotypes were not significant for xylem diameter, CMX number, and root diameter but all genotypes showed the same trend in their performance for all the root anatomical traits measured (Figure 4.3). Hybrid

RA_1 was ranked first among genotypes followed by R_0 for all the traits in the top region of the seminal root while RAD_4 was at the bottom in rankings.

The calculation of correlation coefficients (r) revealed a strong degree of association among traits of root anatomy in the top region of the root (Table 4.2). Stele diameter showed positive correlation with CMX vessel area (0.82), number of xylem poles (0.81), and root diameter (0.84). CMX area was further associated highly with CMX vessel diameter (0.77), number of xylem poles (0.71)), and root diameter (0.86). There was also high correlation between root diameter and number of xylem poles (0.79) (Table 4.3).

Linear regression analysis showed significant R-squared values for all anatomical root traits measured in this study. The number of central metaxylem vessels in top of the root was the best explained trait (51%) due to 1RS dosage. Forty seven percent of the variation of two root traits, CMX area and number of xylem poles, was explained by the linear regression on the number of 1RS dosages in a genotype (Table 4.4). The number of 1RS dosages explained 45% and 38% of the variation in stele diameter and root diameter, respectively. The negative slope of the regression equation showed a negative relationship between root traits and number of 1RS dosages (Table 4.4). This is also evident from the negative correlation coefficients (Table 4.4).

Mid-region of the root (MID)

ANOVA showed no significant differences among genotypes for any of the anatomical root traits in the mid-region of the seminal root except number of xylem poles (data not shown). But, all the traits showed stronger association among themselves except CMX vessel number (Table 4.3). Regression analysis showed negligible dependence of root traits on the number of 1RS dosages (data not shown).

Tip of the root (TIP)

In root tips of the seminal roots, there were significant differences among genotypes for CMX vessel area and CMX vessel diameter. For both the traits, R_0 and RA_1 showed significantly higher mean values than 1RS double dosage genotypes (RA_2 and RD_2) and quadruple dosage of 1RS in RAD_4 genotype (Figure 4.4). Correlation coefficient values of both the above traits also showed highly positive association with each other followed by stele and root diameter (Table 4.3). Again in regression analysis, both the traits, CMX vessel area and CMX vessel diameter, were significantly influenced by different 1RS dosages in the genotypes. Their regression equation showed negative slope and variation explained for CMX vessel area and CMX vessel diameter was 54% and 52%, respectively (Table 4.4).

Figure 4.2: Stained transverse sections of different regions of roots of wheat genotypes. a, f, k -Pavon 76 (R_0); b, g, i - F_1 of Pavon 1RS.1AL × Pavon 76 (RA_1); c, h, m - Pavon 1RS.1AL (RA_2); d, i, n - Pavon 1RS.1DL (RD_2); e, j, o - Pavon 1RS.1AL-Pavon 1RS.1DL (RAD_4). C = cortex, E = endodermis, XP = xylem poles, CMX = central metaxylem vessel. Scale Bar = 100 micron

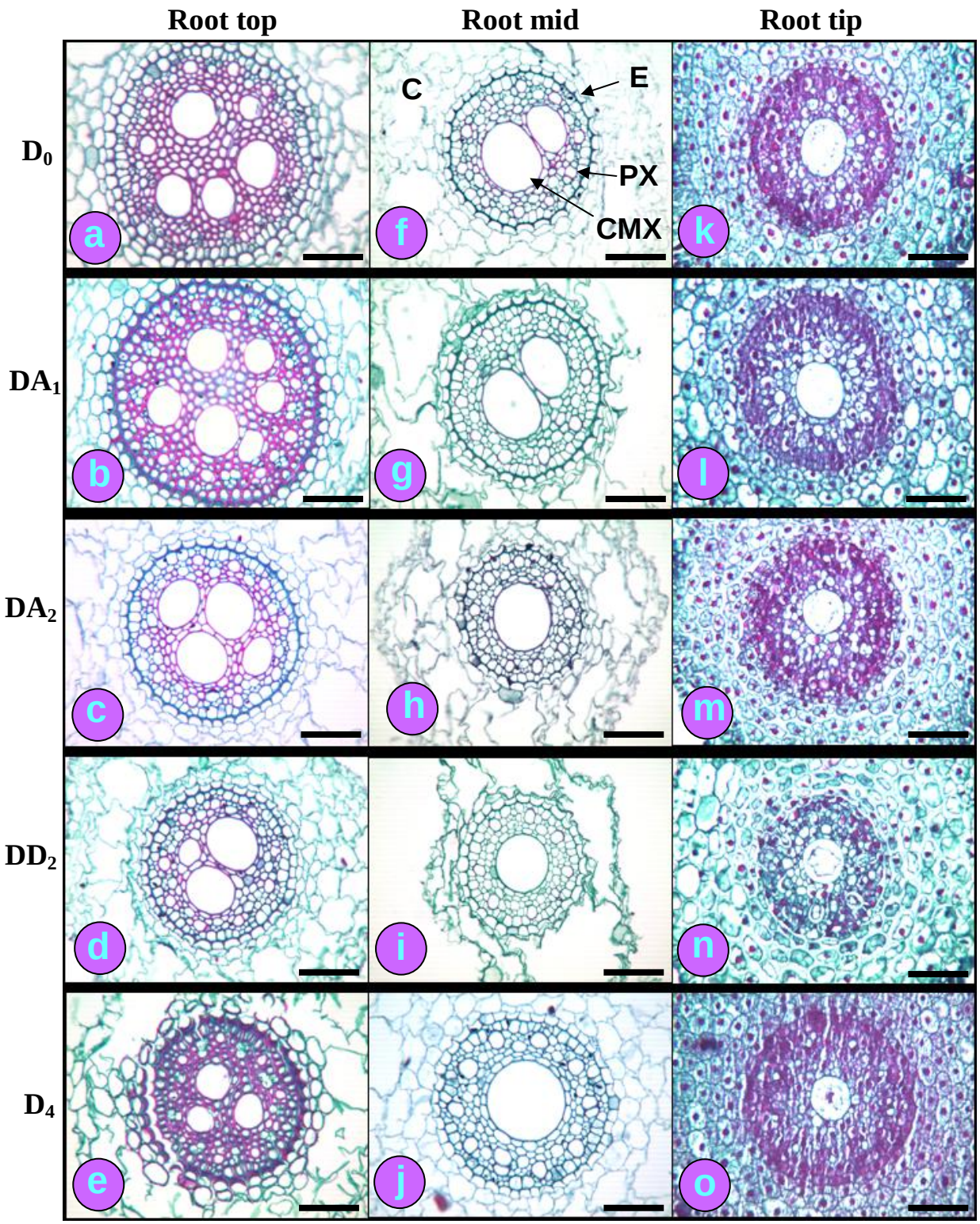
Root top
Root mid
Root tip
D0
DA1
DA2
DD2
D4
C
E
PX
CMX
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o

Table 4.3: Simple correlation coefficients† between different root anatomical traits in different root regions in the genetic background of spring bread wheat 'Pavon 76' with different number of 1RS translocation arms

	Root regions	Root traits				
		Stele diameter	CMX vessel area	CMX vessel diameter	CMX vessel number	Xylem poles
CMX vessel area	TOP	0.82**				
	MID	0.96**				
	TIP	0.68**				
CMX vessel diameter	TOP	0.46*	0.77**			
	MID	0.96**	0.96**			
	TIP	0.73**	0.99**			
CMX vessel number	TOP	0.86**	0.85**	0.68**		
	MID	0.29	0.19	0.21		
	TIP	-	-	-		
Xylem poles	TOP	0.81**	0.71**	0.53*	0.84**	
	MID	0.91**	0.88**	0.89**	0.27	
	TIP	0.14	-0.02	-0.01	-	
Root diameter	TOP	0.84**	0.86**	0.56*	0.84**	0.79**
	MID	0.83**	0.75**	0.78**	0.08	0.84**
	TIP	0.75**	0.59**	0.64**	-	-0.08

† n = 20 for TOP and TIP regions, n = 25 for MID regions
** = significant ($p \leq 0.01$)
* = significant ($p \leq 0.05 > 0.01$)

Table 4.4: Summary of regression analysis. The independent dependent variable is number of 1RS translocation arms in the spring bread wheat 'Pavon 76' genetic background (n = 20)

Dependant variable	**Intercept**	**Slope**	R^2	*F*	*P*
Root Top					
Stele diameter (μm)	363.4	–36.5	0.45	14.66	0.00
Total CMX area (μm^2)	15720	–2540	0.47	16.01	0.00
CMX diameter (μm)	59.0	–4.2	0.21	4.65	0.04
CMX (number)	5.4	–1.0	0.51	18.63	0.00
Xylem pole (number)	12.9	–1.2	0.47	15.78	0.00
Root diameter (μm)	667.7	–44.5	0.38	6.60	0.03
Root Tip					
Stele (μm)	250.8	–5.4	0.10	2.00	0.17
CMX area (μm^2)	6161.4	–773.5	0.54	21.30	0.00
CMX diameter (μm)	87.7	– 6	0.52	19.69	0.00
Xylem pole (number)	8.8	0.1	0.01	0.21	0.65
Root diameter (μm)	655.8	–15.1	0.06	1.19	0.29

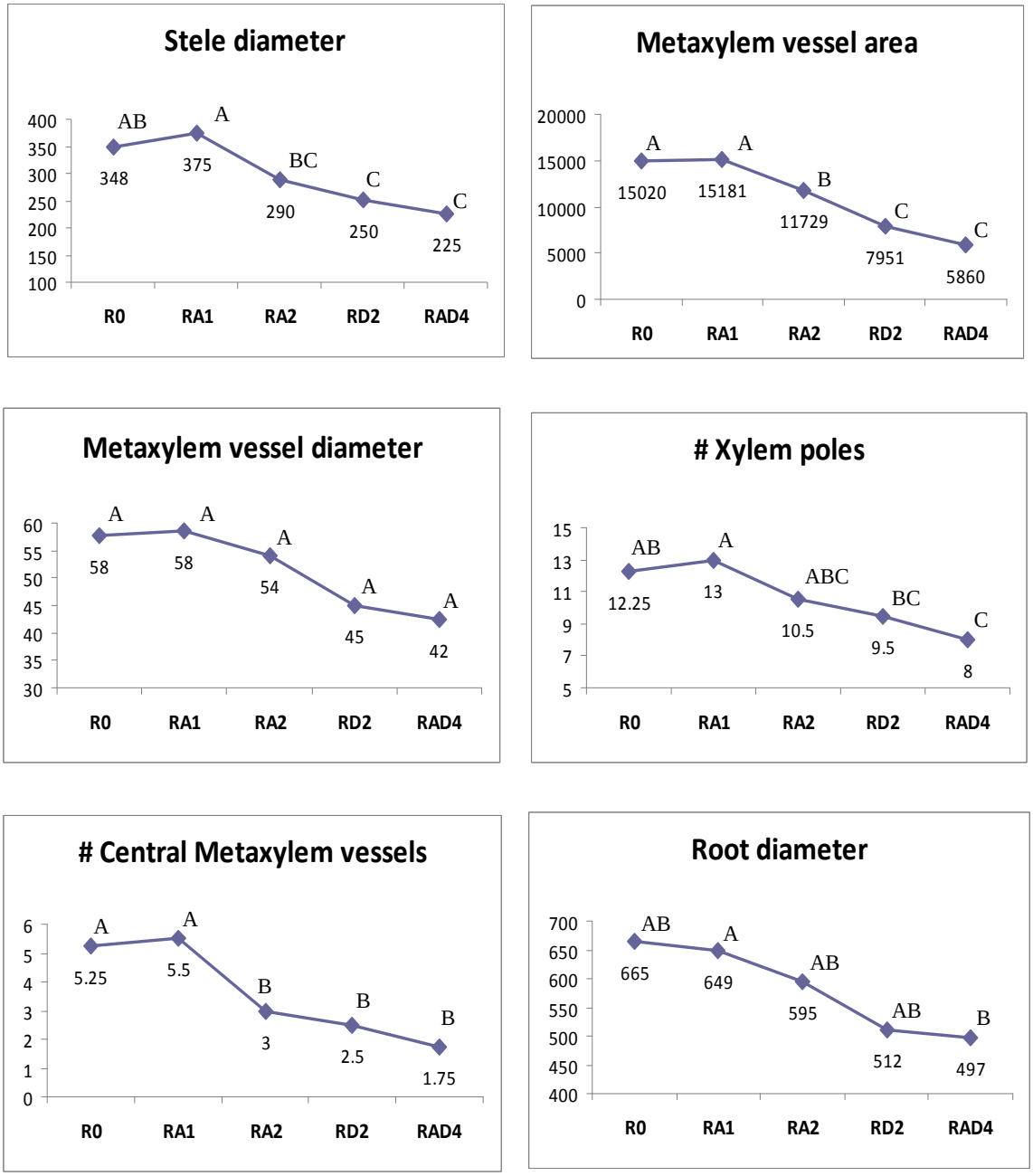

Figure 4.3: Graphical representation of mean performances of anatomical traits measured from top region of the roots of different wheat genotypes with different number of 1RS translocation arms in the genetic background of spring bread wheat 'Pavon 76'. Same capital letters within a graph are not significantly different.

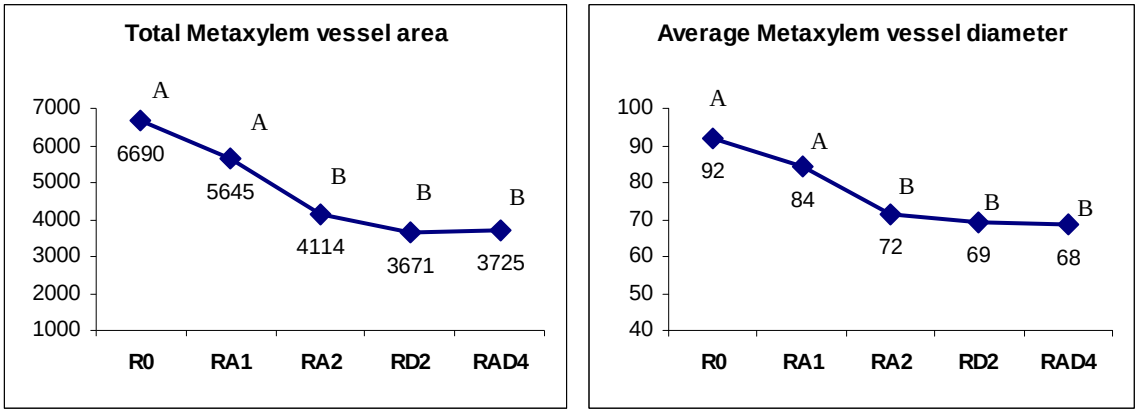

Figure 4.4: Graphical representation of mean performances of anatomical traits measured in root tips of the roots of different wheat genotypes with different number of 1RS translocation arms in the genetic background of spring bread wheat 'Pavon 76'. Same capital letters within a graph are not significantly different.

Discussion

From previous chapters of this dissertation and earlier studies (Ehdaie et al. 2003), it was clear that there was a gene (or genes) present on 1RS chromosome arm which affects root traits in bread wheat. But there was no report on the chromosomal localization of any root anatomical trait in bread wheat. The purpose of this study was to look for variation in root morphology and anatomy among different wheat genotypes and then determine how these differences are related to different dosages of 1RS in bread wheat. During this study, we came to some very interesting conclusions: 1) F_1 hybrids showed a heterotic effect for root biomass and there was an additive effect of the 1RS arm number on root morphology of bread wheat; 2) There was a specific development pattern in the root vasculature from top to tip in wheat roots and 1RS dosage tended to affect root anatomy differently in different regions of the seminal root. Further, the differences in root morphology, and especially anatomy of the different genotypes have specific bearing on their ability to tolerate water and heat stress.

Effect of 1RS dosage on root morphology

The effect of number of 1RS translocation arms in bread wheat was clearly evident from their averaged mean values for root biomass. RA_1 and RAD_4 were ranked highest while R_0 ranked at the bottom (Figure 4.1, Table 4.1). These results supported the previous studies on the performance of wheat genotypes with 1RS translocation where

1RS wheats performed better in grain yield but similar for shoot biomass (Ehdaie et al. 2003, Ehdaie and Waines 2006; Sharma et al. 2009). Genotype RD_2 performed slightly better than R_0 for root biomass because of its poor performance in one season otherwise it showed better rooting ability in the other three seasons. Here, all the genotypes with 1RS translocations showed higher root biomass than R_0 which carried a normal 1BS chromosome arm. Data in this study suggested two types of effects of 1RS on wheat roots. First, an additive effect of 1RS, there was increase in root biomass with the increase in 1RS dosage from zero (R_0) to two (RA_2 & RD_2) and then to four (RAD_4). Second was a heterotic effect of 1RS on root biomass and shoot biomass. MPH and HPH of the F_1 hybrid (RA_1) were higher for root biomass than for shoot biomass (Table 4.2). This further explained the more pronounced effect of 1RS on root biomass than shoot biomass. Significant positive heterosis was observed for root traits among wheat F_1 hybrids and twenty seven percent of the genes were differentially expressed between hybrids and their parents (Wang et al. 2006). The possible role of differential gene expression was suggested to play a role in root heterosis of wheat and other cereal crops (Wang et al. 2006). In a recent molecular study of heterosis, it was speculated that up-regulation of TaARF, an open reading frame (ORF) encoding a putative wheat ARF protein, might be contributing to heterosis observed in wheat root and leaf growth (Yao et al. 2009)

Root anatomy in three dimensions in different doses of 1RS arms

There is large void in root research involving study of root anatomy in wheat as well as other cereal crops. Most of the anatomical literature is either limited to root anatomy near the base of the root (Watt et. al. 2008, Aloni and Griffith 1991) or near the root tip (Huang et. al.1991) in young seedlings (Richards and Passioura 1989). There is still a general lack of knowledge about the overall structure and pattern of whole root vasculature during later stages of the growth in cereals especially in wheat. In the present study, root anatomical traits were studied in the primary seminal root of different wheat genotypes containing different dosages of 1RS translocation arms at mid-tillering stage (around 7 weeks). Root sections were made from three regions along the length of the root, viz. top of the root, middle of the root and root tip, to get an overview of the complete structure and pattern of root histology relative to differences in 1RS dosage. Comparison of different regions of root of a genotype showed a transition for metaxylem vessel number and CMX area from higher in top region of the root to a single central metaxylem vessel in the root tip. Diameter of the stele also became narrower towards the root tip as the plant roots grow into deeper layers of soil. In the root tip only central metaxylem vessel diameter and area were traceable as other cell types were still differentiating. This developmental pattern was consistent across the different wheat genotypes used in this study. Interestingly, there was variation in timing for the transitions in root histology among genotypes and this variation was explained by dosage of 1RS arm in bread wheat. RD_2 and RAD_4 transitioned earlier from having multiple

metaxylem vessels and a larger stele to a single, central metaxylem vessel and smaller stele than did R_0 and RA_1. In the top region, all the root traits were significantly different among genotypes except average CMX vessel diameter and CMX vessel number (Figure 4.3). Here, the average CMX diameter was calculated from the average of diameters of all the CMX number of that subsequent genotype and hence, the number of CMX vessels, was different in each genotype so was the total CMX vessel area. Interestingly, all the root traits in the top region showed negative slope in regression analysis and most of them were significant especially stele diameter, total CMX vessel area, and peripheral xylem pole number. Variation in all the traits was explained by number of 1RS dosages in wheat genotypes and root traits were smaller with higher number of 1RS dosage (Figure 4.2 a-e). Significant positive correlation among almost all the root traits from top-region and mid-region of the roots (except CMX vessel number) suggested their inter-dependences in growth and development. Root diameter could not be measured for all the replicates of each genotype because of the degeneration and mechanical damage to the cortex and epidermis. Earlier, a study on the rate of cortical death in seminal roots was investigated in different cereals. It was found that rate of cortical death was faster in hexaploid wheat and positively associated with root age (Liljeroth 1995).

In the root tip, only two traits, CMX vessel area and CMX vessel diameter, were traceable because of the status of root tip development (Figure 4.4). Negative slope and significant R^2 value in regression analysis explained the effect of 1RS dosage on the CMX vessel area and CMX vessel diameter. This suggested narrow metaxylem vessels

with increase in 1RS dosage (Table 4.4, Figure 4.2 k-o). In roots, central metaxylem vessel is the first vascular element to be determined and differentiate (Luxova and Lux 1974). Here, serial cross sections of the root tips also confirmed it as the first differentiated vascular element in wheat. The other vascular components differentiate thereafter in relation to first formed metaxylem vessel (Feldman 1977). Feldman (1977) first reported that all the metaxylems were not initiated at the same level.

Structural adaptation to stress

Root morphology and root architecture are responsible for the water and nutrient uptake while in root anatomy, xylem vessels are essential for their transportation to the shoots to allow continued photosynthesis. Variations in xylem anatomy and hydraulic properties occur at interspecific, intraspecific and intraplant levels (Zimmermann 1983; Sperry & Saliendra 1994; Jackson et al. 2000). Variations in xylem vessel diameter can drastically affect the axial flow because of the fourth-power relationship between radius and flow rate through a capillary tube, as described by the Hagen–Poiseuille law (Zimmermann 1983; Tyree & Ewers 1991). Thus, even a small increase in mean vessel diameter would have exponential effects on specific hydraulic conductivity (K_s) for the same pressure difference across a segment (McElrone et al. 2004). Xylem diameters tend to be narrower in drought tolerant genotypes (Ou et al. 2005; Yang et al. 2007), and at higher temperature (Huang et al. 1991). Smaller xylem diameters pose higher flow resistance and slower water flow which helps the wheat plant to survive water stressed

conditions. Richards and Passioura (1989) increased the grain yield of two Australian wheat cultivars by selecting for narrow xylem vessels in seminal roots.

The results of this study showed that the presence of 1RS in bread wheat increased the root biomass (Figure 4.1, Table 4.1) and reduced the dimensions of some root parameters especially the central metaxylem vessel area and diameter in the root tip as well as in the top of the root (Figure 4.2, 4.3, 4.4). Manske and Vlek (2002) also reported that wheat genotypes with 1RS translocated chromosome arm had thinner roots and higher root-length density compared with normal wheat with 1BS chromosome arm under field conditions. These results might suggest higher root number or extensive root branching in 1RS translocation wheats. Among 1RS translocation wheats, significant association was observed between root biomass and grain yield under well-watered and droughted environments (Ehdaie et al. 2003). Narrow metaxylem vessels and higher root biomass provide 1RS translocation wheats with better adaptability to water stress and make them better performers for grain yield.

Conclusion

In a recent study on rice root anatomy, QTL for metaxylem vessels were identified on the distal end of the long arm of chromosome 10 of rice (Uga et al. 2008) which showed synteny to the 1RS chromosome arm of rye (Hackauf et al. 2009). These reports also support the present findings and validate the purpose of the current study on

the wheat root anatomy. This study confirms that the short arm of chromosome 1 of rye carries important genes affecting root morphology and root anatomy in bread wheat. The 1RS arm showed heterotic effect and significant dosage effect on root biomass and root anatomy in bread wheat. Thus, higher root biomass and narrow metaxylem vessels in 1RS wheat strongly advocate their inclusion as a selection criterion in wheat breeding programs.

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Chapter 5

General Conclusions

Since the beginning of agriculture, selection for improvement in grain yield was based mostly on observation of visible above ground plant parts which exclude the other half of the plant that is root. Roots provide support and are responsible for water and nutrient uptake for the plant. Although roots have been studied in detail in other crops and many genes have been identified, there is no information on any gene for root traits in bread wheat. The present studies were conducted to fill that void. Centric rye-wheat translocations on chromosome 1 have a grain yield advantage over other wheat genotypes with normal wheat chromosomes. The positive association of grain yield with root biomass strongly indicated the possibility of the presence of a gene(s) on the short arm of chromosome 1 of rye (1RS) which affects root traits. The objectives of this dissertation research were 1) to generate a genetic map of 1RS-1BS recombinant breakpoints, 2) identify different QTL for different root traits in bread wheat, and 3) investigate the effect of different doses of the 1RS chromosome arm on root morphology and root anatomy of bread wheat.

A total of 68 recombinants were screened for 91 EST based primer pairs specific to short arms of chromosome 1 of bread wheat and 16 SSRs/eSSRs specific to 1BS arm of wheat. Eight polymorphic markers showed their specificity to 1BS. Finally, a consensus genetic map of 1RS-1BS recombinant breakpoints was generated which comprised of 20 phenotypic and molecular markers, with an average spacing of 2.5 cM. These 68 recombinant breakpoints distributed among 20 markers provided a resolution of 0.7 cM. Further, a phenotypic study was conducted to identify the chromosomal region of

1RS which may possibly carry the gene(s) for better rooting ability in bread wheat. The statistical analysis showed that the terminal 15% of the rye 1RS arm carried gene(s) for greater rooting ability in wheat. This present finding on root studies prepared a platform to find gene(s)/QTL for root traits on 1RS in bread wheat.

In a first ever attempt to find QTL effects explaining different root traits, a 1RS-1BS arm specific mapping population of 29 recombinants was used in combination with powerful empirical Bayesian (E-Bayes) statistical method. On one hand, this mapping population provided high resolution of QTL detection even in a low progeny experiment and on the other hand, E-Bayes method was advantageous in minimizing the error variance and simultaneous estimation of additive (main) effects of all individual markers along with significant epistatic effects where many may go undetected. A total of 15 QTL effects, including six additive and nine epistatic, were detected for all the root traits on the short arms of 1RS-1BS chromosomes while no QTL effects were detected for shoot traits except two for shoot biomass. Epistatic effects were further partitioned into Inter-genomic (wheat-rye) and Intra-genomic (wheat-wheat or rye-rye) interactions. All the 15 QTL were distributed over four common regions of the 1RS-1BS map and three out of four regions were located in the distal 15% of the chromosomal region. Thus, the QTL detection conformed to our findings in the previous experiment. From the comparison of mean values from these four regions, we conclude that 1RS chromosome arm carries QTL for important root traits.

In order to elucidate the effect of 1RS arm on root morphology and anatomy of bread wheat, a study was carried out involving different bread wheat genotypes in Pavon 76 background which differ in their dosage of 1RS arms from zero to four. The F_1 hybrid with single dose of 1RS and 1AS showed heterotic effect for root biomass and shoot biomass while other genotypes, with 0, 2, and 4 doses of 1RS, showed additive affect on the root biomass. A root anatomical study revealed a specific pattern of transition in vascular development especially central metaxylem vessels from top to tip of the root among all the genotypes. The transition varied from one genotype to another and was explained by the different number of dosages of 1RS arms in bread wheat. Results showed that transition for metaxylem vessel number in two and four dosage 1RS lines was earlier than zero and single dosage 1RS lines. Central metaxylem vessel area and number are found to decrease as the number of 1RS in wheat lines increases. In conclusion, this study suggested that higher number of 1RS lines have morphological and anatomical advantage in combating stress environments over normal bread wheat genotypes.

For the first two goals of this research study, a total of 68 1RS-1BS recombinant breakpoints were used to generate a consensus genetic map of 20 polymorphic markers. Now, there are availabile a total of 91 1RS-1BS recombinant breakpoints which indicate an average resolution of up to 0.5 cM. There is still a possibility to enrich the genetic map with more number of markers so that each breakpoint is saturated with at least one specific marker. This will be a great resource to study 1RS translocations in bread wheat.

The immediate application of the enriched map could be the fine mapping of QTL for different root traits in bread wheat which can further be used for the positional cloning of those QTL. Though there are many other methods to look for polymorphic markers, the Diversity Array Technology (DArT) offers a great potential for the map enrichment because of its sequence information independent genotyping method. The study is underway.

The results from the dosage effect of the 1RS arm on root morphology and anatomy also suggested some possible future research directions. Present findings indicated the presence of gene(s) on 1RS affecting the root structure especially the anatomy. To unravel the candidate gene(s) affecting root anatomy, transcriptome expression analysis of 1RS lines with different dosage number looks promising. The genetic background of these lines is the same except number and location of 1RS in wheat genome which would facilitate the identification of 1RS specific differentially expressed transcripts affecting root traits in bread wheat. In order to identify differentially regulated transcripts which are locally expressed during early differentiation of central metaxylem vessel in the root tip, laser capture micro-dissection (LCM) can be an option for central metaxylem vessel specific trascriptome analysis. Functional characterization of the potential candidate genes will provide insight into the function and development of roots in bread wheat and other cereals.

The overall conclusion and take home message from this dissertation research is that the 1RS translocation carries important genes which affect root properties, both morphological and anatomical, in bread wheat. This explains the advantage in grain yield and better performance under irrigated and stressed environments. The inclusion of 1RS translocations in wheat breeding programs with an emphasis on roots as a selection criterion is strongly recommended.