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Determination of Root Traits in Wild, Landrace and Modern Wheats and Dissection of Quantitative Trait Loci (QTL) for Root Characters in Bread Wheat

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by

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ABSTRACT OF THE DISSERTATION

Determination of Root Traits in Wild, Landrace and Modern Wheats and Dissection of Quantitative Trait Loci (QTL) for Root Characters in Bread Wheat

by

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Doctor of Philosophy, Graduate Program in Plant Biology
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Bread wheat is an allohexaploid crop with a large and complex genome structure. It was one of the first crops domesticated by human beings in the Near East and it had dramatic effects on human history. The amount of energy gained per hour of work from wheat was much higher than hunting and gathering. Ancient farmers continuously selected it to increase seed size, grain yield, and straw yield to feed a growing population; this scenario has not changed for thousands of years. Plant scientists, breeders, and farmers are still working to improve grain yield to support growing demand. Introduction of genetic variation with novel alleles has been a major component of plant breeding, especially after the major genetic bottlenecks of the last century.

I have aimed to retrieve some of the available genetic variation within the *Triticeae* tribe by screening wild wheat relatives, landraces, modern wheats, and a synthetic wheat population. All of the above materials were evaluated to genetically locate and identify root system traits.

Breeding for drought tolerance requires selection for traits that improve water uptake and use efficiency. Root system traits are a major component to improve water acquisition. Here we report significant genotypic variation for root traits within and between wheat wild relatives, bread wheat landraces, modern wheats, and the Synthetic W7984* Opata M 85 doubled haploid (SynOpDH) mapping population. Up to four-fold difference for root size within wild accessions, eight-fold difference within landraces and modern wheats and fourteen-fold difference within the progeny of the SynOpDH population was observed.

This large range in genotypic variation may provide many useful alleles for breeders, especially those who target rain-fed growth conditions in their breeding programs. Even though studying the root system is technically challenging, and time and labor intensive, an urgent need for drought tolerant crops makes it a necessity. There is a major need for research leading to a complete understanding of the genetic control of the wheat root system. Advances in genotyping technologies, marker assisted selection, and fast / accurate phenotyping may provide useful tools to select root traits such as, deep root biomass, number of seminal and nodal roots, and root angle with less labor and time, to develop drought tolerant wheat cultivated varieties.

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General Introduction

Bread wheat (*Triticum aestivum* L.) is a staple crop with maize (*Zea mays* ssp. *mays*) and rice (*Oryza sativa* L.). It is cultivated on every continent except Antarctica from sea level to 4000 m elevation in Tibet. Wheat production reaches 700 million metric tons per year and it has the most acreage planted across the world, which provides approximately 20% of our daily caloric intake (FAOSTAT, 2014). As with any other crop, pressure to increase yield becomes stronger every year. Estimates of human population increase address how urgent the situation is by losing arable land to salinity, flood, drought, erosion and development (Döös, 2002). Wheat yield averages have not even approached the actual potential due to environmental factors including biotic and abiotic stresses especially in rain-fed conditions where an average yield of 2-3 tons / hectare is achieved while the actual potential is 7 tons / hectare (Anderson, 2010, Hawkesford, et al., 2013). Plant breeders face new challenges every year to cope with new strains of biological pathogens and abiotic stresses. Therefore, continuous search for genetic variation and novel allelic diversity among modern cultivars (cv.), landraces, and wild gene pools is important and urgent.

Bread wheat is an allohexaploid crop with three homoeologous genomes; *Aegilops speltoides Tausch* (SS) the “B” genome, *Triticum urartu* Tum. ex. Gandil (A^uA^u) the “A” genome and *Aegilops tauschii* Coss. (DD) the “D” genome donors (Kihara, 1944, Dubcovsky and Dvorak, 2007). Multiple hybridization events

produced an allopolyploid crop with a genome composed of three homeologous genomes totaling 17 GB, five times larger than the human and maize genomes.

Since the domestication of Einkorn wheat approximately 10000 years ago wheat has been selected for many uses such as grain and straw yield. With the domestication of emmer and later bread wheats other quality traits became important selection criteria, such as; high protein content, gluten and bread making quality (Salamini, et al., 2002). Continuous efforts for selection never stopped. The twentieth century recorded pioneering works of Nazareno Strampelli, Orville Vogel, Norman Borlaug and many other plant scientists for their substantial efforts in plant breeding. Orville Vogel introduced 'Norin 10' a Japanese bread wheat variety, the source of *Rht-B1b* (formerly *Rht1*), and *Rht-D1b* (formerly *Rht2*) genes, to USA and later Norman Borlaug worked with 'Norin 10', and Vogel's other hybrids at large scale breeding at CIMMYT headquarters in Mexico (Hedden, 2003). Strampelli introduced the *Rht8* gene into European germplasm from another Japanese variety called 'Aka komugi' in early twentieth century much before Vogel and Borlaug. Strampelli's cv. Mentana spread to Eastern Europe, Western Asia and South America before World War II. This cultivar was not only grown worldwide, it was also frequently used in breeding (Borojevic and Borojevic, 2005, Salvi, et al., 2013). Modern wheats with *Rht-B1b, Rht-D1b*, *Rht8b* and other dwarfing or semi-dwarfing genes caused up to threefold grain yield increase within decades. Wheats that are semi-dwarf, stiff, early maturing and highly efficient at

nutrient use are now grown in more than 70% of the world's wheat production areas (Borlaug, 2007, Pingali, 2012, Salvi, et al., 2013).

In addition to semi-dwarfing genes, day length insensitivity (*Ppd*) genes were also introduced. Day length insensitivity genes were as important as *Rht* genes by allowing modern wheats to be grown anywhere in northern or southern hemispheres. These two important genes, in addition to irrigation and fertilizer applications, were the main reasons for the replacement of old varieties and landraces (Pingali, 2012). It was estimated that domestication and modern breeding of the last century have caused 69% and 75% loss of genetic diversity, respectively (Jaradat, 2013). Since 1970s importance of genetic variation for continuity of plant breeding and plant evolution is widely recognized (Brush, 1995, Jarvis and Hodgkin, 1997, Moghaddam, et al., 1997, Tanksley and McCouch, 1997, Ceccarelli, 2000, Reynolds, et al., 2007, Jaradat, 2008, Jaradat, 2013).

The largest genetic diversity is available in the wild gene pools. Bread wheat's wild ancestors, *Ae. speltoides*, *Ae. tauschii,* and *T. urartu* are the first sources of genetic variation in its gene pool. While all three wild relatives are important, introduction of genetic diversity into wheat has never been easy because of differences in ploidy level, linkage drag and meiotic problems. The only systematic evaluation and selection was done in *Ae. tauschii*, which was used in the development of Synthetic hexaploid wheats. This was achieved by crossing tetraploid wheats with *Ae. tauschii*, followed by subsequent chromosome doubling (Mujeeb-Kazi, et al., 1996) to create synthetic wheats. This hybridization event

aimed to introduce some of the extensive genetic variation available in the *Ae. tauschii* genome into bread wheat germplasm.

The first standard mapping population derived from durum wheat and *Ae. tauschii* crosses was Synthetic W7984 x Opata M85 recombinant inbred population (RIL), so-called International Triticeae Mapping Initiative population (ITMI). The 'Synthetic W7984' line is a manmade amphiploid derived from the durum wheat line 'Altar 84' (*Triticum turgidum* L.) crossed with the accession (219) 'CIGM86.940' of *Ae. tauschii*, the D genome donor of bread wheat (Nelson, et al., 1995). ITMI recombinant inbred (RIL) population was widely used around the world as a resource for breeding programs, genetic marker development, and QTL mapping as listed in Sorrells, et al. (2011). SynOpDH population is the reconstructed / new version of the same population with two forms, doubled haploid (DH) and RIL.

There have been many reports with the aim to introduce genetic variation for quantitative and qualitative traits into modern crops including drought, heat, and salt tolerance, as well as biotic stress factors, such as root rot, nematodes, stripe rust, leaf rust and for many other diseases (Tanksley, 1997, Van den Boogaard and Villar, 1998, Jarvis and Hodgkin, 1999, Assefa and Fehrmann, 2000, Hegde, et al., 2000, Valkoun, 2001, Hegde, et al., 2002, Colmer, et al., 2006, Feuillet, et al., 2007, Reynolds, et al., 2007, Gomez-Becerra, et al., 2010, Sheedy, et al., 2012, Placido, et al., 2013). Mostly *Ae tauschii, T. dicoccoides,* and *T. monococcum* were screened for the above mentioned traits. A total of nine traits

were reported for wheat, most of which were pest and disease resistance related, so-called single gene traits. However, there have not been any reports of the transfer of abiotic stress tolerance / resistance genes from wild gene pools into wheat (Feuillet, et al., 2007, Hajjar and Hodgkin, 2007).

The second largest source for genetic variation is the landrace populations with extensive allelic diversity. Even though landraces are no longer grown by most farmers, they are still cultivated in remote locations such as high elevations or under low-input conditions where modern wheats do not perform or yield well. Landraces are also preferred for high straw yield and yield stability (Karagöz, 2013). Landraces are domesticated, locally adapted varieties, which have been selected over thousands of generations (Ehdaie and Waines, 1989, Harlan, 1992, Jaradat, 2011). Continuous selection and elimination generated a heterogeneous population structure and wide allelic diversity for adaptation to low-input conditions (Harlan, 1992, Zeven, 2000, Zeven, 2002, Jaradat, 2013). Landraces have the potential for many useful traits with an advantage of easy crossing and hybridization for breeders (Reynolds, et al., 2005). Therefore, introgression of alleles from landraces into modern wheats may extend the variation in any target trait including drought and heat tolerance as well as grain yield.

Grain yield has always been the main target for modern plant breeding. Traditional selection criteria measure and eliminate candidate genotypes based on yield and disease resistance. If one accession performs well under given conditions it is selected, if it does not yield well it is eliminated. This approach has

been very effective until recent environmental changes. Many modern wheats did not have the genetic potential to cope with heat, drought or salinity. Traits that were previously ignored, such as drought tolerance, root system traits, high hydraulic resistance, high water uptake and use efficiency, and high nutrient uptake and use efficiency became key traits to prevent or tolerate the disastrous effects of climate change.

Root traits were neglected historically owing to the difficulty of selection and the lack of understanding of root structure and function (Waines and Ehdaie, 2007); most breeding programs targeted high-input growth conditions that do not require an extensive root system (Pingali, 2012). As a result, modern breeding indirectly and negatively affected abiotic stress tolerance. Many traits to improve drought tolerance are related to the root system of the plant. It is important for soil exploration and acquisition of water and nutrients. An extensive and deep root system is associated with drought tolerance, better water uptake, and maintenance of plant water status (Hurd, 1974, Blum, 1996, Blum, 2009, Ehdaie, et al., 2010, Ehdaie, et al., 2012). Modelling estimates demonstrated that every ml of water extracted from the water table during grain filling directly increases grain yield (Manschadi, et al., 2006).

The wheat root system consists of two major parts, seminal roots that emerge early from the seed and develop from scutellar and epiblast nodes of the embryonic hypocotyl of the germinating caryopsis, and the adventitious / nodal roots emerge later from the coleoptilar nodes of the base of the tillers (Manske and

Vlek, 2002). Seminal roots develop to depths of the soil with a strong gravitropic response (Oyanagi, 1994), nodal roots develop later and have more of a lateral growth pattern (Manske and Vlek, 2002). Bread wheats generally have 3 to 5 seminal roots, and many nodal roots based on the number of tillers (Robertson, et al., 1979, Manske and Vlek, 2002).

In an effort to search genetic diversity for root traits, I have evaluated four sets of accessions from domesticated and wild wheat germplasm.

My first chapter surveys *Ae. speltoides, Ae. tauschii, T. urartu, T. monococcum* and *T. dicoccoides* with an aim to uncover some of the genetic potential in the wild *Triticeae* gene pool for root traits. Since all five species evaluated were in the first gene pool of bread wheat they have always been leading candidates for abiotic and biotic stress traits. Unfortunately, there were no previous reports on the evaluation of the root system in these wild relatives. I evaluated 15 accessions that belong to 5 wild species, which were collected from Armenia, Turkey or Syria between 1988 and 1998.

In the second chapter, I evaluated Turkish landrace and modern wheats. Since a significant part of wheat production in Turkey relies on rain and stored soil water, I wanted to evaluate interactions between drought tolerance and root system traits in Turkish wheat germplasm. Five bread wheat landraces, three durum and eleven bread wheat cultivars that represent pre - and post - Green

Revolution germplasm were screened for root and shoot biomass traits, as well as grain yield.

In the third chapter, I evaluated the role of *Rht* genes on the root and shoot traits of the bread wheats from CIMMYT germplasm. Nine accessions that represent some of the parental-tall, early-tall and late generation semi-dwarf Green Revolution wheats, which includes historic parental lines “Marroqui’, ‘Mentana’ and ‘Gabo’ were selected and evaluated.

In the final chapter, I evaluated a bi-parental doubled haploid (DH) standard mapping population (SynOpDH) to locate quantitative trait loci (QTL) associated with root, shoot and grain yield traits. The SynOpDH population was a good candidate because of the diverse genetic background from durum wheat cv. Altar 84, wild accession of *Ae. tauschii* and bread wheat cv. Opata M85. I evaluated a total of 147 lines along with parents under well-watered conditions for two years in 2013 and 2014.

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CHAPTER 1: Characteristics of Root Systems in the Diploid Genome Donors of Hexaploid Wheat

Abstract

Wild crop relatives are of considerable interest in plant breeding and significant efforts have been made to transfer their genetic variation into modern crops. In bread wheat (*Triticum aestivum* L.), of the three diploid progenitors, *Aegilops speltoides*, *Aegilops tauschii* and *Triticum urartu* only *Ae tauschii* has been explored and exploited in a systematic way, and even then only for the above ground characteristics. The three wild progenitors have never been assayed for root traits. Here we report on just such a study, also including *Triticum monococcum* and *Triticum turgidum subsp. dicoccoides.* From the five wild wheat species 15 accessions were selected and tested in the presence of one bread wheat cultivar 'Pavon F76'. Significant variation was observed between and within the taxa. Of all accessions tested, cv. Pavon F76 has the smallest root system at maturity while *Ae. speltoides* had the largest root. Moreover, *Aegilops spp*. had larger mean values for root biomass when compared with *Triticum spp*. These results suggest that there is significant unexplored potential for the use of wheat wild relatives in wheat breeding to improve the root system, or to develop synthetic mapping populations to study root traits.

Introduction

Bread wheat is a hexaploid crop with three homoeologous genomes. The diploid genome donors are *Aegilops speltoides Tausch* (SS) the ancestral “B” genome, *Triticum urartu* Tum. ex. Gandil (A^uA^u) the “A” genome and *Aegilops tauschii* Coss. (DD) the “D” genome (Kihara, 1944, Dubcovsky and Dvorak, 2007). The large genome size and complex polyploid nature make genetic studies in bread wheat challenging. Wheat as one of the major staple crops around the world provides more than 20% of our daily caloric intake (FAOSTAT, 2014). In fact, it has been one of the most important crops since the domestication for food, feed, and other uses. There has been a significant amount of effort towards the improvement of grain yield, abiotic and biotic stress resistance / tolerance. Genetic control of many above ground traits has been reported within the last couple of decades. In contrast our level of understanding of below ground organs, such as roots of the plant, is still limited (Herder, et al., 2010).

Wild and domesticated members of the *Triticeae* tribe have wide distribution around the world. Many wild and weedy races spread with domesticated species of *Hordeum* and *Triticum.* According to Valkoun, et al. (1998) *Ae. speltoides* has distribution mainly in grasslands in south east Turkey and it is missing from Jordan and south Syria. *Triticum urartu* follows the Fertile Crescent from Jordan, through southwest and central Syria, southeast Turkey to Iraq and Iran. *Aegilops tauschii* has wide distribution from Syria to China, mostly as weedy races, spread by the silk trade. Nevertheless, major habitats are in central Syria and eastern Turkey, and West Azerbaijan province of Iran and in the Caspian region of Iran. *Triticum*

dicoccoides spread from Jordan, through southwest and central Syria, Southeast Turkey to Iraq and Iran.

The genetic potential of wild wheat relatives in biotic stress tolerance of bread wheat has been documented since the 1970s (Frankel and Bennett, 1970). Genes that provide resistance to leaf rust, stem rust, and root lesion nematodes (Assefa and Fehrmann, 2000, Sheedy, et al., 2012) have been transferred from wild crop relatives. A total of nine traits have been transferred, most of which were pest and disease resistance related, so called single gene traits. However, there have not been any reports of the transfer of drought tolerance genes from wild crop relatives (Feuillet, et al., 2007, Hajjar and Hodgkin, 2007).

Of the three progenitors, *Ae. taushii* enjoyed the most attention. It is the most recent genome incorporated into bread wheat, and its addition might have extended the range of wheat by providing wide environmental adaptability (Feuillet, et al., 2007). Many *Ae. taushii* accessions have wide distribution around the world from sea shores to sub-tropic areas, roadsides, to the edges of deserts. The adaptability potential and extensive genetic diversity of *Ae. tauschii* is well documented and it is a leading candidate for bread wheat genetic improvement. Genetic variation of *Ae. tauschii* has been explored in a systematic way in the development of synthetic wheats (Mujeeb-Kazi, et al., 1996). These are new amphidiploids created from tetraploid wheats (genomes BBAA) and a large number of *Ae. tauschii* accessions. When these synthetics were incorporated into wheat breeding programs, numerous characters were improved, such as bread

making quality, (Orth and Bushuk, 1973, Kunert, et al., 2007), cold hardiness (Limin and Fowler, 1981), salt tolerance (Schachtman, et al., 1992), drought tolerance (Gororo, et al., 2002, Sohail, et al., 2011), kernel size (Okamoto, et al., 2013), and yield and yield components (Gororo, et al., 2002, Li, et al., 2004, Narasimhamoorthy, et al., 2006, Okamoto, et al., 2013).

Sohail, et al. (2011) reported significant drought tolerance and large root biomass for synthetic wheat lines and *Ae. tauschii* accessions with the former having better stress responses than the latter. Multi gene interactions between B, A and D homoeologous genomes may be the reason for stronger responses of the synthetics. Overall, both the synthetic wheat lines and *Ae*. *tauschii* accessions performed better than commercial cultivars under drought treatment. Similarly, Reynolds, et al. (2007) evaluated four synthetic wheat derived lines and their recurrent parents. They reported an increased partitioning of root biomass to deep soil zones in the synthetic derived lines than recurrent parents. The synthetic lines also had a smaller reduction in root biomass under stress conditions when compared with recurrent parents.

Nutrient uptake and efficiency were evaluated by Górny and Garczyński (2008) of diploid, tetraploid, and hexaploid *Triticum* and *Aegilops* accessions grown in high / low nitrogen, phosphorus and potassium (NPK) conditions. Significant differences within and between *Aegilops spp.* and *Triticum spp*. were reported. *Triticum spp*. were more efficient on NPK utilization than *Aegilops spp.*

except *Ae. tauschii.* On the other hand, *Aegilops spp,* had a high nutrient uptake efficiency and enhanced tolerance to nutrient shortages.

The above reports evaluated the potential to utilize wild crops relatives in plant breeding. Drought tolerance is a complex trait with many genes being involved (Witcombe, et al., 2008). Studying root traits to improve water and nutrient uptake efficiency and maintenance of plant water status may reduce the effect of unfavorable environmental conditions (Manschadi, et al., 2006). The study of root traits such as total root biomass, rooting depth, and number of seminal and nodal roots, xylem diameter has become a necessity. Deep rooting is a desirable trait for high plant water status, especially through grain filling (Passioura, 1983, Blum, 1996). Understanding the root system and modifying roots of modern wheats is a fundamental pre-requisite to increase grain yield in rain-fed and moisture stressed conditions (Reynolds, et al., 2005, Reynolds, et al., 2007).

Unfortunately, none of the diploid ancestors or close relatives of bread wheat have been studied for root system characteristics. On the other hand, a related crop, barley and its wild ancestor did provide useful information on the potential of wild plants in breeding. Bengough, et al. (2004) and Hargreaves, et al. (2009) evaluated seminal root morphology of wild (*Hordeum spontanum* C. Kosh), landrace, and modern barley (*H. vulgare* L.*)* accessions. They demonstrated that wild and landrace barley accession had deeper rooting (expressed as narrower seminal root angles) when compared with modern barleys. Root biomass and root length of wild and domesticated barley seedlings were evaluated by Grando and

Ceccarelli (1995). They reported large seedling root biomass for domesticated barleys, whereas wild barleys had thin and deep roots indicating adaptation to rain-fed growing conditions. Moreover, significant salt tolerance (Alamri, et al., 2013) and extensive genetic variation (Górny, 2001, Tyagi, et al., 2014) were reported for wild barley accessions.

The purposes of this study were to determine the genetic variation of the diploid ancestors of bread wheat and close relatives for root traits. To evaluate the correlation coefficients between and within wild species, and to examine similarities and differences between *Aegilops* and *Triticum* taxa for root system development.

Materials and Methods:

Fifteen wild accessions of *Aegilops* and *Triticum* spp. and cv. 'Pavon F 76' were selected based on previously published data (Hegde, et al., 2000, Moghaddam, et al., 2000, Hegde, et al., 2002). Of these accessions, four were *Aegilops speltoides spp. speltoides,* two *Aegilops speltoides spp. ligustica,* three *Aegilops tauschii*, three *Triticum urartu*, one *Triticum monococcum, and* two *Triticum dicoccoides.* Two of the *Ae. tauschii* accessions were provided by the USDA germplasm collection, the rest of the seeds were from the collection of J. G. Waines, University of California, Riverside. Selected accessions were originally collected from Turkey in 1988, Syria in 1994, and Armenia in 1998. Each accession was selected from a different population to assure genetic variation. (Table 1.1).

Seeds were surface sterilized with 1% sodium hypochlorite (NaCIO) solution for 10 minutes, rinsed in distilled water and germinated in Petri dishes on wet filter paper for five days, transferred into flats of sand and vernalized in a cold room for ten weeks at 2-5º C. After vernalization, seedlings of similar size were transplanted in to 1 m long & 10 cm diameter PVC tubes filled with 10.5 kg #30 grade silica sand in long plastic bags on February 2013 and January 2014. The sand tube technique was that described by Ehdaie and Waines (2006) and Sharma, et al. (2011). Seedlings were planted using a randomized complete block (RCB) design with four replications treated as blocks. Sand filled tubes were brought to the water holding capacity (24%) by watering excessively for two consecutive days before planting. Plants were given sufficient water and half-strength Hoagland's nutrient solution each day to prevent stress until maturity. The amount of water given each day was recorded.

At maturity, spikes and shoots were harvested separately and dried at 65º C for 72 hours. The plastic sleeves were taken out of the PVC tubes and cut lengthwise. The roots were washed out of the sand, their length measured and their images taken. The entire length of the roots was divided into two sections, deep roots below 30 cm and shallow roots between 0 to 30 cm and air dried at 65º C for 72 hours.

During the growing period, number of days from plant transplantation into tubes to booting, to heading, to anthesis, and to maturity were recorded as well as plant height (PH), number of fertile tillers (FT), number of tillers (NT), number of

spikes (NS) per plant flag leaf length (FLL) and flag leaf width (FLW) prior to harvest. After drying at 65º C for three days, data on shoot biomass (SM), shallow root weight (SRM) 0-30 cm, deep root weight (DRM) 30-100 cm, total root biomass (RM) and root to shoot ratio (R / S) per plant were collected.

Statistical Analysis:

Analysis of Variance (ANOVA) was performed to determine the genotypic variation for each trait in each experiment and for each taxon in 2013 and 2014. Normality of data distribution was tested by normal probability plots. Statistical analyses were performed using the Statistix software (Analytical Software; Tallahassee, FL, USA). Correlation of coefficients for accession mean values were performed to determine relationships between the above and below ground traits. Separation of means using the least significant difference (LSD) (Steel, et al., 1997) was performed based on ANOVA results. Combined ANOVA were performed across years to measure the genotype, year and genotype x year interactions.

Results

We observed significant differences for shoot biomass, total root biomass, shallow and deep root weight, number of tillers, plant height and root length between and within *Aegilops* and *Triticum* taxa. The mean values for the

experiment conducted in 2014 were greater than in 2013 but the genotype rankings for most traits did not change across years (Table 1.2 A-B). Genotype, year and genotype x year interactions were significant ($p<0.05$) for most traits, therefore data for experiments conducted in 2013 and 2014 were analyzed separately.

Aegilops spp. had significantly higher mean values for shoot biomass, shallow root weight, deep root weight (57.6 to 117.2% more deep roots), total root biomass, total plant biomass, root length, number of tillers, and number of fertile tillers, in 2013; and deep root weight, total root biomass, root length, plant height, number of tillers and number of fertile tillers in 2014, when compared with *Triticum* spp. (Table 1.2 A - B).

Average shoot biomass ranged between 21.01 g and 45.4 g plant^{-1} in 2013 and between 41.6 g and 78.1 g plant^{-1} in 2014. Genotypes with the largest shoot biomasses were ASL19/1, ASS13, and ASS5/1 in 2013, and ASS13, ASL13, and ASS2 in 2014, with 45.43 g, 43.59 g, 40.90 g plant^{-1} in 2013 and 78.14 g, 77.74 g, and 73.12 g plant^{-1} in 2014, respectively. In contrast, genotypes with the smallest shoot biomasses were 'Pavon F76', TU14 and TU16 in 2013 and TU14 and AT38 in 2014, with means of 21.01 g, 27.91 g, 29.13 g, plant^{-1} in 2013 and 41.63 g, and 50.28 g plant^{-1} in 2014, respectively (Table 1.2 A - B).

Average root biomasses ranged from 1.41 g to 6.8 g plant^{-1} in 2013, and 4.7 g to 14.4 g plant^{-1} in 2014 (Figure 1.1). Genotypes with the largest total root biomasses were AT38, AT5 and TU-Bulk with 6.84 g, 5.15 g and 5.09 g plant^{-1} root

biomass in 2013, and genotypes ASL2, ASL19/1 and TD13/1 with 14.33 g, 12.28 g and 11.02 g plant^{-1} root biomass in 2014, respectively (Table 1.2A - B). On the other hand, the smallest root biomasses belonged to 'Pavon F76' and TM11, with 1.42 g and 2.68 g, plant^{-1} in 2013 and 'Pavon F76' and TU14, with 4.72 g and 5.52 g, plant^{-1} in 2014, respectively. Similar results for deep and shallow root weights are listed in Table 1.2 A - B.

The range for number of fertile tillers was between 57.25 and 77.67 tiller plant^{-1} for *Aegilops* spp. and between 12.25 and 41.75 tiller plant^{-1} for *Triticum* spp. in 2013. Similarly, it was between 57.75 and 91.75 tiller plant^{-1} for *Aegilops* spp. and between 15.75 and 38.00 tiller plant^{-1} for *Triticum* spp. in 2014. Genotypes AT38 (77.6 tillers) in 2013 and AT5 (91.75 tillers) in 2014 had 6 fold more tillers than 'Pavon F76', which had 12.25 and 15.7 tillers in 2013 and 2014, respectively (Table 1.2 A - B).

Significant positive correlation was observed between the number of fertile tillers and deep root weight (0.37), maximum root length (0.39), and shoot biomass (0.33). As well as between root biomass and shoot biomass (0.71), total plant biomass (0.79), deep root weight (0.31), number of tillers (0.41), number of fertile tillers (0.33) and between shoot biomass and total plant biomass (0.99), maximum root length (0.49), and plant height (0.62). A slight negative correlation between plant height and deep root weight (-0.11) was observed (Table 1.3).

Average days to anthesis (flowering) was 76.8 days for wild species and 40.6 days for 'Pavon F76' in 2013, and 82 days for wild species and 77 days for

’Pavon F76’ in 2014. Even though there were phenological differences between ’Pavon F76’ and wild species there were no significant correlations between phenological stages (days to heading, to anthesis and to maturity) and root biomass ($r<0.30$), except the only correlation was between days to booting and root biomass (0.505).

Discussion

This is perhaps the first attempt to evaluate root systems of the three diploid ancestors of bread wheat in a single study. Given the geographical distribution of these species, from Near East to China (Valkoun, et al., 1998) the fifteen accessions tested here cannot possibly be taken as representing the entire range of genetic variation in the root systems of these species. However, given that each species is represented by several accessions, the study offers a general idea of the genetic potential in the germplasm for the root system and sheds light on a neglected group of plants for root studies. Follow up studies with a larger range of accessions may provide a deeper understanding of actual diversity of the germplasm accessions.

The study was replicated in two consecutive years and the mean values for most traits in 2014 were higher than in 2013 (Figure 1.1). We do not know the reason for additional biomass accumulation in 2014. The winter growing season did not appear noticeably cooler or cloudier but the rate of plant growth was clearly slower in 2014. Those additional 16 days of growth before heading in 2014 had

significant effects on biomass accumulation. We observed similar year effects in other experiments conducted in the same seasons. It was possibly a combined effect of seasonal differences for temperature, humidity and day light.

The study shows a significantly higher total root biomass in *Aegilops spp.* relative to *Triticum spp* (Figure 1.1)*. The* number of tillers may be one of the factors that affect root biomass by increasing the number of nodal roots. We have no way of proving this, since counting the number of nodal roots and calculating nodal root biomass of each tiller was not feasible. More tillering may provide more leaf area for photosynthesis which may provide more carbon assimilates for the above and / or below ground biomass. Kara, et al. (2000) observed strong associations between tillering capacity and the number of nodal roots in *Ae. geniculata* and *H. chilense*. However, when they generated an amphiploid between these two wild accessions and *T. durum*, the amphiploids had reduced tillering and reduced root biomass when compared with wild parents. They have also reported much higher projected root area in *Ae. geniculata* and its amphiploid than old and modern durum wheats. Fibrousness of the root system in *Ae. geniculata,* (numerous nodal and seminal roots) may be a major factor for significant difference of the projected root area and biomass. Similarly, we observed a strong correlation between the number of tillers and shallow root weight (0.55). The interactions between root fibrousness, number of roots and root surface area and number of tillers appear to be positive. In a related study on seedlings of barley, Grando and Ceccarelli (1995) observed narrow root angles and thin seminal roots in wild barley accessions,

which was associated with high hydraulic resistance and drought tolerance. Thin roots can be used as easy predictors of narrow xylem vessel diameters. Passioura (1972) along with Richards and Passioura (1989) reported that narrow xylem vessel diameter and high hydraulic resistance are strongly associated. High hydraulic resistance allows the plant to conserve water for the post anthesis period giving higher grain yield and harvest index. Similarly, in this study, we observed thin and long roots in *Ae. speltoides* and *Ae. tauschii* accessions (Figure 1.2). Assuming that narrow root diameter is associated with narrow xylem vessel diameter the wide adaptation ability of *Ae. tauschii* may be associated with its thin root system. Such a root system possibly costs less per individual root than a modern wheat with relatively thick roots. When we compared total plant biomass (root and shoot biomass combined) per tiller (Total plant biomass / number of tillers), we observed 2 g to 3.75 g biomass per tiller for check cv. 'Pavon F76', 0.6 g to 1 g for wild *Aegilops*, and 1 g to 1.8 g for wild *Triticum spp*. This indicates that the total cost of each tiller and the roots associated with it is much lower in *Aegilops*, and wild *Triticum* than in a domesticated one. Total soil coverage of the root system increases with a relatively lower cost to the plant so that the plant can reach more nutrients and water without sacrificing any additional carbon assimilates for biomass accumulation. Therefore, a root system that can cover large soil profile for water and nutrient uptake, but with a reduced carbon cost, seems to be possible. Especially, thinking of the fact that, many *Aegilops tauschii* accessions growing from Syria to China may rely entirely on relatively low amounts

of seasonal rain. Can we introduce rooting characteristics of wild *Aegilops* in to modern wheats? These findings suggest that root characteristics such as fibrousness, and increased total root surface area may be feasible and cost effective for the improvement of the root system in modern wheats. The evolution of wild crops for root morphological and anatomical traits may provide novel allelic diversity for a relatively small or moderate sized root system with large coverage in the soil profile.

In our study we observed relatively small root and shoot systems in two wild species, *T. urartu* (accessions TU14 and TU16) and *T. monococcum* when compared with *Ae. speltoides* and *Ae. tauschii* (Figure 1.1, Table 1.4). A plausible and interesting explanation for these results comes from previous observations in these species. Manske and Vlek (2002) and Lehmann, et al. (2012) reported more mycorrhizal colonization and dependency in landraces and wild plants than modern wheats. Similarly, according to Hetrick, et al. (1992) *T. urartu* and *T. monococcum* gave 110% and 39.9% positive growth response to mycorrhizae inoculation and they were 52.6 % and 28.5 % mycorrhizae dependent, respectively. In our study we did not inoculate growth media with mycorrhizae. So we do not know if mycorrhizae dependency of these two species were the reason for their lower root and shoot biomass accumulation. In a related study, Hetrick, et al. (1992) also reports a negative correlation between fibrousness of the root system and mycorrhiza dependency. We can probably assume that a fibrous-thin root system with extensive soil coverage may not need an expensive symbiotic

relationship. In order to prove this idea, there is a need for the evaluation of root diameter along with total root surface area and mycorrhizae associations with a wide range of wild plants from the *Triticeae* tribe.

Correlation coefficients of the accession means indicate that positive association of root and shoot biomass accumulation was shared over six different species from the *Triticeae* tribe. Regardless of the differences in growth patterns, numbers of tillers and leaf size, correlation of the above and below ground organs continued. A similar observation was reported by Kara, et al. (2000) with different test material. Therefore positive and competitive growth patterns of above and below ground organs seems to be universal at least within the *Triticeae* tribe. This interaction may partially explain extensive above and below ground biomass in landraces and the opposite in modern wheats. In our experiments, we observed an increase in root and shoot size from wild plants to landraces and a decrease from landraces to modern wheats (unpublished data). Similarly, Burton, et al. (2013) observed significant increases in root dry weight on landraces of maize when compared with Teosinte. Modern wheats (post Green Revolution), have a higher harvest index, which means reduced above and / or below ground biomass relative to grain yield. This characteristic makes high yield possible, as long as water and nutrient input is sufficient. However, wheats with high harvest index do not usually yield well under low-input conditions, especially under terminal drought. Shallow root length and reduced soil coverage of modern wheats (approximately

1 m) may not reach the stored water, and if it does not rain at the right time in the season they suffer from terminal drought.

It is a well-known fact that root biomass accumulation slows or even reduces after anthesis in domesticated wheats. A major part of the carbon assimilates are reserved for grain production after anthesis by switching off the sinks from root and shoot biomass accumulation. But we do not have much knowledge of the changes in carbon sinks in wild plants. There is a need for the evaluation of the changes in carbon sinks in wild relatives, in order to understand if they are similar to domesticated crops or did domestication or breeding change source sink relationships? Moreover, the role of phenology on the carbon sinks is also an area that needs extensive evaluation. There are more questions to be answered in the management of carbon assimilates through season and their effect on the plants response to stress factors and seed formation. Since spike and kernel biomass per tiller is much lower in wild plants, carbon cost of seed development may not be as high as in crops with larger seeds. Therefore shoot and root growth may continue for a longer time in wild plants than in domesticated ones. If this is true, wild plants may have more time and sources for a deep and dense root systems. As it widely observed in winter wheats, the longer the vegetative period the larger and longer the root system (Thorup-Kristensen, et al., 2009, Akman and Topal, 2014). In our study we observed 16 days of extra vegetative growth in 2014 compared to 2013, and observed more vigorous growth (Table 1.2 A - B). However, this strategy may not be possible for spring wheats

with relatively short vegetative growth and limited carbon supply. Breeding for intermediate type wheats that are similar to spring habit but with a longer vegetative growth phase may be the answer for vigorous growth. This approach may give the plant an ability to grow deeper roots to benefit from stored water. This limited observation brings light to the importance of the duration of the vegetative growth phase and its role in biomass accumulation and grain yield. Of course, this strategy may backfire if the amounts of accessible stored water are limited as the excessive vegetative growth will not be translated into higher grain yield.

Wild wheat relatives, such as *Aegilops* species deserve to be investigated in depth for root anatomical and morphological features such as root thickness, number and length of roots in order to get a better understanding of the genetic potential in this group. Understanding the relationships between phenology and root-shoot biomass accumulation may be useful to select specific ideotypes for target conditions like short season, long season, spring or winter growth habit.

Conclusions

Wild relatives of crops have served as a pool of genetic variation for decades. In every emergency, such gene pools are screened and almost always the desired variation is found. Here we show that the diploid progenitors of hexaploid wheat also harbor wide variation for the characteristics of the root system. Both the B and D-genome progenitors have much larger, deeper and more extensive root systems than cultivated wheat. Whether this variation can be

exploited for practical benefit in agriculture remains to be seen, but the example of the so-called synthetic wheats (synthetized from cultivated durum wheat and *Ae. tauschii*) implies that much breeding progress can be achieved.

Genotype	Origin	Abbreviation
	Syria	ASS13
Aegilops speltoides ssp. speltoides	Syria	ASS2
	Turkey	ASS19/1
	Turkey	ASS5/1
Aegilops speltoides ssp. ligustica	Syria	ASL13
	Turkey	ASL15/1
	Armenia	AT5
Aegilops taushii	Turkey	AT38
	Turkey	AT56
	Syria	TU14
Triticum urartu	Syria	TU16
	Turkey	TUBulk
Triticum monococcum	Turkey	TM11
Triticum dicoccoides	Turkey	TD13/1
	Turkey	TD7/2
Triticum aestivum 'Pavon F76'	Mexico	Pavon F76

Table 1.1: List of *Aegilops* and *Triticum* genotypes evaluated in glasshouse under well-watered conditions in 2013 and 2014.

GENOTYPE	RM	SRM	DRM	SM	FT	PH	RL	DTH
ASL 13	2.96^{ef}	2.51^{efg}	0.45^{bcdef}	32.23^{efg}	59.75^{abcd}	101.00^{ab}	88.00^{bcd}	68.25^{cde}
ASL 15/1	3.21^{de}	2.93^{def}	0.28^{ef}	33.38^{ef}	57.25^{bcd}	88.63^{abcd}	79.88^{d}	79.00^{bcde}
ASS 13	4.64^{bcd}	3.99^{bcde}	0.66^{b}	43.59^{ab}	59.75^{abcd}	100.00^{ab}	99.25^{ab}	79.50^{bcde}
ASS 19/1	4.64^{bcd}	4.08^{bcd}	0.57^{bcd}	45.43^{a}	74.25^{ab}	87.00^{bcde}	99.00^{ab}	94.75^{ab}
ASS 2	4.76^{bcd}	4.15^{bcd}	0.61^{bc}	38.94^{bcd}	61.00^{abc}	102.25^{ab}	95.25^{ab}	67.75^{cde}
ASS 5/1	4.10^{bcde}	3.42^{bcdef}	0.68^{b}	40.90^{abc}	58.75^{bcd}	94.00^{abc}	94.75^{abc}	82.75^{bcd}
AT 5	5.15^{b}	4.65^{ab}	0.50^{bcde}	38.82^{bcd}	57.75^{bcd}	71.25^{ef}	96.25^{ab}	89.67^{de}
AT 38	6.84^{a}	5.82^{a}	1.02^{a}	40.68^{abc}	77.67^{a}	65.00^{f}	100.67^{a}	77.75^{bcde}
AT 56	4.86^{bc}	4.46^{abc}	0.40^{cdef}	36.27^{cde}	69.25^{ab}	77.00^{cdef}	94.25^{abc}	76.25^{bcde}
TU 14	3.40^{cde}	3.15^{cdef}	0.25^{fg}	27.91^{g}	34.75^{e}	86.25^{bcde}	89.75^{abcd}	61.00^{e}
TU 16	3.96^{bcde}	3.67^{bcdef}	0.29^{ef}	29.13^{fg}	34.25^{e}	94.00^{abc}	83.00^{cd}	69.50^{cde}
TU BULK	5.09^{b}	4.77^{ab}	0.32^{ef}	38.59^{bcd}	43.00^{cde}	95.25^{ab}	88.75^{abcd}	86.75^{bc}
TM 11	2.68^{ef}	2.47^{fg}	0.21^{fg}	37.41^{cde}	28.75^{ef}	73.25^{def}	80.75^{d}	109.25^{a}
TD 13/1	2.84^{ef}	2.48^{fgh}	0.36^{def}	34.93^{de}	41.75^{de}	93.75^{abc}	91.75^{abcd}	66.25^{de}
TD 7/2	3.41^{cde}	3.06^{cdef}	0.35^{def}	39.99^{bcd}	31.50^{e}	104.25^{a}	88.25^{bcd}	82.00^{bcd}
PAVON F76	1.42^{f}	1.38^{g}	0.04^{g}	21.01^{h}	12.25^{f}	73.00^{def}	83.00^{cd}	35.00^{f}
LSD	1.575	1.496	0.244	5.197	17.522	16.510	11.996	11.469
P values	0.000	0.000	0.0001	0.000	0.000	0.000	0.0077	0.000

Table 1.2 A: Mean values per plant for total root biomass (RM), shallow root weight (SRM), deep root weight (DRM), shoot biomass (SM), root to shoot ratio (R / S), number of fertile tillers (FT), plant height (PH) and days to heading (DTH) for ASL; *Aegilops speltoides* spp. *ligustica*, ASS; *Aegilops speltoides* spp. *speltoides*, AT; *Aegilops tauschii*, TD; *Triticum dicoccoides*, TU; *Triticum urartu*, TM; *Triticum monococcum* and 'Pavon F76', bread wheat cultivar evaluated in 1 m tubes under well-watered conditions in 2013. Means followed by a different letter are significantly different at p≤0.05 level using an LSD test.

GENOTYPE	RM	SRM	DRM	SM	FT	PH	RL	DH
ASL 13	11.53^{abc}	9.93^{a}	3.91^{ab}	77.74^{a}	68.25^{bc}	125.00^{bc}	100.00^{a}	94.25^{ab}
ASL 15/1	9.86^{bcde}	7.24^{bcd}	2.62^{bcde}	55.17^{cde}	90.50^{a}	105.00^{def}	100.00^{a}	101.00^{ab}
ASS 13	8.89^{bcdef}	6.23^{bcde}	2.66^{bcde}	78.14^{a}	77.25^{abc}	125.00^{bc}	100.00^{a}	90.00^{abc}
ASS 19/1	12.28^{ab}	7.81^{abc}	4.48^{a}	58.29^{cd}	61.00^{c}	112.50^{cd}	100.00^{a}	98.00^{ab}
ASS 2	14.33^{a}	10.72^{a}	3.61^{abc}	73.12^{ab}	84.50^{ab}	122.50^{bc}	97.50^{a}	96.25^{ab}
ASS 5/1	7.28^{defg}	4.10^{ef}	3.18^{abcd}	62.28^{bcd}	30.50^{ef}	105.00^{def}	100.00^{a}	109.00^{a}
AT 5	6.50^{efg}	4.90^{cdef}	1.61^{efg}	72.04^{ab}	91.75^{a}	98.00^{def}	100.00^{a}	95.25^{ab}
AT 38	6.67^{efg}	4.90^{cde}	1.77^{efg}	50.28^{de}	57.75^{cd}	97.00^{ef}	100.00^{a}	101.00^{ab}
AT 56	9.01^{bcdef}	6.69^{bcde}	2.32^{cdef}	57.82^{cd}	91.75^{a}	95.00^{fg}	91.00^{a}	98.50^{ab}
TU 14	5.52^{fg}	4.52^{def}	1.00^{fg}	41.63^{e}	31.00^{ef}	111.25^{cde}	69.75^{b}	97.50^{ab}
TU 16	7.37^{defg}	5.98^{bcdef}	1.39^{efg}	55.33^{cde}	28.^{25ef}	131.25^{a}	94.75^{a}	90.25^{abc}
TU BULK	9.28^{bcdef}	7.35^{bcd}	1.93^{defg}	67.03^{abc}	38.00^{de}	130.00^{b}	87.00^{ab}	90.25^{abc}
TM 11	6.63^{efg}	4.82^{cdef}	1.81^{efg}	57.14^{cd}	22.50^{ef}	157.50^{a}	83.25^{ab}	104.50^{a}
TD 13/1	11.02^{bc}	8.68^{ab}	2.35^{cdef}	60.693^{bcd}	28.00^{ef}	126.25^{bc}	87.00^{ab}	81.75^{bc}
TD 7/2	9.71^{bcde}	7.21^{bcd}	2.51^{cdef}	59.46^{bcd}	29.50^{ef}	121.25^{bc}	100.00^{a}	94.25^{ab}
PAVON F76	4.72^{g}	3.52^{f}	1.20^{fg}	59.80^{bcd}	15.75^{f}	81.25^{g}	88.25^{ab}	70.25^{c}
LSD	3.314	3.344	1.491	9.758	21.242	15.000	19.466	21.266
P values	0.000	0.0041	0.0008	0.000	0.000	0.0000	0.1067	0.000

Table 1.2 B Mean values per plant for total root biomass (RM), shallow root weight (SRM), deep root weight (DRM), shoot biomass (SM), root to shoot ratio (R / S), number of fertile tillers (FT), plant height (PH) and days to heading (DTH) for ASL; *Aegilops speltoides* spp. *ligustica*, ASS; *Aegilops speltoides* spp. *speltoides*, AT; *Aegilops tauschii*, TD; *Triticum dicoccoides*, TU; *Triticum urartu*, TM; *Triticum monococcum* and 'Pavon F76', bread wheat cultivar evaluated in 1 m tubes under well-watered conditions in 2014. Means followed by a different letter are significantly different at $p \leq 0.05$ level using an LSD test.

Correlations for Accession Means

	SM	SRM	DRM	RM	RL	PH	NT	FT	TM	DTH
SRM	**0.52**									
DRM	**0.67**	**0.59**								
RM	**0.68**	**0.86**	**0.90**							
RL	**0.65**	**0.40**	**0.67**	**0.64**						
PH	**0.40**	**0.38**	0.29	**0.34**	-0.14					
NT	**0.40**	**0.55**	**0.68**	**0.72**	**0.73**	-0.27				
FT	**0.44**	**0.57**	**0.62**	**0.69**	**0.69**	-0.20	**0.97**			
TM	**0.99**	**0.63**	**0.74**	**0.77**	**0.67**	**0.42**	**0.47**	0.51		
DTH	0.23	0.00	-0.12	-0.04	0.01	0.28	-0.10	-0.06	0.20	
DTA	0.11	-0.03	-0.23	-0.13	-0.10	0.14	-0.17	-0.20	0.06	**0.67**

Table 1.3: Correlation coefficients (r values) between shoot biomass (SM), shallow root weight (SRM), deep root weight (DRM), total root biomass (RM), root length (RL), plant height (PH), number of tillers (NT), fertile tillers (FT), total plant biomass (TM), days to booting (DTB), days to heading (DTH), and days to anthesis (DTA number of) for AS; *Aegilops speltoides* AT; *Aegilops tauschii*, TU; *Triticum urartu*, TM; *Triticum monococcum,* TD; *Triticum dicoccoides*, and *Triticum aestivum* ‘Pavon F76’, evaluated in 1 m tubes under well-watered conditions in 203 and 2014.
0.30 > r > 0.50 Significant correlation at p <0.05
r>0.50 Highly significant correlation at p <0.01.

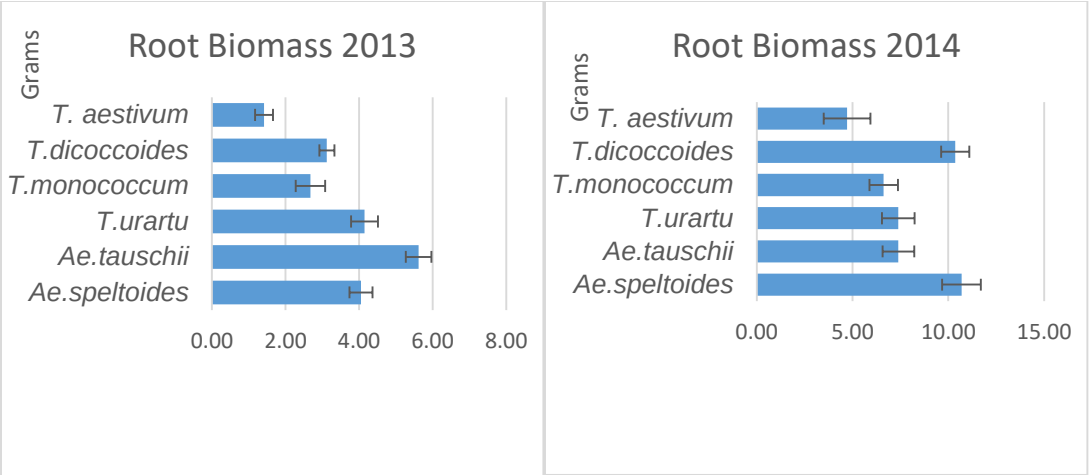
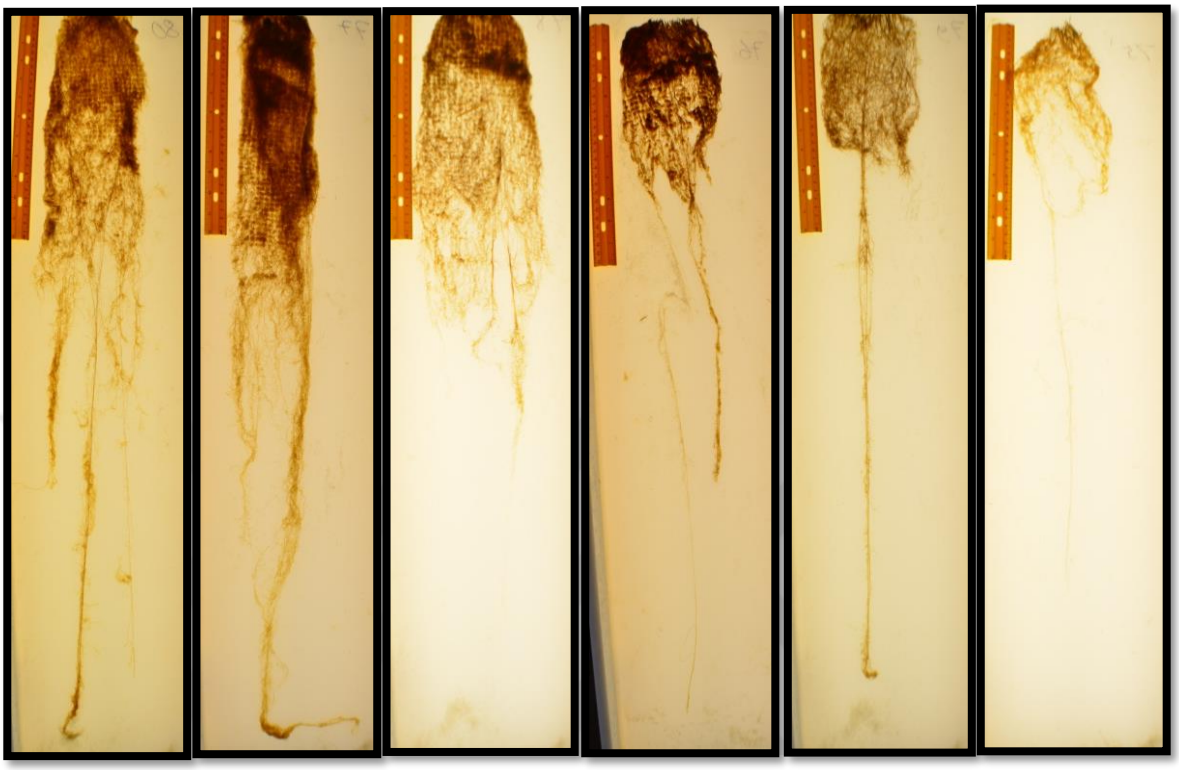


Figure 1.1: Mean values of root biomass for *Aegilops speltoides, Aegilops tauschii*, *Triticum urartu*, *Triticum monococcum, Triticum dicoccoides* from *Triticum aestivum* 'Pavon F76' grown in 1 m tubes under well-watered conditions in 2013 and 2014.

Year	Genotype	Min. Value	Mean Root Biomass	Max. Value
2013	*Ae. speltoides*	1.57	4.05	7.52
	Ae. tauschii	3.59	5.62	6.84
	Triticum urartu	2.26	4.15	6.39
	Triticum monococcum	2.01	2.68	3.73
	Triticum dicoccoides	2.1	3.12	3.79
	Triticum aestivum	0.95	1.42	2.05
2014	*Ae. speltoides*	4	10.69	17.68
	Ae. tauschii	4.39	7.40	11.27
	Triticum urartu	3.96	7.31	11.32
	Triticum monococcum	5.27	8.12	12.55
	Triticum dicoccoides	6.37	10.37	13.19
	Triticum aestivum	2	4.72	7.64

Table 1.4: Mean values and ranges of total root biomass per plant for AS; *Aegilops speltoides,* AT; *Aegilops tauschii,* TU; *Triticum urartu,* TM; *Triticum monococcum*, TD; *Triticum dicoccoides, and Triticum aestivum* 'Pavon F76'; grown in 1 m tubes under well-watered conditions in 2013 and 2014.



Ae.speltoides *Ae. tauschii,* *T. urartu,* *T. monococcum,* *T. dicoccoides,* *T. aestivum*

Figure 1.2: Roots system architecture for *Ae.speltoides, Ae. tauschii, T. monococcum, T. urartu, T. dicoccoides,* and *Triticum aestivum* 'Pavon F76', respectively. Plants were grown under well-watered conditions in 1 m PVC tubes (Samples from 2013).

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CHAPTER 2: Root and Shoot Traits of Turkish Wheat Landraces and Cultivars Evaluated in the Glasshouse Under Well-Watered Conditions

Abstract

The search for genetic diversity in plant gene pools and germplasm collections is an important part of plant breeding. Genetic variation is needed for any breeding progress, in order to break current grain yield barriers. Breeding for resistance to abiotic and biotic stresses will not only lead to better survival of a plant, but may also improve grain yield. It has been suggested that exploring landraces could expand the genetic diversity of modern wheats. Five Turkish bread wheat landraces and 14 modern durum and bread wheat cultivars were evaluated for root and shoot biomass and grain yield for two years in three separate experiments. Root and shoot traits were measured in plants grown in 1 m and 1.5 m PVC tubes in a glasshouse. Significant genotypic differences were found within and between landraces and modern wheats. In both years, landraces had much higher root biomass than modern wheats. Shoot biomass, total root biomass, shallow root weight (roots developed 0 to 30 cm soil depth) deep root weight (roots developed below 30 cm), root / shoot biomass ratio, number of tillers plant^{-1}, and plant height were significantly greater in landraces compared to modern wheats. Correlation coefficients were positive between root biomass and shoot biomass (0.78), and the number of fertile tillers (0.76). Plant height and shallow and deep root weights as well as the total root biomass were positively correlated. However,

semi-dwarf (75-90 cm) and mid-height (90-100 cm) cultivars had greater grain yield. The growth habit also had a significant effect with winter wheats having significantly greater harvest index, whereas intermediate wheats had greater shallow root weights and total root biomass. Results of these experiments raise several interesting questions on the mode of adaptation in landraces to water stress and suggest that landraces may be a valuable resource in breeding for altered root architecture in wheat.

Introduction

Bread wheat (*Triticum aestivum* L.) is among the top three staple crops, with almost 700 million metric tons produced per year. Projections estimate that the human population will be over 9 billion by 2050 and food consumption will increase by at least 50% (FAOSTAT, 2014); with no additional land available for farming purposes (Döös, 2002) a steady annual increase of grain yield is needed to meet the growing demand.

The Green Revolution breeders developed semi-dwarf high yielding wheat and rice cultivars that provided food security as well as self-sufficiency for developing countries (Borlaug, 2007). Two to three-fold increases in grain yield were achieved, as compared to what had been produced before. The demand for higher grain yield created by the need for additional food promoted high-input farming. Many modern wheats have been bred and selected for under these high-input systems. Although mono-genotypic modern wheats perform well under high input conditions, the loss of genetic variation in crop germplasm reduced valuable resources for future crop improvement (Feuillet, et al., 2007, Newton, et al., 2010). It is estimated that as much as 75% of the genetic diversity has been lost within the last century (Pingali, 2012, Jaradat, 2013). Pressure for grain yield improvement grows bigger every year, whereas it becomes harder to achieve under water deficit, salinity, and nutrient deficiencies. New cultivars with tolerance to the above stress factors are needed and genetic diversity is the key to achieve progress in breeding. In a search for resistance to any biotic stress, germplasm

collections are screened, usually quite successfully. The same strategy applies to salinity tolerance, drought tolerance, and any other target trait. Limited genetic diversity in modern wheats is a well-known fact, and germplasm collections are the only resources to extend the allelic diversity (Tanksley and McCouch, 1997). Given the limitations of water and arable land, high-input growth conditions are not cheap anymore (Döös, 2002). Any new released cultivar must perform well with less fertilizer and water, similar to landraces, which were grown without any fertilizer and irrigation for thousands of years.

Wheat landraces are domesticated, locally adapted traditional varieties selected by farmers through ten thousand years since wheat domestication (Ehdaie and Waines, 1989, Harlan, 1992, Jaradat, 2011). They were not specifically selected for high grain yield; for the priority has always been yield stability under local conditions. Since landraces had to cope with many stress factors, they must have evolved and adapted to them. They have survived with heterogeneous and changing population structure with ongoing evolution. Heterogeneity and continuous evolution allowed them to survive better in changing environmental conditions, while also leading to a dynamic, genetically complicated population (Harlan, 1992, Zeven, 2000, Zeven, 2002, Jaradat, 2013). Landraces are still preferred over modern wheats in many parts of world, mainly for their stable yields in low input conditions, prized end use qualities, and high straw yield. Farm size, lack of machinery, and lack of fertilizer are also important limitations to growing modern wheats (Karagöz, 2013). All of the above qualities and extensive

genetic diversity make landraces a valuable resource for many traits leading to mitigation of abiotic and biotic stresses (Ehdaie, et al., 1988, Ehdaie and Waines, 1989, Reynolds, et al., 2005, Feuillet, et al., 2007, Reynolds, et al., 2007, Jaradat, 2013, Jaradat, 2014). Introgression of alleles from landrace populations into modern wheats may extend the drought and heat tolerance of the latter and may help to gain high grain yield in rain-fed and / or low input growth conditions. To do so, such alleles must first be identified.

Turkey, located in the center of the Fertile Crescent and having a large diversity of germplasm collections including thousands of wheat landraces, has been a major source of genetic diversity in wheat. More than 90% of wheat grown in Turkey is rain-fed and annual rainfall amounts are low; spells of drought are common (FAOSTAT, 2014). Therefore, selection pressure for drought tolerance over many generations probably generated well adapted wheats (Karagöz and Zencirci, 2005, Karagöz, 2013).The first exploration for genetic diversity in Turkey started with Zhukovsky in the early 1920s, followed by Gökgöl (1939) and continued with the collections of Harlan from the 1940s to 1960s. The first modern wheat breeding and selection effort started in Turkey in 1925 at the “Islahi büzür”, the Seed Breeding Institute where landraces were screened and recommended for commercial distribution http://www.turktob.org.tr/tr/turktob-dergisi (Shrewry, 2001, Atay, 2006). Evaluations were in field trials with emphasis on high yield, and the first registered landrace ‘Ak 702’ (Ak basak) was released in 1931 (Atay, 2006, ECP/GR, 2014). Later, in addition to Ak 702, foreign bread wheat cultivars such as

Italian cv. ‘Mentana’, (Strampelli 1913), a bread wheat with *rht8a* from Akakomugi), (Zeven, et al., 1976), Russian cv. Bezostaja 1 and Mexican CIMMYT wheat germplasm were successfully used in Turkish breeding programs (TTSM, 2014) (Table 2.1). A national wheat research and training project was established and the Turkish “Green Revolution” was started in 1967. Within a decade of breeding Turkish wheats with CIMMYT germplasm, Turkey doubled its wheat production with new cultivars and high levels of pesticides and fertilizer application.

Crop improvement throughout domestication and thousands of years of on-farm selection all the way to modern plant breeding, has focused on the above-ground traits such as shoot biomass, straw yield, leaves, flowers and grain yield. Root traits were neglected owing to the difficulty of selection below the soil surface and lack of understanding of root structure and function (Waines and Ehdaie, 2007). However, the root system is important for soil exploration and acquisition of water and nutrients. An extensive and deep root system is associated with drought tolerance, better water and nutrient uptake, and maintenance of plant water status (Hurd, 1974, Blum, 1996, Blum, 2009, Ehdaie, et al., 2010, Ehdaie, et al., 2012). According to a modelling estimate demonstrated by Manschadi, et al. (2006), water use efficiency under terminal drought was 55 kg per hectare more for each one millimeter of water extracted from the water table. The next step in plant breeding is to include root traits and water and nutrient uptake efficiencies in the selection procedure to find the best candidates to cope with drought stress.

In this study we compared five Turkish bread wheat landraces with fourteen modern wheat cultivars. The main objectives of this study were; to determine the effect of pre and post-Green Revolution breeding on root, shoot and yield traits, to quantify the genetic variation for root traits within and between landraces and modern wheats and to determine the relationship between root traits (shallow and deep root weight and total root biomass) and yield components and shoot traits.

Materials and Methods

Nineteen wheat accessions from Turkish wheat germplasm were selected with an aim to survey landraces, early and late generation Green Revolution bread and durum wheats (*T. turgidum* L. ssp *durum* Desf.). Historically important accessions such as ‘Bezostaja 1’ and ‘Ak 702’ were included. A minimum of three widely cultivated and genetically diverse accessions were selected to represent the above groups. Therefore five bread wheat landraces, three durum wheats, four early and seven late generation Green Revolution wheats were grown in glasshouse conditions to evaluate root and shoot traits. Seeds were provided by Dr. Emin Donmez of the Field Crops Central Research Institute, Turkish Ministry of Agriculture, Ankara, Turkey. Each line was carefully selected in an effort to explore maximum genotypic variation. The landraces were ‘Kose’, ‘Uveyik’, ‘Yayla’, ‘AK 702’, and ‘Sivas 111/33’; durum wheat cultivars were ‘Kiziltan 91’, ‘Altin 40-98’ and ‘Cesit 1252’, and the bread wheat cultivars were ‘Gerek 79’, ‘Gun 91’, ‘Kate A1’, ‘Bezostaja 1’ (Russian wheat), ‘Turkmen’, ‘Atay 85 ’, ‘Mizrak 98’, ‘Demir

2000', 'Ikizce 96', 'Kirkpinar 79', and 'Uzunyayla' (Table 2.1). 'Pavon F76', a CIMMYT bread wheat cultivar was used as check. The three durum wheat accessions were released in the post Green-Revolution era; they are not necessarily representative of all Turkish germplasm. In all tests they performed similarly to modern bread wheats and their data were combined with those of with modern bread wheats and in the remainder of this paper all bread and durum wheat cultivars will be called "modern wheats". 'Bezostaja 1' is a Russian wheat cultivar released in 1959; it carries the semi-dwarfing gene *Rht8c*. Even though 'Bezostaja 1' was released before the Mexican Green Revolution, it shows many of the characteristics of the post Green Revolution cultivars, with reduced biomass and tillering. More importantly, it was one of the major parental lines used extensively in Turkish wheat breeding. Therefore it is also included in the modern wheat group.

Seeds were surface sterilized with 1% sodium hypochlorite (NaCIO) solution for 10 minutes, rinsed with distilled water, germinated on wet filter paper in Petri dishes for five days, planted in flats and vernalized for 10 weeks at 2-5°C. Vernalized seedlings were transplanted into sand tubes on February 2013 and January 2014 and grown to maturity as described by Ehdaie and Waines (2006). Seedlings were selected to be of similar size and planted in PVC tubes of 1 meter by 10 cm in diameter filled with 10.5 kg #30 grade silica sand in long plastic bags. Two small holes were made at the bottom of the plastic bags to allow proper drainage. The 1 m tubes were too shallow to determine the maximum root length.

To determine maximum root length and distribution in soil zones, ten genotypes were evaluated in a separate experiment in 2014, in 1.5 m long tubes filled with 15.5kg of #30 grade sand.

Tubes with seedlings were arranged in a randomized complete block design (RCB) with four replications. For the 1 m tube experiments four replications were used, with three replications for the 1.5 m tube experiments. Sand-filled tubes were brought to the full water holding capacity (24%) by watering excessively for two consecutive days before planting (Maheepala, et al., 2014). Each tube had a single plant. Plants were regularly watered with sufficient water-nutrient solution to prevent drought stress.

Plants were grown until maturity. Spikes and shoots were harvested separately and dried in a hot air oven at 65°C for 72 hours. The plastic sleeves were taken out of the PVC tubes and cut lengthwise, the sand was washed out and the maximum root length was measured. Total roots were divided into sections: two for the 1 m tubes, (deep roots, below 30 cm soil depth and shallow roots, between 0 to 30 cm soil depth and three sections for the 1.5 m long tubes (from 0 to 30 cm, 31 to 100 cm and 101 to 150 cm). Root sections were first air dried in a glasshouse and then transferred to a hot air oven for 72 hours at 65°C. For all plants, notes were taken on the amount of water applied, days to booting (DTB), to heading (DTH), to anthesis (DTA), and to maturity (DTM) (calculated from the day seedlings were transplanted into tubes). At harvest plant height (PH), number of fertile tillers (FT), number of tillers (NT) and number of spikes (NS) plant^{-}

^{1}, flag leaf length (FLL) and flag leaf width (FLW) were measured/counted. Based on the measurements the following parameters were created: shoot biomass (SM), shallow root weight (SRM), and deep root weight (DRM), total root biomass (RM), total plant biomass (TM), grain yield (GY), and 1000 grain weight (1000gW), number of seeds per spike (SS), number of seeds plant^{-1} (NS), harvest index 1 (ratio of grain yield to shoot biomass ratio, expressed in %) (HI 1), harvest index 2 (ratio of grain yield to total plant biomass ratio, expressed in %) (HI 2), the ratio of root to shoot biomass (R / S), total water used (TWU), water use efficiency (WUE) and evapotranspiration efficiency (ETE). The water use efficiency (WUE) and the evapotranspiration efficiency (ETE) were calculated as follows ETE = TM/TWU, and WUE = (TM/TWU) x (GY/TM).

Statistical Analyses

The Analysis of variance (ANOVA) was performed for each trait in each experiment. Normality of distribution was tested by normal probability plots. Statistical analyses were performed using the Statistix software (Analytical Software; Tallahassee, FL, USA). Means were compared using the least significant difference (LSD) (Steel, et al., 1997) based on the ANOVA results. The relationships between pairs of traits were determined using simple correlation analysis. Contrasts between landraces, modern wheats and between winter, intermediate and spring wheats; and between tall, mid-height and semi-dwarf wheats, were evaluated based on mean values for each group.

Combined ANOVA was performed across years to measure the main effect of the year and the genotype as well as the magnitude of the genotype × year interaction. Highly significant or significant main effects for year, and for genotype and for genotype × year interaction were observed for all traits except root length. The data for all three experiments conducted in 2013 and 2014 were analyzed separately as a result of significant genotype × year interactions.

Results

Mean differences within and between landraces, early and late generation Green Revolution wheats and durum wheats were calculated. There was no significant difference for many traits between all three modern wheat groups (early and late generation Green revolution wheats and durum wheats). However, mean values for landraces were significantly different when compared to all modern wheats. Therefore we have concentrated our analysis on the differences between modern wheats and landraces. Experiments in both years and both in short and long tubes, demonstrated large and highly significant differences in almost all root parameters measured. The highest root biomass and the longest roots were observed among landraces and the smallest among modern cultivars. The same range of variation was observed for all parameters of the above-ground parts of plants and in fact the two were highly correlated (0.70).

In 2013, root biomass per plant ranged between 1.38 g (Pavon F76) and 11.25 g (Yayla, landrace), with an average of 6.02 g plant^{-1}. The largest root

biomasses were 11.25 g, 11.10 g, and 8.95 g plant^{-1} belonging to landraces ‘Yayla’, ‘Ak 702’ and ‘Uveyik’, respectively. In contrast, the smallest root biomasses were 1.38 g, 1.9 g, and 2.6 g plant^{-1} belonging to modern wheats ‘Pavon F76’, ‘Ikizce 96’, and ‘Kate A1’, respectively (Table 2.2 A). In 2014, root biomass plant^{-1} ranged between 2.8 g (Ikizce 96) and 12.5 g (Uveyik, landrace) with an average root biomass of 6.93 g plant^{-1}. The large root biomasses were 12.5 g, 11.4 g and 10.2 g plant^{-1} for ‘Uveyik’, ‘Kose’, and ‘Uzunyayla’, respectively, two landraces and one modern wheat. The smallest root biomasses were 2.8 g, 3.3 g and 3.6 g plant^{-1} for ‘Ikizce’, ‘Gerek-79’, and ‘Bezostaja 1’, respectively (Table 2.2 B).

The largest deep root weights in 2013, were 2.1 g, 1.8 g and 1.6 g plant^{-1} belonging to ‘Yayla’, ‘Altin 40-98’ and ‘Uzunyayla’, respectively while the smallest deep root weights were 0.08 g, 0.25 g and 0.36 g plant^{-1} belonging to ‘Pavon F76’, ‘Bezostaja 1’ and ‘Ikizce’, respectively. Similarly, in 2014 the largest deep root weights were 1.99 g, 2.3 g and 2.7 g, belonging to ‘Atay 85’, ‘Kose’ and ‘Uveyik’, respectively. Modern wheats with the smallest deep root weights were ‘Gerek 79’, ‘Kirkpinar 79’ and ‘Kate A1’, with 0.38 g, 0.52 g, and 0.53 g plant^{-1}, respectively (Fig. 1). Greater shallow root weights belonged to ‘Ak 702’, ‘Yayla’ and ‘Uveyik’ in 2013, and ‘Uveyik’, ‘Kose’, and ‘Uzunyayla’ in 2014, with root weights of 9.5 g, 9.1 g, 7.6 g, and 9.8 g, 9.1 g and 8.6 g plant^{-1}, respectively. Of the six genotypes with large shallow root weights, five were landraces. Modern wheats with the smallest shallow root weights were ‘Pavon F76’, ‘Ikizce 96’, and ‘Gerek 79’ in 2013, and

‘Ikizce 96’, ‘Bezostaja 1’ and ‘Gerek 79’ in 2014 with root biomasses of 0.95 g, 1.54 g, 1.97 g, and 2.5 g, 2.6 g, 2.9 g plant^{-1}, respectively (Table 2.2 A - B, Fig. 2.1).

In 2013 grain yields and harvest indexes of modern wheats were higher than those of landraces. Modern wheats ‘Demir 2000’, ‘Gerek 79’, and Turkmen in 2013 and ‘Atay 85’, ‘Kirkpinar 79’ and ‘Uzunyayla’ in 2014 had greater grain yield, with 22.3 g, 20.6 g, 18.8 g, and 40.7 g, 38.6 g and 35.12 g plant^{-1}, respectively. Landraces ‘Sivas 111/33’, ‘Kose’ and ‘Yayla’ in 2013 and modern wheats ‘Altin 40-98’, ‘Bezostaja 1’ and ‘Ikizce 96’ in 2014 had low grain yield, with 6.0 g, 6.2 g, 7.6 g, and 19 g, 21 g, and 21.3 g plant^{-1}, respectively. The Genotype × year interaction was significant for grain yield. In 2014, on average landraces yielded better than in 2013 (Table 2.2 A - B), and the average grain yield per landrace was slightly higher than per modern wheat. The average harvest index 1 in 2014 was greater than in 2013. Landraces had a lower average harvest index 1 than modern wheats only in 2013. Landraces ‘Sivas 111/33’, ‘Kose’, and ‘Yayla’ in 2013, and cultivar ‘Altin 40-98, and landraces ‘AK 702’, and ‘Uveyik’ in 2014 had 0.09, 0.09, 0.10 and 0.30, 0.31 and 0.32 harvest index, respectively. Modern wheats with greater harvest index were ‘Gerek 79’, ‘Kate A1’, and ‘Ikizce 96’ in 2013 and ‘Kate A1’, ‘Ikizce 96’, and ‘Gerek 79’ in 2014 with 0.49, 0.44, 0.42 and 0.56, 0.53 and 0.53, respectively. Overall, modern wheats produced more grain with reduced plant stature and the number of tillers per plant, thus they possessed higher harvest index.

Landraces produced more tillers than modern wheats and the number of seeds / spike was lower. In 2013 Landraces ‘Yayla’, ‘Uveyik’ and ‘Sivas 111/33’ had 29.7, 28.5, and 27.0 tillers plant^{-1}, modern wheats ‘Pavon F76’, ‘Gun 91’ and ‘Turkmen’, had 8.5, 8.75 and 10.5 tillers plant^{-1}, respectively while in 2014 landraces ‘Ak 702’, ‘Yayla’ and ‘Sivas 111/33’ had 34.75, 23 and 22.75 tillers plant^{-1} in 2014, respectively (Table 2.2A - B).

The average shoot biomass of landraces ranged between 66.6 g plant^{-1} and 95.9 g plant^{-1} whereas for modern wheats the range was 49.8 g plant^{-1} to 69.5 g plant^{-1} in 2013 and 2014, respectively (Table 2.4). Genotypes with greater shoot biomasses were ‘Yayla’, ‘Uveyik’, and ‘Uzunyayla’ in 2013 and ‘Uzunyayla’, ‘Yayla’ and ‘Sivas 111/33’ in 2014, which had 71.4 g, 70.8 g, 67.8 g, and 102.18 g, 101.12 g, and 98.02 g plant^{-1} shoot biomasses, respectively. On the other hand, the smallest shoot biomasses belonged to ‘Kate A1’, ‘Ikizce 96’ and ‘Pavon F76 in 2013 and ‘Ikizce 96’, ‘Kate A1’, and ‘Bezostaja 1’ in 2014 with 34.3 g, 37.5 g, 39.4 g, and 39.9 g, 45.93 g, and 49.94 g plant^{-1}, respectively. Two of the three genotypes with the highest shoot biomass were landraces and the third one was ‘Uzunyayla’, a modern wheat (Table 2.2 A - B, Fig. 2.1). Shoot biomass showed a strong positive correlation with root biomass (0.78), number of fertile tillers (0.60) and number of tillers (0.47), root length (0.59) and plant height (0.56) (Table 2.3).

Water use efficiency (WUE) and evapotranspiration efficiency (ETE) are indicators of the photosynthetic potential. We calculated WUE for landraces and modern wheats in both years. Landraces had lower WUE in 2013, whereas they

did not differ from modern wheats in 2014. On the other hand, landraces had 29.11 % and 25.86 % higher ETE when compared with modern wheats in 2013 and 2014, respectively. Since the total biomass accumulation in 2014 was higher than in 2013, WUE and ETE in 2014 were also higher than in 2013.

Days to anthesis and days to maturity are important phenological periods known to affect the above-ground traits and likely also below-ground traits. There was a 45 day and a 55 day difference between the earliest and the latest maturing genotypes in 2013 and 2014, respectively. Three genotypes with long days to maturity were ‘Uzunyayla’, ‘Altin 40-98’, and Cesit 1252’; the three earliest maturing genotypes were ‘Pavon F76’, Ikizce 96’ and ‘Gerek 79’ in 2013 and ‘Pavon F76’, ‘Demir 2000’ and ‘Gerek 79’ in 2014. The mean grain yield for three longest maturing wheats were 12.21 g and 27.4 g plant^{-1} in 2013 and 2014, whereas the mean grain yield for the three early maturing wheats were 17.13 g and 25.54 g plant^{-1} in 2013 and 2014, respectively.

The long tube experiment; the maximum root length

The long tube experiment aimed to evaluate the effect of rooting depth on biomass accumulation and grain yield. Mean values for root and shoot biomass traits did not change much when compared with the 1 m tube experiment of 2014. However, we saw a clear positive interaction between deep rooting and grain yield. Other than that, similar to the main experiments, landraces had higher mean values for most traits including grain yield. The genotypic differences for shallow

root weight (0 – 30 cm), and intermediate root weight (31 – 100 cm), shoot biomass, number of tillers, number of fertile tillers, and grain yield were significant ($p<0.05$) except, for the maximum root length and root weight below 101 cm. Eight out of ten genotypes had root lengths between 116 and 150 cm, the shortest root lengths belonged to modern wheats ‘Gun 91’ and ‘Kate A1’, 93.67 and 98.33 cm, respectively. Most genotypes that had high grain yield in 1 m tubes, also yielded high in 1.5 m tubes, and they had the longest root lengths. Genotypes ‘Kirkpinar 79’, and ‘Atay’ had the largest root biomasses, followed by two landraces. The landrace ‘Yayla’ and cv. ‘Kirkpinar 79’ had highest grain yield of 26.157 g and 21.483 g plant^{-1} and root length of 150 cm and 129 cm, respectively (Table 2.5). Correlation coefficients between root, shoot and grain yield traits were significant. Grain yield was positively correlated with shallow, intermediate and deep root weights (0.33 to 0.45), total root biomass (0.43), plant height (0.51), shoot biomass (0.66), and root length (0.46). Harvest index was negatively correlated with all biomass traits (-57 to -33). Most above and below ground traits were positively correlated similar to 1 m tubes. The highest correlation was between shoot biomass and total root biomass (0.80).

Discussion

In this study, we report the first evaluation of root traits in a sample of Turkish bread wheat landraces and an association of such root traits with above ground characteristics. Landraces and modern wheats showed significant

differences for traits such as total root biomass, grain yield and number of tillers per plant. Significant genotypic variation was found within and between groups (Table 2.4). On average, root biomass of landraces was larger than that of modern wheats which was in agreement with Siddique, et al. (1990) and Waines and Ehdaie (2007). The number of tillers, number of fertile tillers, shallow root weight, deep root weight, total root biomass, root to shoot ratio and plant height and evapotranspiration efficiency were significantly greater in landraces ($P<0.01$) (Table 2.4). On the other hand, harvest index 1, seeds / spike, water use efficiency (only in 2013) and number of seeds plant^{1} were greater in modern wheats ($P<0.01$) (Table 2.4).

Grain yields of modern wheats were higher only in 2013; in 2014 the landraces produced slightly more grain than modern wheat. This was somewhat surprising and no good explanation can be offered at this time. In many experiments, landraces produced less grain than modern cultivars, not only in wheat, Ehdaie, et al. (1988), Ehdaie and Waines (1989), Denčić, et al. (2000) and Zeven (1998). Higher grain yields of landraces in 2014 indicated a strong positive yield response with a strong genotype × environment interaction. A similar effect was reported by Ninou, et al. (2014). Interestingly, in 2014 the water use efficiency of landraces was slightly higher than that of modern wheats, so the increase in the water use efficiency together with high evapotranspiration efficiency appears to increase grain yield and harvest index in the landraces tested. The water use efficiency of landraces changed significantly from 2013 to 2014, but the ETE did

not change, it was higher in landraces in both years. Therefore, as suggested by Ehdaie and Waines (1993), selection for high ETE and harvest index may to be more efficient. It is also important to note that, water use efficiency or lower water use, may lead to smaller plant size, small leaves, and lower grain yield (Blum, 2005). Under stress conditions, plants with high water use efficiency may not produce higher grain yield, whereas plants with roots that can reach to stored water in deep soil zones may result in high or effective water use and high yield. Therefore, effective water use, instead of water use efficiency may lead to a better selection for high grain yield in water limited conditions (Blum, 2009). Transpiration and biomass accumulation are directly related, and breeding for maximum moisture capture from the soil must be targeted for yield improvement under drought stressed or rain-fed conditions. This implies that using landraces with large and long root systems with high ETE in breeding may increase drought tolerance without grain yield penalty.

Previous studies identified candidate landraces for yield stability (Koutis, et al., 2012), quality traits (Dotlačil, et al., 2010), the effect of planting density on yield (Ninou, et al., 2014), and yield potential (Denčić, et al., 2000). Bread wheat landraces and modern wheats were evaluated by Ninou, et al. (2014) in densely spaced and wide-spaced plots. In dense plots modern wheats out yielded landraces, whereas landraces yielded better in wide-spaced plots. Therefore, experimental conditions do have an effect on the results and correct identification of potential candidate genotypes. Our experiments were conducted under well-

watered conditions to get a better understanding of root and shoot traits, and their effect on grain yield. The root system by its nature is very plastic in response to changes in environmental conditions, resulting in dramatic changes in its size and architecture. Testing new material for the first time requires an understanding of root structure under optimum growth conditions. After evaluating without any environmental limitations throughout the season, additional tests under different irrigation regimes are needed to understand the range of plasticity of these genotypes. Therefore future research with more genotypes and multiple environment tests is needed.

Our data demonstrate much larger root systems in landraces. This is similar to the findings of Waines and Ehdaie (2007) (Table 2.4 & Figure 2.1). For a landrace, grown under rain-fed conditions, hence frequent water stress, a preferred root system would be large enough and well distributed throughout the soil profile to capture nutrients and water effectively throughout the season (Hurd, 1974, Blum, 1996). Additionally, fine roots with narrow xylem vessel diameter that have high hydraulic resistance and surface area would be desirable (Comas, et al., 2013). At the same time, the roots cannot draw excessive resources from the above ground parts of the plant. On the other hand, a large root system with wide xylem vessel diameter may not be beneficial under the terminal stress conditions because the plant would capture and consume a significant amount of water early in the season leaving little left for grain filling (Richards and Passioura, 1989). Grando and Ceccarelli (1995) compared wild barley (*Hordeum vulgare subsp.*

spontaneum L.), landraces and modern barley accessions (*Hordeum vulgare* L.) and reported that fine, thin roots and narrow root angles in wild barley and landraces may be associated with strong hydraulic resistance. Narrow-root angles in wild and landrace barleys were also reported by Bengough, et al. (2004) and Hargreaves, et al. (2009). Steinemann, et al. (2015) evaluated two wheat cvs. 'RAC875' and 'Kukri', and observed smaller xylem vessel diameter in the drought tolerant cv. 'RAC875'. Further studies are needed to evaluate genotypic variation of xylem vessel diameter in landraces and other germplasm accessions. Landraces may have the genetic potential to transfer various root traits into modern wheats for drought tolerance and for low input rain-fed growth conditions.

Total root biomass may be a quick indicator of the total root surface area. A large root biomass may indicate more seminal and / or nodal roots and root hairs. Both 'Ak 702' and 'Yayla' had large shallow root weight, a significant portion of which comes from nodal roots. A large shallow root system becomes very important under nutrient deficiency and especially around grain filling (Mattsson, et al., 1993, Manske and Vlek, 2002). It may also be important to capture precipitation from light rains at the end of the growing season. And may help to reach non-mobile nutrients. Engineering a root system that can capture water and nutrients in shallow soil layers and water from deep soil zones at a high rate may be ideal, but may not be easy to achieve. Breeding different cultivars for different conditions / limitations might be more feasible, but may create cultivars with very

narrow adaptation to soil conditions, unable to cope with any deviation from specific conditions.

In the 1 m-tube experiments, landraces had 81.5% and 11.5% more root weight below 30 cm soil depth in 2013 and 2014, respectively, when compared with modern wheats. Deep rooting is important for better water uptake and to reach mobile and non-mobile nutrients in the soil under limiting conditions (Waines and Ehdaie, 2007). By the equation of Passioura (1983) yield = water used x water-use efficiency x harvest index. Any additional water captured from the soil has a direct positive affect on grain yield (Manschadi, et al., 2006). By this token, the deep rooting characteristics of landraces (Table 2.5) are crucial for rain-fed farming conditions.

The landraces tested here showed much higher tiller numbers than modern wheats. Extensive biomass accumulation by additional tillers may have a positive feedback mechanism on the biomass and size of the root system. Large above ground biomass may provide extensive carbon allocation for a large root system with greater root length density in soil (Blum, 1996). As seen in this evaluation, the yield penalty of a large above and / or below ground biomass may not be as great as is thought. Kebrom and Richards (2013) reviewed the non-tillering mutant (*tin*) in wheat, and suggested that grain yield might be increased by reduced number of tillers in order to save carbon assimilates for grain filling. However, the extreme phenotype of the *tin* mutant is not stable over various environmental conditions. Its limited nodal root development may be a problem in nutrient deficiency soils.

Yield stability in various conditions is a pre-requisite for cultivar release. The general characteristics of Green Revolution wheats were high grain yields in high input conditions with reduced stature and reduced tillers compared to old landraces, but they tended to fail under low input conditions. Breeding new cultivars with moderate to high grain yields under such conditions is more likely to be possible using landraces that historically have been selected for stable yields over millennia. Nonetheless, shoot and root biomass above a certain threshold may not always be beneficial resulting in a low harvest index and possibly lower grain yield. It is important to identify an optimum plant height and biomass for target environment conditions which can provide enough carbon assimilates for high grain yield and an ideal root system.

The effect of maximum root length

In the 1.5 m tube experiment positive correlations between root weight below 31 cm and grain yield (0.455) and root weight below 101 cm and grain yield (0.459) indicate the importance of deep roots for yield and grain filling. These results are in agreement with Manschadi, et al. (2006) and Kong, et al. (2013). In a simulation study Hammer, et al. (2009) reported that different root architectures such as narrow or wide root angle, which lead to deep or shallow roots, had more effect on biomass accumulation and yield than canopy architecture and light capture. Additionally, we have observed strong positive correlations between shoot biomass and root biomass (0.80), shoot biomass and plant height (0.71),

shoot biomass and shallow, intermediate and deep root weights (0.64 to 0.82), grain yield and number of fertile tillers (0.63), and between grain yield and maximum root length (0.46). As we have observed in 1 m tube experiments and here in 1.5 m tube experiment, deep and dense root systems may be able to support large above ground biomass with high grain yield.

The effect of growth habit

Out of the 20 accessions evaluated; eight were winter wheats, eight were intermediate, and four were spring wheats including the check cultivar 'Pavon F76'. To assess the effect of growth habit on root, shoot and grain yield traits, we compared mean differences between winter, intermediate and spring wheats. The intermediate wheats had a greater average shoot biomass, shallow root weight, total root biomass and more days to anthesis than winter and spring wheats. On the other hand, winter wheats had a higher harvest index. Intermediate wheats reached anthesis approximately 10 days later than winter wheats, which might have contributed to higher biomass accumulation. There were no significant differences for the number of tillers and plant height between winter, intermediate and spring wheats (data not shown). The test may indicate a positive effect of longer vegetative period on biomass accumulation and a possible negative effect of that additional biomass on harvest index. Further studies with more genotypes and similar maturity days are needed to evaluate the effect of growth habit on

biomass accumulation in wheat. A study of lines isogenic for various vernalization genes / alleles would be particularly informative.

The effect of plant height

Many of the modern wheats had plant heights between 75 cm to 90 cm with some reaching between 91 cm and 100 cm, while all landraces were taller than 101 cm (Table 2.2 A - B). In an effort to analyze any possible effect of plant height on root, shoot and grain yield traits, genotypes were evaluated in three major groups; semi-dwarf (75 cm – 90 cm), mid-height (91- 100 cm) and tall (>101 cm). The mean values of tall genotypes for shoot biomass, shallow and deep root weight, and total root biomass, and root / shoot ratio were greater than mid-height and semi-dwarf genotypes. Harvest index 1 was highest in the mid-height genotypes. Moreover, average total root biomass of tall genotypes was 86 % and 71 % greater than those of the mid-height and semi-dwarf genotypes, respectively. The average deep root weight of tall genotypes was 100 % and 83 % greater than those of semi-dwarf and mid-height genotypes, respectively (Table 2.7). Tall plant stature can cause lodging and yield loss (Borlaug, 2007); however, tall plants are still preferred wherever straw yield is as important as grain yield (Annicchiarico and Pecetti, 2003). In this study, plant height was positively correlated with root biomass and shoot biomass (Table 2.3). We do not know all the dwarfing genes present in the tested lines (Table 2.1) but semi-dwarf accessions had significantly reduced root and shoot biomass when compared to the tall ones in the Turkish

wheat germplasm, which was in agreement with results reported by Waines and Ehdaie (2007), Siddique, et al. (1990) and Gale and Youssefian (1985). In spite of this, there is still a need for further evaluation of germplasm accessions in order to understand the effect of semi-dwarfing genes and tall genes on root and shoot biomass traits in wheat.

Conclusions

The results presented here are based on two experiments conducted in a controlled glasshouse, in 2013 and 2014, in 1 m tubes with four replications and 20 bread wheat landraces and modern wheats, and an additional experiment in 2014 using 1.5 m tubes with three replications and 10 accessions of landraces and modern wheat genotypes. The results of the three experiments suggest that Turkish landraces have significantly higher mean values for most of the root biomass and shoot biomass traits. Interestingly, landraces had low grain yield in 2013 but out yielded modern wheats in 2014 in the 1 m-tube experiment. This was an unexpected result since landraces have not been selected directly for high grain yield, under conditions similar to these well-watered experiments. Additionally, in 2014 while grain yields were significantly greater than in 2013, the above and below ground biomass was greater as well. We do not know what conditions in 2014 caused the difference. We have observed similar year effects in other independent studies. Average days to anthesis in 2014 were 16.57 days more than

in 2013 and this may account for higher biomass production. As observed in intermediate wheats with longer vegetative growth, this additional 16.57 days maybe the reason for additional biomass accumulation. Even though a glasshouse is technically controlled for temperature and humidity, it is completely dependent on many outside conditions. Seasonal differences on factors such as temperature, relative humidity may have extended vegetative growth as well as biomass accumulation.

A large above and below ground biomass may not always be a negative factor for grain yield formation. This implies that landraces may hold many interesting alleles for grain yield as well as for maximum root length, shallow root weight, deep root weight, total root biomass, number of fertile tillers and shoot biomass. Landraces have been tested over thousands of years by farmers in diverse growing conditions, and only the best ones with stable yield in any given condition were retained. Landrace yield loss in low-input or stress conditions may not be as sharp as of modern wheats.

This study indicates that a moderately sized shoot and root system with extensive deep root distribution / biomass may increase water capture from deep soil zones with minimum or no penalty in grain yield and harvest index. Given the insights into current limitations with the present system of measuring root system traits, we plan to use improved phenotyping and high throughput genotyping to investigate genes / loci responsible for differences in root traits. The present study

provides a general outline for future research to find candidate genotypes to be used in wheat breeding programs.

CULTIVAR/ VARIETY	RELEASE	STATUS	RHT GENES	HABIT	PEDIGREE	REFERENCE
Uveyik	-	LV	-	S	LV	(Gökgöl, 1939, ECP/GR, 2014, GRIS, 2014)
Kose	-	LV	-	I	LV	(Gökgöl, 1939, ECP/GR, 2014, GRIS, 2014)
Yayla	-	LV	-	W	LV-East-TUR	(Gökgöl, 1939, ECP/GR, 2014, GRIS, 2014)
Ak 702	1931	LV-released	Rht-B1a, Rht-D1a	I	(S)LV-Anatolia; (S)Local-club-wheat	(Gökgöl, 1939, Yediay, et al., 2011, ECP/GR, 2014, GRIS, 2014)
Sivas 111/33	1937	LV-released	Rht-B1a, Rht-D1a	S	LV-Central-Anatolia	(Gökgöl, 1939, Zencirci, 1998, Yediay, et al., 2011, ECP/GR, 2014, GRIS, 2014)
Bezostaja 1	1959	CV**	Rht-B1a,Rht-D1a,Rht8c,	W	Lutescens-17/Skorospelka-2	(Auvuchanon, 2010, ECP/GR, 2014, GRIS, 2014)
Kirkpinar 79	1979	CV	Rht-B1b, Rht-D1a	I	Hyslop/Siete-Cerros-66; 63-11/66-2/Siete-Cerros-66	(Yediay, et al., 2011, ECP/GR, 2014, GRIS, 2014)
Gerek 79	1979	CV	Rht-B1a, Rht-D1a	I	Mentana/Mayo-48//4-11/3/Yayla-305	(Auvuchanon, 2010, Yediay, et al., 2011, ECP/GR, 2014, GRIS, 2014)
Atay 85	1985	CV	Rht-B1b, Rht-D1a	I	Hyslop/Siete-Cerros-66	(Auvuchanon, 2010, Yediay, et al., 2011, ECP/GR, 2014, GRIS, 2014)
Kate a1	1998	CV	Rht-B1a, Rht-D1a	W	Hebros/Bez-1	(Yediay, et al., 2011, Olgun, et al., 2013, ECP/GR, 2014, GRIS, 2014)
Gun-91	1991	CV	-	I	F-35-70/Mochis-73	(ECP/GR, 2014, GRIS, 2014)
Ikizce 96	1996	CV	Rht-B1a, Rht-D1a	W	Arthur*2/Siete-Cerros-66//Brill	(Yediay, et al., 2011, ECP/GR, 2014, GRIS, 2014)
Uzunyayla	1998	CV	Rht-B1a, Rht-D1a	W	Hyslop/Siete-Cerros-66//Yayla-305/6/ Nadadores-63/CO652643/4/ Nainari-60/Mayo-54//Nainari-60/ Kadas/3/NS-220/5/Hyslop/Siete Cerros-66	(Yediay, et al., 2011, ECP/GR, 2014, GRIS, 2014)

Mizrak 98	1998	CV	Rht-B1a, Rht-D1a	W	Ycross//C126–15/C47–6/3/Yesilkoy1978–79–7	(Yediay, et al., 2011, ECP/GR, 2014, GRIS, 2014)
Turkmen	1998	CV		W	ECVD-12/Bezostaya-1	(ECP/GR, 2014, GRIS, 2014)
Demir 2000	2000	CV	Rht-B1a, Rht-D1a	W	21031/CO-6552142//Mara/Scout/3/PAI-YU-PAO[3670]	(Yediay, et al., 2011, ECP/GR, 2014, GRIS, 2014)
Kiziltan 91	1991	CV-durum	-	I	Uveyik-162/61-130//Barrigon-Yaqui-Enano*2/TE	(ECP/GR, 2014, GRIS, 2014)
Cesit 1252	1999	CV-durum	-	S	61-130/KUNDURU-414-44//377-2	(ECP/GR, 2014, GRIS, 2014)
Altin 40-98	1998	CV-durum	-	I	Barrigon-yaqui-Enano/2*Tehuacan-60//2B//Longshanks/3/Berkmen-469	(ECP/GR, 2014, GRIS, 2014)
Pavon f76	1976	CV	Rht-D1b	S	Vicam-71//Ciano-67/Siete-Cerros-66/3/Kalyansona/Bluebird	(ECP/GR, 2014, GRIS, 2014)

Table 2.1: List of tetraploid and hexaploid wheat accessions evaluated in glasshouse conditions for two years over three separate experiments under well-watered conditions; release year, type (cultivar, landrace), previously known *Rht*-dwarfing genes, growth habit (W: winter, S: spring, I: intermediate) and known pedigree information. CV: Released cultivar, LV; Landrace variety

Genotype	RM	SRM	DRM	SM	GY	FT	PH	HI 1
Uveyik	8.95^{b}	7.67^{bc}	1.27^{bcde}	70.8^{a}	8.3^{hi}	28.5^{a}	115.5^{a}	0.12^{gh}
Kose	8.1^{bc}	7.25^{cd}	0.85^{efghi}	64.3^{abc}	6.2^{i}	24.5^{ab}	112.2^{ab}	0.09^{h}
Yayla	11.24^{a}	9.1^{ab}	2.14^{a}	71.4^{a}	7.6^{hi}	29.7^{a}	112.7^{ab}	0.10^{h}
Ak 702	11.1^{a}	9.52^{a}	1.57^{abcd}	61.2^{abcd}	12.2^{efg}	25.2^{ab}	104.2^{bcd}	0.19^{efg}
Sivas 111/33	8.14^{bc}	7.05^{cd}	1.08^{cdef}	65.3^{ab}	6.06^{i}	27^{a}	107.7^{abc}	0.09^{h}
Bezostaja 1	3.8^{fgh}	3.55^{gh}	0.25^{ij}	45.4^{efgh}	13.8^{def}	11.5^{fghi}	84.7^{ghi}	0.31^{cd}
Kirkpinar 79	5.88^{de}	5.28^{ef}	0.59^{fghij}	66.4^{ab}	15.6^{cde}	16^{cdef}	82.2^{ghij}	0.23^{de}
Gerek 79	2.84^{ghi}	1.97^{hi}	0.87^{efghi}	42.3^{efgh}	20.6^{ab}	13.2^{efghi}	92.2^{efg}	0.49^{a}
Atay 85	8.26^{b}	7.34^{cd}	0.92^{efgh}	60.3^{abcd}	12.8^{def}	14.7^{defg}	100^{cde}	0.20^{ef}
Kate A1	2.6^{hi}	2.17^{hi}	0.43^{ghij}	34.3^{h}	15.1^{cde}	11.2^{fghi}	78^{ij}	0.44^{ab}
Gun 91	3.8^{fgh}	3.39^{gh}	0.41^{hij}	47.5^{efg}	18.1^{bc}	8.7^{hi}	95.5^{def}	0.38^{bc}
Ikizce 96	1.9^{i}	1.54^{i}	0.36^{hij}	37.5^{gh}	15.3^{cde}	11.7^{fghi}	95.5^{def}	0.42^{ab}
Uzunyayla	8.76^{b}	7.13^{cd}	1.62^{abc}	67.8^{a}	16.3^{cd}	18.7^{cd}	97.2^{def}	0.23^{de}
Mizrak 98	5.26^{ef}	4.6^{efg}	0.59^{fghij}	49.9^{defg}	15.7^{cde}	18^{cde}	89^{fgh}	0.33^{c}
Turkmen	5.21^{ef}	4.25^{efg}	0.95^{defgh}	46.5^{ef}	18.7^{abc}	10.5^{ghi}	88.7^{fgh}	0.42^{ab}
Demir 2000	4.79^{ef}	3.96^{fg}	0.83^{efghi}	52.8^{cde}	22.3^{a}	11.5^{fghi}	111^{ab}	0.42^{ab}
Kiziltan 91	6.39^{cde}	5.33^{ef}	1.06^{cdefg}	54.1^{bcde}	7.7 ^{hi}	20.7^{bc}	87.7^{fghi}	0.14^{fgh}
Cesit 1252	4.65^{efg}	3.96^{fg}	0.69^{efghij}	50.9^{def}	9.05^{ghi}	14^{defgh}	74.5^{j}	0.17^{efgh}
Altin 40-98	7.54^{bcd}	5.72^{de}	1.81^{ab}	52.4^{cde}	11.3^{fgh}	15.2^{defg}	72.5^{j}	0.22^{ef}
Pavon F76	1.38^{i}	0.95^{i}	0.08^{j}	39.4^{fg}	15.5^{cde}	8.5^{i}	80.5^{hij}	0.41^{ab}
LSD 0.05	2.14	1.90	0.30	15.02	4.52	6.55	12.26	0.099
P values	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 2.2 A : Mean values per plant for total root biomass (RM), shallow root weight (SRM), deep root weight (DRM), shoot biomass (SM), grain yield (GY), number of fertile tillers (FT), plant height (PH), and harvest index 1 (HI 1) for landrace and modern wheat genotypes evaluated in 1 m tubes under well-watered conditions in 2013. In each column, means followed by the different letter are significantly different at $p \leq 0.05$ level according to LSD test.

Genotype	RM	SRM	DRM	SM	GY	FT	PH	HI 1
Uveyik	12.58^{a}	9.86^{a}	2.72^{a}	92.67^{abc}	29.97^{cdef}	22.50^{bc}	121.2^{bc}	0.32^{ijk}
Kose	11.47^{ab}	9.17^{ab}	2.30^{ab}	92.36^{abc}	34.66^{ab}	22.75^{bc}	142^{a}	0.37^{efghi}
Yayla	9.00^{bcd}	7.62^{abc}	1.38^{bcdefg}	101.12^{a}	37.65^{ab}	23^{b}	118.2^{bc}	0.36^{fghij}
Ak 702	9.89^{abc}	8.42^{ab}	1.46^{bcdef}	95.46^{abc}	30.10^{cdef}	34.75^{a}	109.5^{cde}	0.31^{jk}
Sivas 111/33	7.47^{cdefg}	5.26^{cdef}	2.21^{abc}	98.02^{ab}	32.74^{bcd}	22.75^{bc}	127^{b}	0.33^{ijk}
Bezostaja 1	3.66^{hi}	2.62^{f}	0.78^{efgh}	49.94^{fgh}	21.05^{gh}	13^{fgh}	99.5^{efg}	0.43^{de}
Kirkpinar 79	8.17^{cd}	7.71^{abc}	0.46^{fgh}	94.41^{abc}	38.66^{ab}	18.25^{bcdef}	83^{hij}	0.40^{efg}
Gerek 79	3.30^{hi}	2.92^{f}	0.38^{gh}	51.03^{fgh}	25.02^{efgh}	15.25^{defgh}	88.7^{fghij}	0.49^{bc}
Atay 85	9.72^{abc}	7.72^{abc}	1.99^{abcd}	95.89^{abc}	40.76^{a}	15.75^{defgh}	93.7^{fghij}	0.42^{de}
Kate A1	3.78^{hi}	3.26^{f}	0.52^{fgh}	45.93^{gh}	26.2^{defgh}	12.75^{gh}	80.2^{j}	0.56^{a}
Gun 91	4.61^{fghi}	3.96^{ef}	0.65^{efgh}	58.72^{fg}	29.29^{cdef}	14.75^{efgh}	92.5^{fghij}	0.49^{bc}
Ikizce 96	2.83^{hi}	2.56^{f}	0.26^{h}	39.99^{h}	21.35^{gh}	12.5^{gh}	97.2^{efg}	0.53^{ab}
Uzunyayla	10.28^{abc}	8.68^{ab}	1.60^{bcde}	102.18^{a}	35.12^{abc}	20^{bcd}	116^{bcd}	0.34^{hijk}
Mizrak 98	7.82^{cdef}	6.32^{bcde}	1.49^{bcdef}	82.22^{cd}	34.14^{abc}	15^{defgh}	93^{fghij}	0.41^{ef}
Turkmen	7.47^{cdefg}	6.37^{bcde}	1.10^{defgh}	76.72^{de}	31.99^{bcde}	18.75^{bcde}	96.7^{efgh}	0.43^{de}
Demir 2000	4.29^{ghi}	3.77^{ef}	0.52^{fgh}	58.69^{fg}	27.7^{cdefg}	12.25^{gh}	102.5^{def}	0.47^{cd}
Kiziltan 91	5.89^{defgh}	5.07^{cdef}	0.99^{defgh}	81.26^{cd}	28.79^{cdef}	19.75^{bcde}	86.5^{ghij}	0.35^{ghijk}
Cesit 1252	8.00^{cde}	6.96^{bcd}	1.03^{defgh}	83.79^{bcd}	28.3^{cdefg}	17.5^{cdefg}	81^{j}	0.33^{ijk}
Altin 40-98	6.04^{defgh}	4.68^{def}	1.36^{bcdefg}	63.09^{ef}	19.03^{h}	11.5^{h}	82.2^{ij}	0.30^{k}
Pavon F76	4.72^{efghi}	3.51^{ef}	1.20^{cdefg}	59.80^{fg}	23.9^{fgh}	15.75^{defgh}	81.2^{j}	0.39^{efgh}
LSD 0.05	3.33	2.83	1.07	14.78	7.71	5.48	13.42	0.056
P values	0.000	0.000	0.0002	0.000	0.000	0.000	0.000	0.000

Table 2.2 B : Mean values per plant for total root biomass (RM), shallow root weight (SRM), deep root weight (DRM), shoot biomass (SM), grain yield (GY), number of fertile tillers (FT), plant height (PH), and harvest index 1 (HI 1) for landrace and modern wheat genotypes evaluated in 1 tubes under well-watered conditions in 2014. In each column, means followed by the different letter are significantly different at p≤0.05 level according to LSD test.

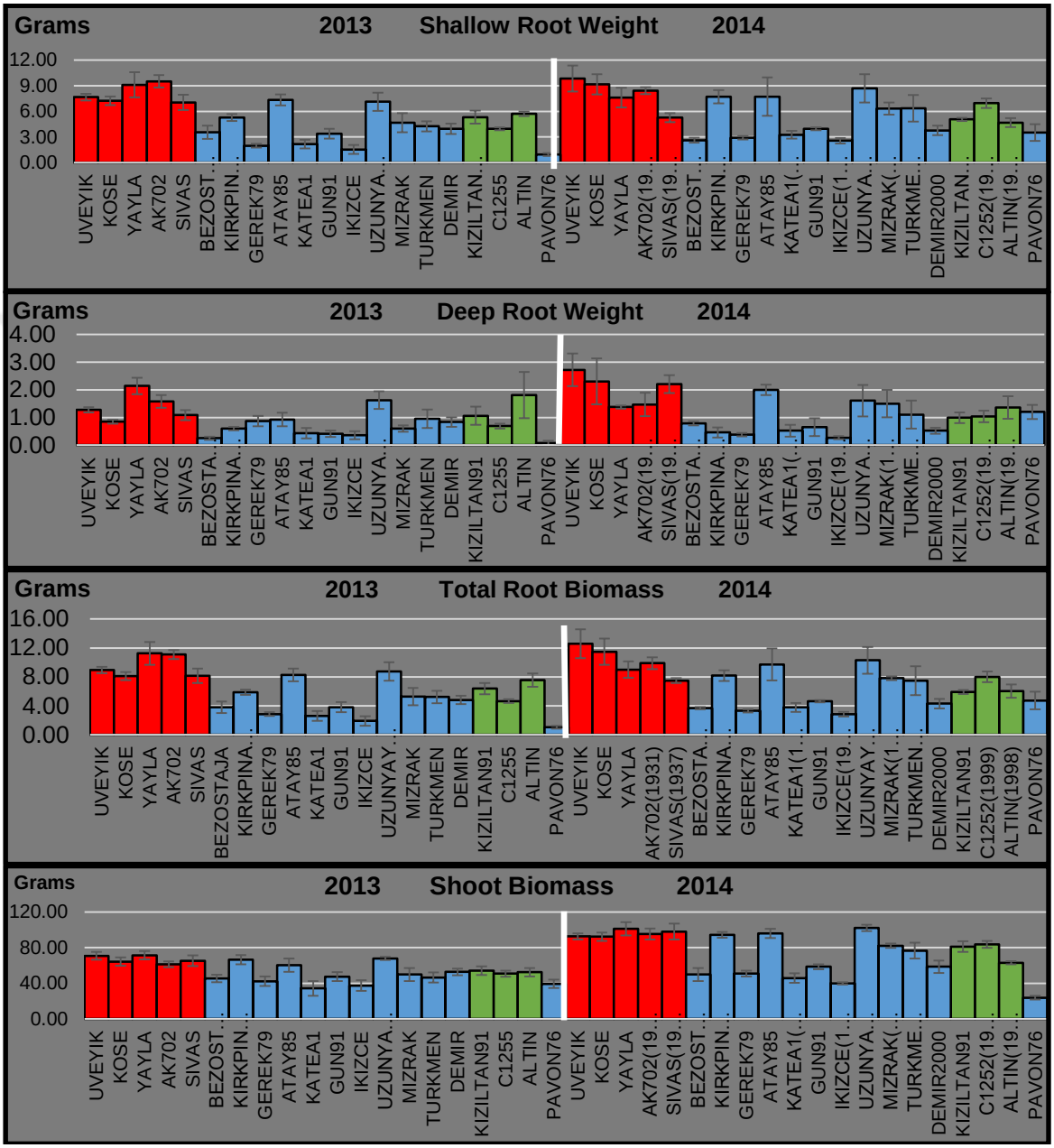

Figure 2.1 Mean values per plant for shallow root weight, deep root weight, total root biomass and shoot biomass of 19 bread and durum wheat genotypes and check cv. 'Pavon-F76' evaluated in glasshouse under well-watered conditions. Red: Landrace; Blue: Bread wheat Cultivar; Green: Durum wheat cultivar.

	GY	SM	DRM	SRM	RM	RS	TM	RL	PH	HI1	DTA	DTM	FT
SM	**0.608**												
DRM	0.263	**0.645**											
SRM	0.196	**0.782**	**0.750**										
RM	0.218	**0.786**	**0.838**	**0.989**									
RS	**0.321**	0.233	**0.629**	**0.759**	**0.765**								
TM	**0.609**	**0.962**	**0.724**	**0.830**	**0.842**	**0.335**							
RL	**0.299**	**0.593**	**0.442**	**0.577**	**0.573**	**0.347**	**0.638**						
PH	0.23	**0.560**	**0.583**	**0.564**	**0.595**	**0.319**	**0.562**	0.282					
HI1	**0.643**	-0.163	**-0.328**	**-0.521**	**-0.501**	**-0.684**	-0.198	-0.231	-0.177				
DTA	**0.441**	**0.822**	**0.687**	**0.789**	**0.799**	**0.429**	**0.89**	**0.713**	0.335	**-0.324**			
DTM	**0.692**	**0.827**	**0.485**	**0.547**	**0.558**	0.046	**0.783**	**0.626**	0.266	0.103	**0.775**		
FT	-0.011	**0.602**	**0.587**	**0.774**	**0.766**	**0.590**	**0.654**	**0.426**	**0.59**	**-0.577**	**0.621**	**0.322**	
NT	-0.174	**0.476**	**0.555**	**0.720**	**0.716**	**0.662**	**0.528**	**0.404**	**0.484**	**-0.673**	**0.532**	0.195	0.938

Table 2.3 Correlation coefficients (r values) between grain yield (GY), shoot biomass (SM), deep root weight (DRM), shallow root weight (SRM), total root biomass (RM), root to shoot ratio (R/S), total plant biomass (TM), root length (RL), plant height (PH), harvest index (HI1), days to anthesis (DTA), days to maturity (DTM), number of fertile tillers (FT), and number of tillers (NT) for landrace and modern wheat genotypes evaluated in 1 tubes under well-watered conditions in 2013 and 2014. Overall means for each accession were used for correlation analysis.
0.30 > r > 0.50 Significant correlation at p <0.05
r>0.50 Highly significant correlation at p <0.01.

	2013		2014	
	DRM (g plant^{-1})	**GY (g plant^{-1})**	**DRM (g plant^{-1})**	**GY (g plant^{-1})**
	L: 1.38^{a}	M: 15.23^{a}	L: 2.01^{a}	L: 33.02^{a}
	M: 0.76^{b}	L: 8.10^{b}	M: 0.95^{b}	M: 28.76^{b}
LSD	0.34	2.34	0.42	3.81
	SRM (g plant^{-1})	**SM (g plant^{-1})**	**SRM (g plant^{-1})**	**SM (g plant^{-1})**
	L: 8.12^{a}	L: 66.65^{a}	L: 8.07^{a}	L: 95.92^{a}
	M: 4.08^{b}	M: 49.88^{b}	M: 5.07^{b}	M: 69.57^{b}
LSD	1.08	6.48	1.34	9.96
	RM (g plant^{-1})	**FT plant^{-1}**	**RM (g plant^{-1})**	**FT plant^{-1}**
	L: 9.50^{a}	L: 27.00^{a}	L: 10.08^{a}	L: 25.15^{a}
	M: 4.85^{b}	M: 13.63^{b}	M: 6.043^{b}	M: 15.51^{b}
LSD	0.12	2.65	1.56	2.48
	HI (%)	**PH (cm)**	**HI (%)**	**PH (cm)**
	M: 0.32^{a}	L: 110.50^{a}	B: 0.42^{a}	L: 123.60^{a}
	L: 0.12^{b}	M: 88.67^{b}	L: 0.34^{b}	M: 91.62^{b}
LSD	0.059	6.10	0.038	6.75

Table 2.4: Mean values per plant for deep root weight (DRM), shallow root weight (SRM), total root biomass (RM), harvest index 1 (HI 1), grain yield (GY), shoot biomass (SM), number of fertile tillers (FT), and plant height (PH) for landraces (L) and modern wheats (M), in 2013 and in 2014. For each trait, means followed by the different letter are significantly different at $p \leq 0.05$ level according to the LSD test.

Genotype	SM (g plant^{-1})	RM (g plant^{-1})	RM 0-30 cm	RM 31-100 cm	RM 101-150 cm	RL cm	GY (g plant^{-1})	FT	PH cm	HI %
Atay 85	74.367^{a}	6.960^{ab}	4.810^{a}	1.730^{abc}	0.420^{ab}	140.00^{ab}	19.367^{abcd}	17.667^{bc}	82.67^{abcd}	0.260^{ef}
Cesit 1252	59.910^{ab}	5.643^{abc}	3.196^{abc}	2.096^{ab}	0.350^{abc}	150.00^{a}	9.190^{e}	17.000^{bcd}	75.33^{cde}	0.152^{g}
Gun 91	36.500^{c}	2.950^{d}	1.943^{c}	1.006^{cd}	0^{c}	93.67^{ab}	12.250^{cde}	13.000^{cd}	78.00^{bcde}	0.332^{bcde}
Kate A1	26.530^{c}	2.443^{d}	1.800^{c}	0.593^{d}	0.050^{bc}	98.33^{ab}	13.183^{bcde}	11.000^{cd}	69.33^{cde}	0.496^{a}
Kirkpinar 79	64.280^{a}	7.540^{ab}	4.733^{ab}	2.306^{a}	0.500^{a}	129.33^{ab}	21.483^{ab}	16.667^{bcd}	75.33^{cde}	0.333^{bcde}
Kiziltan 91	42.067^{bc}	3.670^{cd}	2.260^{c}	1.226^{bcd}	0.183^{abc}	90.00^{b}	12.683^{bcde}	11.000^{cd}	53.67^{e}	0.288^{cdef}
Mizrak 98	58.607^{ab}	5.816^{abc}	3.410^{abc}	2.220^{a}	0.186^{abc}	116.67^{ab}	21.020^{abc}	17.667^{bc}	76.33^{bcde}	0.351^{bcd}
Uzunyayla	78.220^{a}	7.710^{a}	4.846^{a}	2.586^{a}	0.276^{abc}	125.00^{ab}	15.543^{bcde}	22.333^{ab}	91.33^{abc}	0.199^{fg}
Sivas 111/33	71.310^{a}	5.003^{bcd}	2.913^{c}	1.796^{abc}	0.293^{abc}	132.33^{ab}	19.023^{abcd}	26.667^{a}	104.67^{a}	0.266^{def}
Yayla	760427^{a}	6.173^{abc}	3.543^{abc}	2.313^{a}	0.316^{abc}	150.00^{a}	26.157^{a}	26.000^{a}	101.00^{ab}	0.343^{bcde}
LSD	8.955	1.139	1.002	0.556	0.362	31.860	5.529	4.392	7.190	0.0611
P values	0.000	0.000	0.0008	0.0001	0.0056	0.1878	0.0034	0.0001	0.000	0.000

Table 2.5: Mean values per plant for shoot biomass (SM), total root biomass (RM), root weight 0-30 cm (RM 0-30), root weight 31-100 cm (RM 31-100), root weight 101-150 cm (RM 101-150), root length (RL), grain yield (GY), number of fertile tillers (FT), plant height (PH), and harvest index 1 (HI 1) for landrace and modern wheat genotypes evaluated in 1.5 m tubes under well-watered conditions in 2014 . In each column, means followed by the different letter are significantly different at $p \leq 0.05$ level according to LSD test.

	GY	HI	FT	NT	RM 0-30	RM 31-100	RM 101-150	DTM	PH	RM	SM
HI	0.207										
FT	0.631	-0.356									
NT	0.462	-0.493	0.840								
RM 0-30	0.332	-0.455	0.424	0.496							
RM 30-100	0.455	-0.571	0.664	0.744	0.698						
RM 100-150	0.459	-0.339	0.465	0.359	0.583	0.548					
DTM	0.156	-0.560	0.318	0.367	0.360	0.415	0.228				
PH	0.512	-0.372	0.793	0.73	0.274	0.464	0.402	0.256			
RM	0.431	-0.537	0.568	0.631	0.951	0.875	0.685	0.407	0.392		
SM	0.665	-0.55	0.824	0.783	0.665	0.825	0.647	0.428	0.716	0.8004	
RL	0.460	-0.333	0.584	0.415	0.414	0.520	0.552	0.263	0.392	0.518	0.646

Table 2.6: Correlation coefficients(r values) of the accession means for; grain yield (GY), harvest index 1 (HI 1), number of fertile tillers (FT), number of tillers (NT), root biomass 0-30 cm (RM 0-30), root weight 31-100 cm (RM 31-100), root weight 101-150 cm (RM 101-150), days to maturity (DTM), plant height (PH), total root biomass (RM), and shoot biomass (SM) for landrace and modern wheat genotypes evaluated in 1.5 m tubes under well-watered conditions in 2014.
0.30 > r > 0.50 significant correlation at $p < 0.05$
r>0.50 highly significant correlation at $p < 0.01$.

Trait	Growth Habit		Plant Height	
Shoot Biomass	I	68.698[a]	Tall	78.858[a]
	S	65.993[ab]	Mid-height	58.102[b]
	W	60.230[b]	Semi-Dwarf	57.962[b]
Shallow Root weight	I	6.0893[a]	Tall	7.5162[a]
	W	4.9804[b]	Semi-Dwarf	4.4828[b]
	S	4.9448[b]	Mid-height	4.0155[b]
Deep Root weight	I	1.1458[a]	Tall	1.5624[a]
	S	1.1284[a]	Mid-height	0.8599[b]
	W	0.9375[a]	Semi-Dwarf	0.7894[b]
Total Root Biomass	I	7.2285[a]	Tall	9.0762[a]
	S	6.0649[ab]	Semi-Dwarf	5.2942[b]
	W	5.9369[b]	Mid-height	4.8600[b]
Root / shoot ratio	I	0.1060[a]	Tall	0.1175[a]
	W	0.0965[ab]	Semi-Dwarf	0.0903[b]
	S	0.0865[b]	Mid-height	0.0812[b]
Harvest Index	W	0.3873[a]	Mid-height	0.3919[a]
	S	0.3184[b]	Semi-Dwarf	0.3563[a]
	I	0.3074[b]	Tall	0.2649[b]
Days to Anthesis	I	76.254[a]	Tall	78.482[a]
	S	66.587[b]	Semi-Dwarf	67.438[b]
	W	63.924[b]	Mid-height	60.845[b]

Table 2.7: Mean values for growth habit (winter, intermediate, and spring) and plant height (tall, mid-height, and semi-dwarf) groups per plant for 2013 and 2014 combined data. I; intermediate wheat, W; winter wheat, S; spring wheat. Tall; >101 cm, mid; 91 cm-100 cm, semi-dwarf; <90 cm height. In each column, means followed by the different letter are significantly different at $p \leq 0.05$ level according to LSD test.

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CHAPTER 3: Root and Shoot Traits in Parental, Early and Late Generation Green Revolution Wheats Evaluated Under Well-Watered Conditions

Abstract

Introduction of stem-dwarfing genes had a major impact on wheat breeding and production. It is estimated that 70 to 90% of modern wheats carry one or more such genes. These genes were the cornerstone of the Green Revolution. They solved the lodging problem by reducing stem height thus permitting a marked increase in mineral fertilizer use. They also reduced biomass accumulation and allowed more carbon assimilates to be stored as grain. With heavy fertilization and irrigation plants had little use for an extensive and expensive root system for uptake of water and nutrients. However, with climate change and limited water and nutrient sources there is a need to remodel modern wheats and this requires genetic variation. In this study we evaluated nine accessions of wheat representing gene pools of parental, early-tall and late-semi-dwarf Green Revolution wheats for root and shoot biomass and grain yield under well-watered conditions in a glasshouse. Significant genotypic variation was found for total root biomass and its distribution in the soil profile as well as for plant height and days to anthesis. There were no consistent differences over two years for grain yield, harvest index 1, deep root weight and the number of tillers between the early and late generation Green Revolution wheats. Strong positive correlations between above and below

ground biomasses and negative correlations between harvest index and root biomass were observed. Modern wheats had reduced root systems relative to their predecessors. This may be the effect of the dwarfing genes, or an indirect effect of the selection process in modern plant breeding but it is clear that the wheat root system may have become smaller within the last century.

Introduction

The Green Revolution wheat breeding program was established in the mid-twentieth century at what is now the International Maize and Wheat Improvement Center (CIMMYT) in Mexico, to increase grain production for Mexico and other developing countries (Borojevic and Borojevic, 2005, Borlaug, 2007). The main aims of this program were to improve grain yield and disease resistance (Borlaug, 1968). This extensive breeding project concentrated on disease resistance at first, but with increasing grain yields, lodging became a major problem. To solve the lodging problem, short and stiff-stemmed wheats were needed.

Semi-dwarf and dwarf wheats have been grown in Korea and Japan since the 3rd century (Borojevic and Borojevic, 2005). Nazareno Strampelli of Italy introduced the dwarfing genes (*Rht* for Reduced height) to Europe in the early twentieth century. Under the Italian national wheat breeding program (so called Wheat Battle), Strampelli crossed Japanese variety “Akakomugi” (source of *Rht8* and *Ppd-D1* genes) with his Wilhelmina Tare x Rite hybrid in order to introduce short straw and earliness. This eventually lead to the release of many successful cultivars including “Mentana” by 1918 (Borojevic and Borojevic, 2005, Salvi, et al., 2013). Cv. Norin 10, another dwarf Japanese wheat cultivar (source of *Rht-B1* and *Rht-D1* genes) was first introduced to the US after World War II where it was crossed with local wheat cv. Brevor 51 by Orville Vogel at Washington State University (Waines and Ehdaie, 2007). Cv. Gaines resulting from this cross successfully dominated wheat production in the Pacific Northwest in the late 1960s

(Borojevic and Borojevic, 2005). Cv. Gaines, Norin 10, and Brevor 51 were introduced to the CIMMYT breeding program by Norman Borlaug to reduce plant height and tillering and to increase grain yield by changing the harvest index. Ultimately, reduction of plant height solved the lodging problem and currently 70 to 90 % of wheats worldwide carry dwarfing genes (Borlaug, 1968, Waines & Ehdaie, 2007).

Dwarfing genes *Rht-B1 (*formerly *Rht1)* and *Rht-D1* (formerly *Rht2)* are gibberellin (GA)-insensitive, whereas some others, such as *Rht8,* are GA-sensitive, that is, responsive to GA application. Each of the *Rht* genes has multiple alleles, and they are coded with 'a' recessive, and 'b, c; d, e etc.' dominant alleles. Li, et al. (2013) discovered novel allelic variation for *Rht-B1* genes, as well as new alleles for tall phenotypes in addition to the common *Rht-B1a (*formerly *rht1)* allele. Phenotypic effects of *Rht* genes / alleles are significantly different from one another but all are a part of the DELLA gene family that represses GA-responsive growth, which limits the inter-nodal growth (Hedden, 2003, Wojciechowski, et al., 2009).

Plants can respond to environmental fluctuations by changing their root length, density, architecture and root to shoot ratio depending on their genetic potential and background. These changes can give plants a better chance to survive under drought and nutrition stress conditions (Manske and Vlek, 2002). Hurd (1964) and Hurd (1974) evaluated root systems of wheat genotypes in response to environmental changes. He reported distinct patterns of root growth in different moisture conditions, and extensive rooting ability in drought tolerant

genotypes. Manschadi, et al. (2006) demonstrated that drought tolerant cv. Seri M82 responded to drought by extracting more water from subsoil layers with long seminal roots. Reynolds, et al. (2007) reported a 15% biomass increase with 8.4 mm of additional extracted water. Ehdaie, et al. (2010) reported that deep rooting improved N, P, K uptake rates, and reduced N leaching. All these studies imply that an extensive root system well distributed in the soil profile is associated with drought tolerance and maintenance of acceptable plant water status. Optimum uptake of water and nutrients are crucial to increase grain yield (Bengough, et al., 2011).

Previous studies demonstrated that landraces and pre Green Revolution wheats respond to drought better than modern wheats (Ehdaie, et al., 1988, Reynolds, et al., 2007, Trethowan and Mujeeb-Kazi, 2008) by extracting more water from deep soil layers. A reason for this may be the conditions in which Green Revolution wheats were selected. They were selected under high fertilizer and irrigation conditions, with emphasis on high grain yield and little concern for water and nutrient-use efficiencies. The characteristics of the root system were not among the selection criteria, and under optimum growing conditions genotypes more susceptible to stress would not even be identified. It is not implausible that selection for high yields under optimum conditions worked against any (many) characteristics associated with drought tolerance. An extensive root system requires additional resources which are diverted from grain. As a consequence,

this direction of selection reduced root system size in wheat (Waines and Ehdaie, 2007, Pingali, 2012).

Interactions between the *Rht* genes and the wheat plant root system were reported many times under variable conditions and in different backgrounds. MacKey (1973), Siddique, et al. (1990) and Waines and Ehdaie (2007) reported that the late generation Green Revolution wheats with semi-dwarfing genes had less root biomass than the tall early-generation wheats and landraces. On the other hand, Lupton, et al. (1974), Ehdaie and Waines (1994), Ehdaie and Waines (1996) with isogenic lines in pots, Miralles, et al. (1997), Bush and Evans (1988) and Wojciechowski, et al. (2009) reported quite the opposite results. Wojciechowski, et al. (2009) demonstrated that lines isogenic for the *Rht* genes gave contrasting phenotypes for root traits in gel and soil media at the seedling stage. Lines with semi-dwarfing genes had 40% longer roots in gel media but 24-33 % shorter roots in soil. These findings demonstrate a need for additional research on root / shoot biomass traits and their interactions with different *Rht* genes / alleles of Green Revolution wheats.

The main objectives of this study were; to determine the effect of pre and post-Green Revolution breeding on root, shoot and grain yield traits, to quantify the genetic variation for root traits within and between parental, early and late generation wheats and to determine the relationship between root traits (shallow and deep root weight and total root biomass) and yield components and shoot traits. Additionally, other objectives of this study were to investigate root system

characteristics in sand-tube culture instead of sand-pot culture that was used by Ehdaie and Waines (1994, 1996 and 2007). In contrast to pots, tubes allow the observer to divide the root system into shallow and deep root weight, whereas pot culture does not. The total soil surface is much greater in the sand-tube system than in the sand-pot system. Also, some of the results reported in Waines and Ehdaie (2007) had only one good year of observation. Therefore, the same or similar accessions that were previously tested in pot culture by Ehdaie and Waines (1996 and 2007) were used for comparison and to investigate the role of experimental design (tube vs. pot) on the root system architecture and distribution of roots (deep and shallow) in soil.

Materials and Methods

Nine accessions with recessive-tall or dominant-semi-dwarf *Rht* alleles were selected from CIMMYT germplasm to evaluate the effects of *Rht* alleles on root, shoot and grain yield traits at maturity in PVC sand tubes in a glass house. Three common parental lines: landrace 'Marroqui', cvs. Mentana, and Gabo; two early generation Green Revolution wheats with recessive *rht-B1a* and *rht8a* alleles, cvs. Lerma 52, and Nainari 60; and four cultivars with dominant *Rht* alleles released after 1970: 'Pavon F76' 'Bacanora 88', 'Rayon 89' and 'Pastor', were selected to survey root system traits (Table 3.1). The early generation Green Revolution CIMMYT cv. Nainari 60 was included in the germplasm set in order to

compare this cultivar's response to sand-tube culture and compare it with pot culture results of Ehdaie and Waines (1996).

Seeds of similar size were sterilized with 1% sodium hypochlorite (NaCIO) solution, rinsed with distilled water and germinated in Petri dishes for five days. Seedlings were transplanted into sand tubes on January 2012 and February 2013 and grown to maturity as described by Ehdaie and Waines (2006) and Sharma, et al. (2011). PVC tubes of 80 cm long and 10 cm in diameter were filled with 8.5 kg #30 grade silica sand in plastic bags. Plants were watered daily with half-strength Hoagland's nutrient solution. At maturity spikes and shoots were harvested separately and dried in a hot air oven at 65°C for 72 hours. The plastic sleeves were taken out of the PVC tubes and cut lengthwise, the roots were washed out and their length was measured. Total roots were divided into two fractions: deep roots (below 30 cm soil depth) and shallow roots (0 to 30 cm soil depth). The two fractions were air dried in the glasshouse and transferred to a hot air oven at 65°C for 72 hours. For all plants notes were taken on days from plant emergence to booting (DTB), to heading (DTH), to anthesis (DTA), and to maturity (DTM); at harvest plant height (PH), the number of tillers (ST) and the number of fertile tillers (FT), were noted for each plant. Shoot biomass (SM), the shallow root weight (SRM), and the deep root weight (DRM), total root biomass (RM), grain yield (GY), harvest index 1 (ratio of grain yield to shoot biomass, expressed in %) (HI), harvest index 2 (ratio of grain yield to total biomass, expressed in %) (HI2) and the ratio of root to shoot biomass (R / S), plant^{-1} was calculated for each genotype.

Statistical Analysis

Analysis of variance (ANOVA) was performed to determine genotypic variation for each trait in each experiment. Statistical analyses were performed using the Statistix software (Analytical Software; Tallahassee, FL, USA). Normality of distribution was tested by normal probability plots. Separation of means using least significant difference (LSD) (Steel, et al., 1997) was performed based on ANOVA results. Relationships between pairs of traits for accession means were determined using correlation coefficients. Contrasts between parental, early, and late generation wheats were evaluated. Combined ANOVA was performed across years to measure the main effect of year and genotype and the magnitude of genotype × year interaction.

Results

Significant genotypic variation was observed consistently over two years for the total root biomass, shallow root weight, days to anthesis and plant height ($p<0.05$). Additionally, significant variation was observed for grain yield, harvest index, and root length, in 2012 and for days to maturity in 2013. Genotype × year interactions were significant for more than half of the traits so data for each year were evaluated separately.

Mean values for shoot biomass were significant only at ($p<0.10$) in both years. Genotypes with the highest mean values for shoot biomass were ‘Mentana’ and ‘Pastor’ in 2012 and ‘Mentana’ and ‘Marroqui’ in 2013 with 25.90 g and 25.52 g, and 50.52 g, and 46.66 g plant^{-1}, respectively. On the other hand, the smallest shoot biomasses belonged to ‘Marroqui’ and ‘Bacanora 88’ in 2012 and ‘Bacanora 88’ and ‘Rayon 89’ in 2013 with 19.63 g, 19.51 g, 35.24 g, and 28.96 g plant^{-1}, respectively (Table 3.2).

Genotypes with the largest total root biomass were ‘Mentana’ and ‘Pastor; with 4.01 g and 3.46 g plant^{-1} in 2012 and Mentana’ and ‘Marroqui’ with 6.93 g, 4.52 g plant^{-1} in 2013, respectively. The smallest root biomasses belonged to ‘Lerma 52’ and ‘Rayon 89’ with 2.68 g and 2.57 g plant^{-1} in 2012 and ‘Bacanora’ and ‘Rayon 89’ with 2.78 g and 1.84 g, plant^{-1} in 2013, respectively. Both genotypes with large total root biomass are tall with *rht-B1a* and *rht8a* alleles, whereas small total root biomasses belonged to semi-dwarf cultivars with *Rht-B1b* and *Rht-D1b* alleles except ‘Lerma 52’. Genotypes with the largest deep root weights were ‘Mentana’ and ‘Gabo with 1.49 g and 1.11 g plant^{-1} in 2012 and ‘Nainari 60’ and ‘Marroqui’ with 0.98 g, and 0.91 g plant^{-1} in 2013 respectively. Similarly, genotypes with the largest shallow root weights were ‘Mentana’ and ‘Pastor’ in 2012 and ‘Mentana’ and ‘Marroqui’ in 2013 with 2.52 g, 2.44 g, 6.06 g, and 3.61 g plant^{-1}, respectively (Table 3.2).

Statistically significant differences for grain yield were observed in 2012 but not in 2013. However, cvs. Mentana and Pastor had the highest grain yield in both

years, in addition to ‘Pavon F76’ which had high grain yield in 2013. Differences in harvest index 1 (grain yield / shot biomass) were not significant but harvest index 2 (grain yield / total biomass) were higher in late generation cultivars (Table 3.2).

Grain yield was positively correlated with shallow root weight (0.41), root biomass (0.37), shoot biomass (0.70), and number of fertile tillers (0.79). Other significant correlations were between days to anthesis and root length (0.47), days to anthesis and root biomass (0.76); shoot biomass and deep root weight (0.81), and shoot biomass and total root biomass (0.82). We observed negative correlations between harvest index 1 and deep root weight (-0.69), harvest index 1 and total root biomass (-0.58), harvest index 1 and shoot biomass (-0.42) and harvest index 1 and plant height (-0.54) (Table 3.5).

Root and Shoot Characteristics of Tall and Semi-Dwarf Wheats

We examined differences between mean values of the five tall and four semi-dwarf accessions tested. Tall accessions had significantly higher ($p<0.05$) mean values for plant height, for the total root biomass and shallow root weight. The mean plant height was 100.9 cm in tall accessions and 81.78 cm in semi-dwarfs. The mean value for the total root biomass was 4.15 g plant^{-1} in tall wheats, and 3.32 g plant^{-1} in semi-dwarf wheats. Similarly, shallow root weight was 3.26 g plant^{-1} in tall wheats and 2.50 g plant^{-1} in semi-dwarfs. There was no significant difference between tall and semi-dwarf wheats for shoot biomass, deep root

weight, root / shoot ratio, grain yield, harvest index 1 and days to anthesis (Table 3.3).

Root and Shoot Characteristics of Parental, Early and Late Generation Wheats

Analysis of mean differences between the parental-tall, early-tall and late-semi-dwarf wheats revealed significant differences. Total root biomass was largest in the parental lines (4.16 g plant^{-1}) and the smallest in the late generation wheats (2.98 g plant^{-1}). The same rank order was observed for deep and shallow root weights, with the parental genotypes being the largest and the late generation genotypes being the smallest. Plant height was 99 cm for the parents, 92.1 cm for the early and 74 cm for the late generation wheats. We did not observe any differences between the three groups for the shoot biomass, root / shoot ratio, grain yield, harvest index 1, and days to anthesis (Table 3.4).

Discussion

This study shows significant variation for root biomass among the accessions tested, and its reduction between parental and early generation wheats with *rht8a, rht-B1a* alleles at the standard dwarfing loci on the one hand and late generation wheats with *Rht-B1b* and *Rht-D1b* alleles on the other. Similarly, shoot biomass and total plant biomass was the highest in the parental accessions and other accessions with highest root biomass and lowest in the accessions with the lowest root biomass. However, some above ground traits did not show consistent differences over the years. Differences in grain yield, harvest index 1 and deep root weight were significant only in 2012, and number of tillers only in 2013. Possible reasons for this are that the number of genotypes and plants per genotype evaluated was limited and that degrees of freedom for any given trait were low, producing large standard deviations. The examined groups of lines were heterogeneous, with different pedigrees and different composition at the *Rht* loci, with a relatively small number of accessions within each group. However, these types of experiments are technically taxing which precludes testing large numbers of lines at the same time. On the other hand, as this study demonstrates, seasonal variation makes comparisons among different experiments risky. However, even though the results did not indicate consistent differences for the traits measured, significant differences for shoot biomass, total root biomass and shallow root weight were observed over the years. It seems that genotypic variation and possibly an extensive plastic response to minor differences in growing conditions

yielded statistically significant differences in root traits. Therefore, even with relatively low differences in grain yield and harvest index, we still observed wider and significant differences in root traits.

In this study, differences in root systems cannot be explained solely by phenology or the number of tillers. We observed positive correlations between the numbers of fertile tillers and the total root biomass (0.71) and grain yield (0.79), as well as between total root biomass and grain yield (0.37). In this sense, cv. ‘Mentana’ and ‘Pastor with the largest root biomasses had some of the highest grain yields in both years. So, under our experimental conditions there was no grain yield penalty in growing larger roots. Correlation analysis and (Figure 3.2) clearly show a symmetrical and positive pattern between grain yield, biomass traits and days to anthesis with most accessions, which was consistent over years.

A question this experiment raises is, how did accessions with large total biomass accumulation also have high grain yield and hence, almost the same harvest index as the semidwarf accessions? Ninou, et al. (2014) and Sukumaran, et al. (2015) suggest that old cultivars and landraces perform better in reduced inter plot competition, whereas modern wheats are less sensitive to changes in density. Landraces and old cultivars respond to higher input and reduced planting densities with increased total biomass and grain yield. Experimental conditions with each plant in separate tubes, amply watered and fertilized, were similar to a reduced density (noncompetitive) planting. The result was similar to those of Ninou et al (2014) and Sukumaran et al (2015): parental lines and the early - tall wheats

produced similar or even higher grain yield levels (eg; Mentana) as the late generation wheats, along with extensive root biomass. In this sense total biomass production, including shoot and root, of the early wheats was higher. The only exception was ‘Pavon F76’, which had the highest total plant biomass in 2013 and this higher biomass translated into a higher grain yield when compared with lower total biomass and yield in 2012. Similarly, increased grain yield with increased root biomass was observed in late generation cv. Pastor which had high total root biomass and grain yield in 2012 and 2013. ‘Pavon F76’ is known for its capability to respond to environmental fluctuations by significantly changing its root biomass (Ehdaie, et al., 2012). However, even with that plastic response, the total root biomass of ‘Pavon F76’ was much lower than that of landraces or early accessions. This observation suggests that plasticity alone may not be enough to lead to the formation of an extensive root system. Proper genetic make-up for a deep and dense root system needs to be introduced in to modern wheats.

All accessions tested consistently produced the same pattern: larger shoot biomass was correlated with higher grain yield and with larger root biomass, and larger root biomass is associated with a higher number of fertile tillers. The experiment, however, cannot answer the question as to which character drives the other: is it that a larger root permits more growth of the above ground biomass, and these two generate higher grain biomass, or is it that higher above ground biomass permits more root growth. In other words, we do not know which is the dependent and which the independent variable. One would think that under the

experimental conditions used here, with ample watering and fertilization, and no competition, any investment in an extensive root system would be wasteful and its reduction could have led to even higher grain yield. But this also raises the question: can the root size be extended without a penalty in grain yield or harvest index? The experiment clearly shows that extending the period of vegetative growth (days to anthesis) by one to two weeks, allows plants to develop much deeper and denser root systems. Photosynthetic potential is high (heading – anthesis) and grain filling is not yet consuming carbon assimilates, leaving additional carbon assimilates to be invested in an extensive root and shoot system. In this study, those additional 10.25 days in 2012 and 21 days in 2013 between the earliest and the latest plants to reach anthesis significantly increased biomass accumulation and grain yield. The number of days to anthesis was positively correlated with shallow root weight (0.76) and deep root weight (0.58) as well as the total root biomass (0.74) (Table 3.5). Only a week longer vegetative growth may allow higher root and shoot biomass accumulation and it may promote grain yield. This effect was evident both in tall cv. Mentana and in semi-semi-dwarf cv. Pastor. Here again the maximum range for days to anthesis was 21 days, and it was enough for significant differences in biomass accumulation. However, the solution to the yield issue in wheat by extending its vegetative growth by a week or two is not practical, especially under the terminal stress conditions. While such an extension increases root depth, there may insufficient water left, even in deeper soil layers, to sustain the entire grain filling period, and grain quality may suffer.

Another way to select for certain characteristics of the root system is to find novel alleles such as for narrow root angle and deep root biomass, present in old varieties or landraces as a form of adaptation to water stress. Root angle affects the distribution of roots in the soil profile, with wide angles generating a shallow root system, close to the soil surface; a narrow root angle generates a deep root system that reaches into deep soil levels. The former may be a form of adaptation to irrigated conditions; the latter to drought. In a parallel study we observed up to an eight-fold difference in total root biomass between modern wheats and landraces (unpublished data). Clear segregation for root angles was observed by C. Hohn in wheat mapping populations (pers. comm.). Significant differences in seminal root angle was reported in barley landraces and wild accessions by Bengough, et al. (2004) and Hargreaves, et al. (2009), while Manschadi, et al. (2007) reported wide seminal root angles for drought susceptible cultivars. It is beyond dispute that observed differences in root biomass and previous reports of root angle variation are consequences of differences in genetic architectures of old and modern wheats. Similarly, Sukumaran, et al. (2015) suggest that, extensive response of old varieties to planting density must be a genetic factor that can be amenable to manipulation. It seems like some genetic adaptation that was lost through modern breeding is causing the differences in root biomass and architecture between modern and old wheats. In order to locate and re-gain these characteristics, germplasm collections needs to be evaluated systematically for root characteristics.

The Effect of Plant Height

In an effort to dissect the effects of different alleles of the *Rht* genes on root biomass traits, tall and semi-dwarf accessions were grouped and their mean values compared. Accessions with recessive alleles of *rht-B1a* and *rht8a* had larger mean root biomasses when compared with accessions carrying dominant alleles of *Rht-B1b* and *Rht8b*. Interestingly, 'Mentana' with its *rht8a* allele, had larger roots than 'Lerma 52' with *rht-B1a*. However, it is difficult to clearly quantify the effects of *rht-B1a* and *rht8a* genes without sets of isogenic lines. The difference between 'Mentana' and 'Lerma 52' may be a consequence of several other genetic factors or genotype x environment interactions. Be it as it may, this study shows a clear distinction between *rht-B1a* and *rht-8a* genes for root biomass. These two genes (*Rht-B1* and *Rht8*) have completely different responses to exogenous GA application, perhaps they may have different responses to environmental factors as well. It is also possible that differences may be caused from other factors such as additive or epistatic interactions.

The Role of Breeding

We evaluated mean differences between parental, early and late generation Green Revolution wheats for root / shoot and yield traits. The trend was for reduced rooting as expressed in total biomass, as well as its components, deep and shallow root weights (Figure 3.1) with the highest mean values for total root biomass

observed in the parental accessions (Table 3.4). A similar effect was observed by Siddique, et al. (1990) and Waines and Ehdaie (2007), with larger root systems in landraces and pre-Green Revolution accessions than in modern Green Revolution wheats. Given the conditions in a modern breeding program such as that of CIMMYT, with adequate amounts of water and fertilizer generally available in the shallow soil layers. The root system tends to adapt maximum uptake of available resources from the easiest pathway. Therefore, a shallow root system with reduced biomass and soil coverage is an expected outcome, especially under selection pressure for high harvest index. If conditions change and resources become scarce, many modern wheats perform poorly even if they are capable of outstanding yields under optimum conditions. This must be a consequence of selection methods and management practices tailored for high input – high grain yield conditions.

The effects of only *Rht* genes on the wheat root system were reported with many other conflicting results (for review see Gale and Youssefian (1985) Youssefian, et al. (1992) suggested that the primary effect of *Rht* genes is to reduce stem elongation and vegetative biomass accumulation. Similarly Richards (1992) reported that every 10 cm reduction in plant height causes 4.4% reduction in plant biomass. Here we would like to extend this argument beyond the *Rht* genes themselves. A multigenic adaptation through selection pressure and environmental conditions must be the reason for dramatic changes in root and shoot structure. It seems that *Rht* genes have a significant role in root system size,

perhaps because of the changes in the shoot system. In addition, the genetic background of the accession seems important. Cv. Pastor a semi-dwarf with *Rht-B1b* allele only 77 cm tall has extensive above and below ground biomass under well-watered conditions. This cv. behaved quite differently from other semi-dwarfs tested. 'Pastor' had higher above and below ground biomass and grain yield consistently over two years when compared with other semi-dwarfs. Even though 'Pavon F76' did have higher mean values in 2013, it was not consistent two years, and probably had a more of a plastic response. The genetic background of 'Pastor' in addition to *Rht-B1b* gene allowed higher biomass accumulation under well-watered conditions, in addition to high grain yield. Similar characteristics were observed with tall accessions in this experiment and with landraces in other experiments (unpublished data), both of which tend to produce a higher biomass under well-watered conditions when compared with a modern wheat.

The effect of sand tube culture

Ehdaie and Waines (1996) had earlier found in sand-pot culture that the semi-dwarf *Rht* alleles had larger root biomass than the tall *rht* allele in an isogenic series. This was confirmed in an isogenic series with the Brazilian cv. Maringa, where again semi-dwarf *Rht* alleles had larger root biomass than the tall *rht* allele isogenic line. However, in sand-tube experiment we found completely opposite results. Semi-dwarf accessions with *Rht-B1b and Rht-D1b* had smaller root biomass than tall accessions with *rht-B1a* and *rht8a* alleles. First of all, the tube

system gives plant roots a greater growth potential and less penetration problem for root tips. It seems that cv. Nainari 60, which is an early generation tall CIMMYT accession and other tall accessions responded to the tube system with a larger root growth. There may be many reasons for this differential response; first, old tall accessions show a greater increase in biomass and grain yield accumulation when competition is reduced (Ninou et al 2007). The tube system, with greater soil surface compared to small pots, might have given this change. Additionally, each cv. has a unique pedigree, we observed pedigree specific responses in accessions with the same or similar *Rht* contribution. Therefore, conclusions drawn from near-isogenic lines may have pedigree specific effects as well as *Rht* genes. We observed this effect in cv. Pastor with *Rht-B1b*, which had greater grain yield and root biomass than other three accessions with semi-dwarf *Rht genes.* Genotype specific interactions needs to be included when a plant trait is evaluated even in near isogenic studies. But with our limited knowledge on multi gene interactions pure evaluation of a gene / allele seems hard and inconclusive at this time.

Conclusion

Here we report a survey of a nine wheats representing a temporal cross section of the Green Revolution wheat germplasm. We screened tall, parental lines and compared them to the early-tall and late-semi-dwarf cultivars from CIMMYT. Our observations suggest that in the process of breeding the size of the root and shoot systems was reduced. This reduction was a consequence of the introduction of the *Rht* genes and strong selection pressure for high harvest index. This

produced spectacular results under high water – high fertilization conditions. Unfortunately, with harsher growing conditions modern wheats with small above and below ground biomass have limited adaptability. This may call for a return to old cultivars and landraces for the incorporation of genes for extensive rooting and for better water acquisition as well as large biomass production.

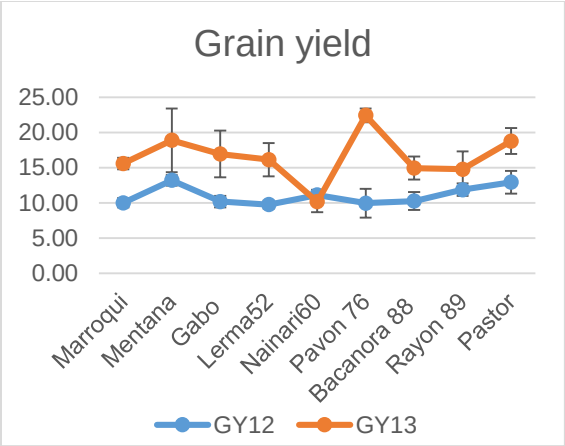
Cultivar	Release Date	Rht Genes	Pedigree	TYPE	Reference
Marroqui “Florence Aurore”	-	*-rht?*	Lv-Morocco	L.-Tall	(GRIS, 2014)
Mentana	1913	*rht8a*	Rieti/Wilhelmina(sel.21)//Akakomugi	Cv.-Tall	(GRIS, 2014)
Gabo	1942	*Rht8b*	Bobin(s)/(tr.dr)Gaza//(s)Bobingular/(tr.dr)Gaza//Gular bobin*2/Gaza Bobin-w-39//Bobin-w-39/Gaza	Cv.-SD	(GRIS, 2014)
Lerma 52	1952	*rht-B1a*	Mentana*3/Kenya-rf-324	Cv.-Tall	(GRIS, 2014)
Nainari 60	1960	*rht8a*	Supremo/Mentana//Gabo-55/3/Thatcher/Queretaro//Kentana/5/Gabo-55 Supremo/Mentana//Gabokenya/3/Thatcher/Queretaro//Kenya/Mentana/4/Gabo-Kenyathatcher/Queretaro/Gabo/3/Thatcher/Queretaro//Kenya/Mentana/4/Gabo	Cv.-Tall	(GRIS, 2014)
Pavon F76	1976	*RhtD1b*	Vicam-71//Ciano-67/Siete-Cerros-66/3/Kalyansona/Bluebird	Cv.-SD	(GRIS, 2014)
Bacanora 88	1988	*RhtB1b*	Jupateco-73/(SIB)Bluejay//Ures-81	Cv.-SD	(GRIS, 2014)
Rayon 89	1989	*RhtB1b*	Ures-81*2/Parula	Cv.-SD	(GRIS, 2014)
Pastor	1993	*RhtB1b*	Pfau/Seri-82(CID-93300//Bobwhite	Cv.-SD	(GRIS, 2014)

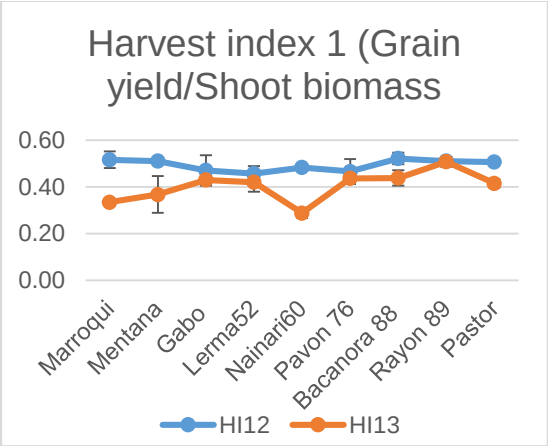
Table 3.1: List of bread wheat accessions evaluated in a glasshouse for two years under well-watered conditions; release year, previously known dwarfing genes (*Rht*), known pedigree information and type (Cv., Cultivar, L., Landrace, SD., semi-dwarf).

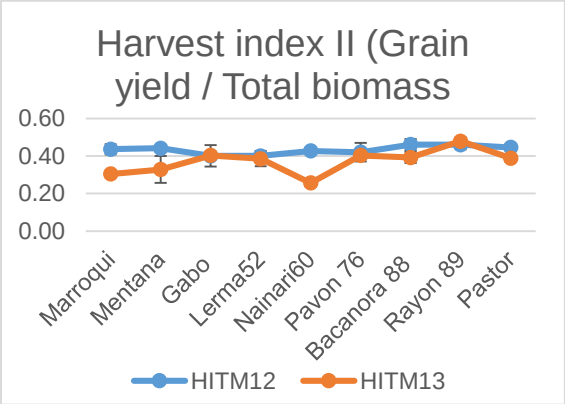
Year	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Genotype	GY		HI1		HI2		DRM		SRM		RM		SM	
Marroqui	10.01	15.59	0.52	0.33	0.44	0.30	0.98	**0.91**	2.30	3.61	3.28	4.52	19.63	46.66
Mentana	**13.22**	**18.89**	0.51	0.37	0.44	0.33	**1.49**	**0.87**	**2.52**	**6.06**	**4.01**	**6.93**	**25.90**	**50.52**
Gabo	10.19	16.95	0.47	0.43	0.40	0.40	1.11	0.54	2.17	2.44	3.27	2.98	22.11	39.05
Lerma52	9.75	16.13	0.46	0.42	0.40	0.39	0.91	0.41	1.77	3.68	2.68	4.09	21.63	37.76
Nainari60	11.12	10.16	0.48	0.29	0.43	0.26	1.00	**0.98**	2.03	3.40	3.03	4.38	22.96	35.23
Pavon 76	9.96	22.43	0.47	0.44	0.42	0.40	0.68	0.70	2.33	3.15	3.00	3.85	20.66	51.66
Bacanora 88	10.25	14.94	0.52	0.44	**0.46**	**0.39**	0.70	0.28	2.01	2.50	2.71	2.78	19.51	35.24
Rayon 89	11.88	14.77	0.51	0.51	**0.46**	**0.48**	0.72	0.26	1.86	1.58	2.57	1.84	23.18	28.96
Pastor	**12.94**	**18.78**	0.51	0.42	**0.45**	**0.39**	**1.02**	**0.87**	**2.44**	**2.80**	**3.46**	**3.67**	**25.52**	**44.58**

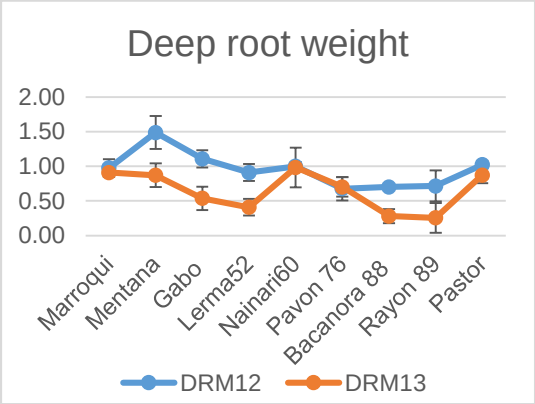
Year	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Genotype	TM		DTA		DTM		PH		FT		NT		RL	
Marroqui	22.91	51.18	62.25	48.00	111.00	95.50	**106.50**	**101.00**	6.50	19.00	7.00	19.25	88.75	81.25
Mentana	**29.90**	**57.45**	**69.00**	**66.50**	112.00	99.50	**110.75**	**93.00**	8.00	**21.50**	8.00	**21.50**	96.50	77.50
Gabo	25.39	42.03	58.75	45.50	120.50	97.25	93.50	89.50	9.00	16.25	9.75	16.75	90.75	76.50
Lerma52	24.30	41.85	60.50	48.75	108.00	96.25	**107.50**	**95.75**	5.00	15.50	5.00	15.50	78.25	65.33
Nainari60	25.99	39.62	66.25	53.00	120.50	100.25	82.75	82.75	8.00	11.50	8.00	11.75	90.50	77.25
Pavon 76	23.66	**55.51**	65.25	**58.25**	118.00	98.75	78.25	83.00	8.00	**18.25**	8.25	18.25	87.00	82.50
Bacanora 88	22.22	38.03	64.00	46.00	116.00	102.50	70.25	66.25	6.25	13.25	6.50	13.25	88.50	76.25
Rayon 89	25.75	30.80	67.00	45.50	118.83	98.25	76.25	67.25	8.50	11.75	8.50	11.75	92.50	74.25
Pastor	**28.99**	48.26	**68.25**	50.50	117.00	101.00	78.75	76.75	8.00	17.50	8.00	17.75	97.25	78.25

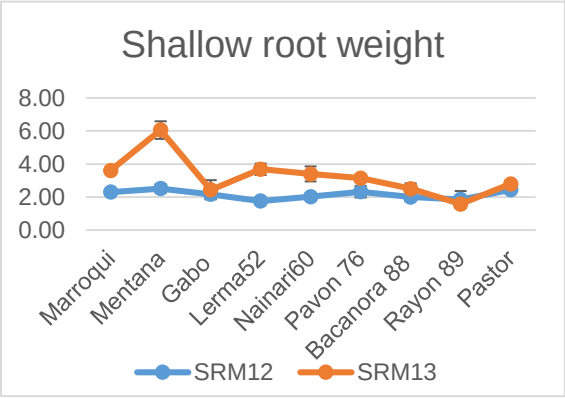
Table 3.2: Accession mean values per plant for 2012 and 2013 for grain yield (GY), harvest index 1 (HI1), harvest index 2 (HI2), deep root weight (DRM), shallow root weight (SRM), total root biomass (RM), shoot biomass (SM), total plant biomass (TM), days to anthesis (DTA), days to maturity (DTM), plant height (PH), number of fertile tillers (FT), number of tillers (NT), and root length (RL) for CIMMYT's parent, early, and late generation wheats evaluated in 80 cm tubes under well-watered conditions. Highlighted values are significantly different than others based on ANOVA results at $p<0.10$ or <0.05.

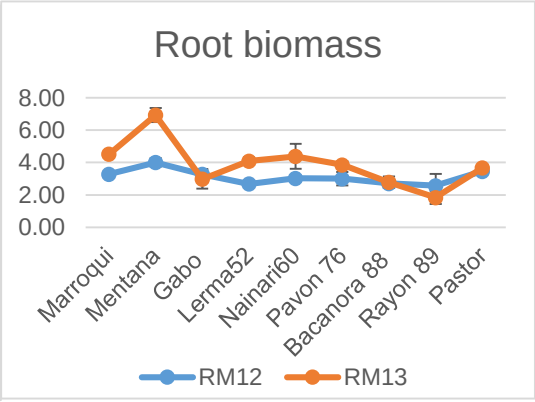

Grain yield
25.00
20.00
15.00
10.00
5.00
0.00
Marroqui
Mentana
Gabo
Lerma52
Nainari60
Pavon 76
Bacanora 88
Rayon 89
Pastor
GY12
GY13


Harvest index 1 (Grain yield/Shoot biomass
0.60
0.40
0.20
0.00
Marroqui
Mentana
Gabo
Lerma52
Nainari60
Pavon 76
Bacanora 88
Rayon 89
Pastor
HI12
HI13


Harvest index II (Grain yield / Total biomass
0.60
0.40
0.20
0.00
Marroqui
Mentana
Gabo
Lerma52
Nainari60
Pavon 76
Bacanora 88
Rayon 89
Pastor
HITM12
HITM13


Deep root weight
2.00
1.50
1.00
0.50
0.00
Marroqui
Mentana
Gabo
Lerma52
Nainari60
Pavon 76
Bacanora 88
Rayon 89
Pastor
DRM12
DRM13


Shallow root weight
8.00
6.00
4.00
2.00
0.00
Marroqui
Mentana
Gabo
Lerma52
Nainari60
Pavon 76
Bacanora 88
Rayon 89
Pastor
SRM12
SRM13


Root biomass
8.00
6.00
4.00
2.00
0.00
Marroqui
Mentana
Gabo
Lerma52
Nainari60
Pavon 76
Bacanora 88
Rayon 89
Pastor
RM12
RM13

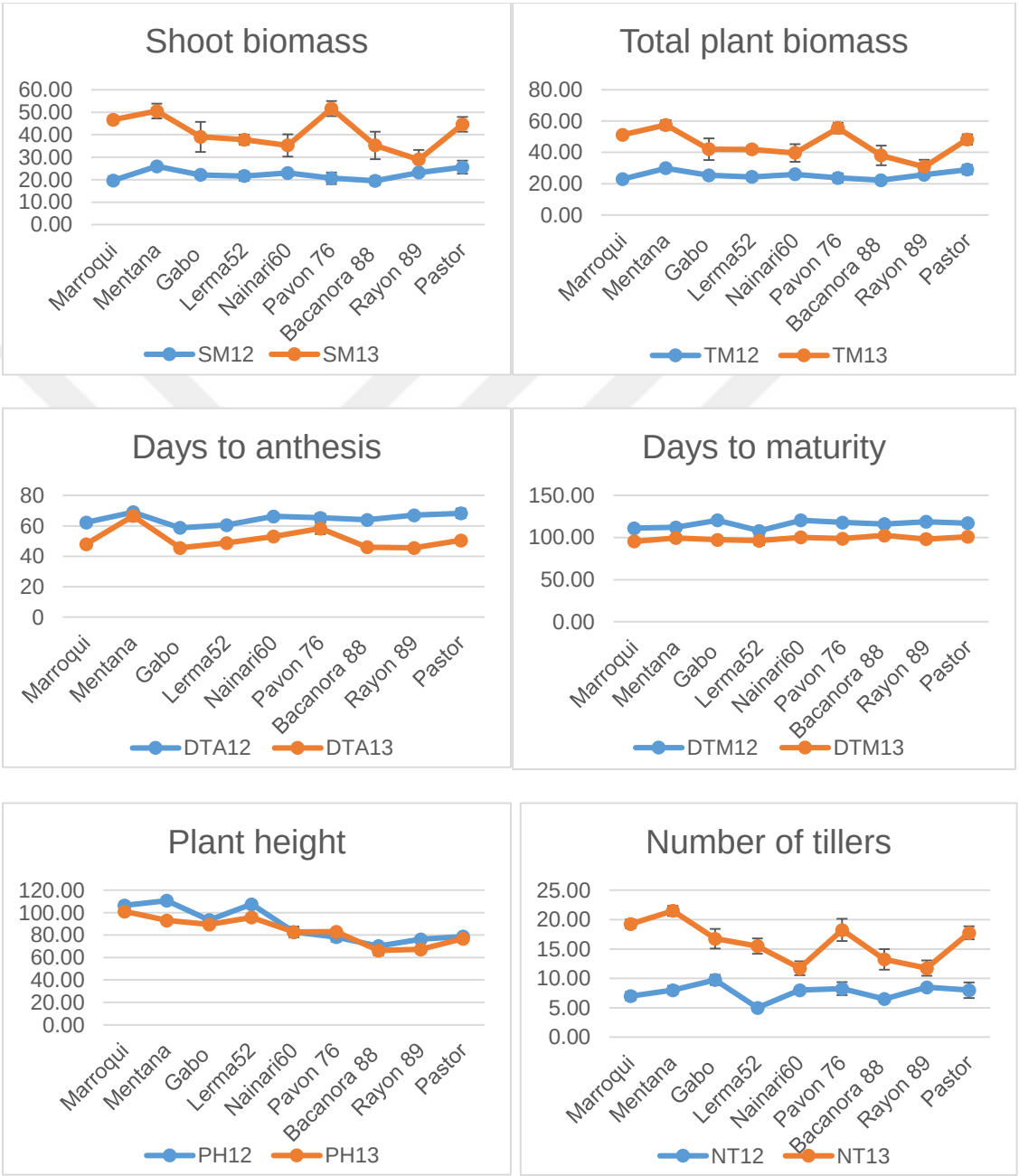
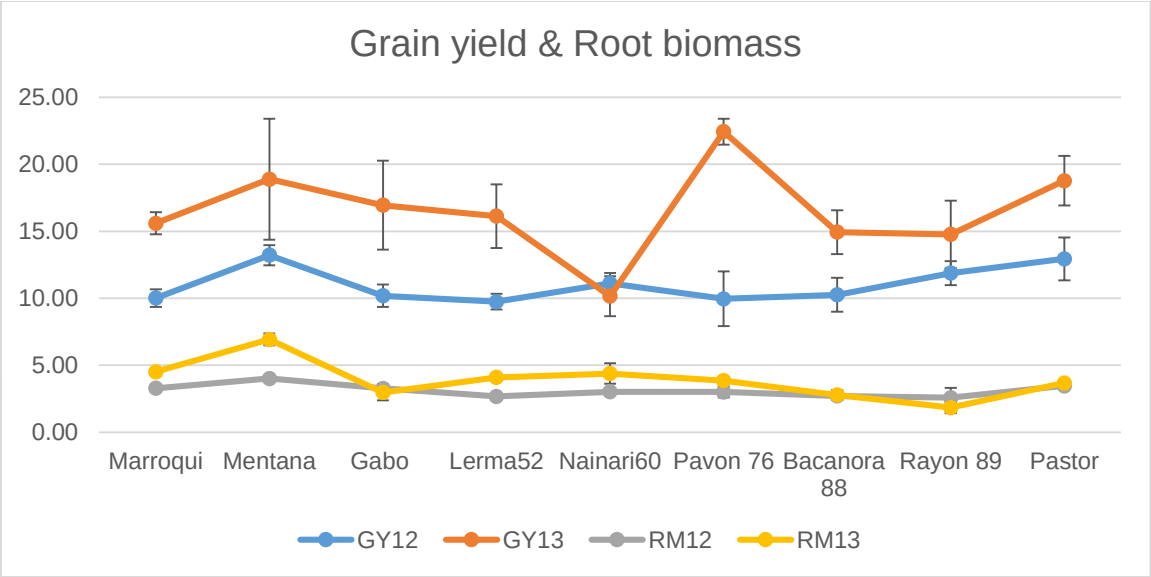


Figure 3.1: Mean values per plant of bread wheat for grain yield, harvest index 1, harvest index 2, deep root weight, shallow root weight, total root biomass, shoot biomass, total plant biomass, days to anthesis, days to maturity, plant height and number of tillers for 2012 (blue) and 2013 (orange). Genotypes are ordered as; parental (1-3), early (4, 5) and late generation (6-9) accessions.
*: Statistically significant differences observed consistently over two years.



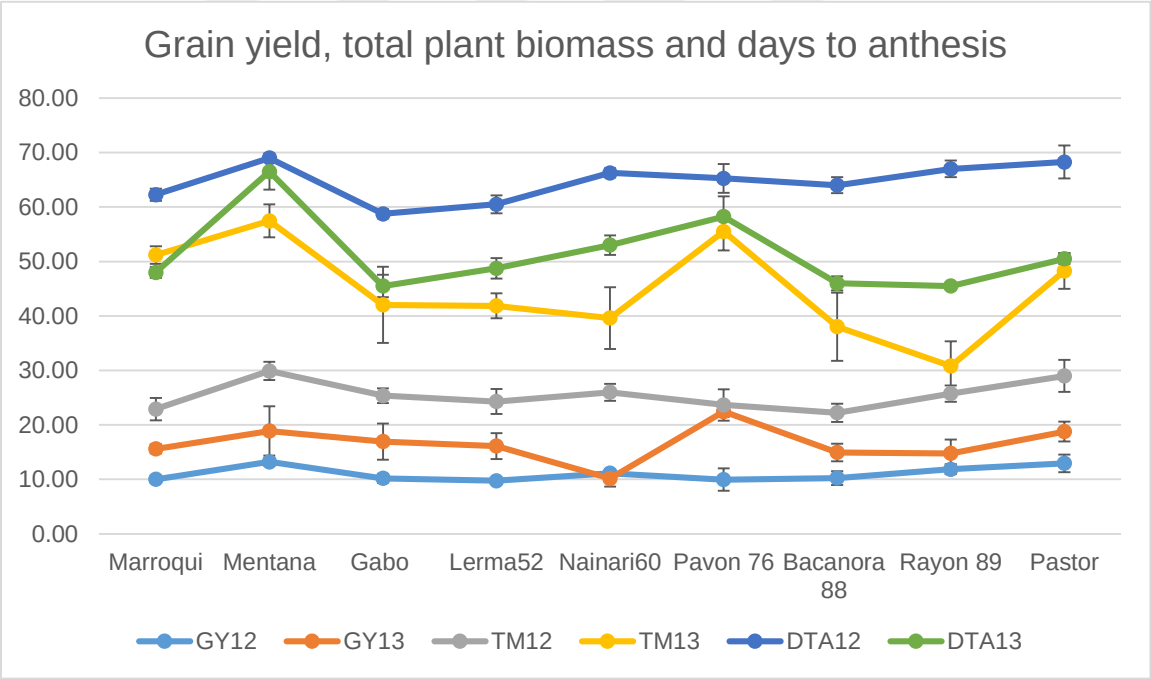


Figure 3.2: Comparison of the interactions between grain yield and total root biomass in bread wheat; and between grain yield, total plant biomass and days to anthesis. Genotypes are ordered as; parental (1-3), early (4, 5) and late generation (6-9) accessions. **A:** Bottom two lines are grain yield, top two lines are total root biomass, **B:** Bottom two lines are grain yield , mid two lines are total plant biomass and top two lines are days to anthesis for 2012 and 2013.

	SRM	DRM	RM
Tall	3.266[a]	0.8877[a]	4.154[a]
Semi-dwarf	2.504[b]	0.8161[a]	3.320[b]
LSD	0.554	0.2012	0.5906
P value	0.0117	0.0091	0.0013
	SM	**R / S**	**PH**
Tall	33.254[a]	0.124[a]	100.91[a]
Semi-dwarf	31.578[a]	0.114[a]	81.78[b]
LSD	6.1128	0.0057	5.5952
P value	0.2717	0.0565	0.000
	DTA	**GY**	**HI**
Tall	59.243[a]	13.835[a]	0.435[a]
Semi-dwarf	57.201[a]	13.888[a]	0.453[a]
LSD	4.5864	2.4895	0.0444
P value	0.8075	0.6612	0.4570

Table 3.3: Comparison of mean values per plant (two years combined) for four tall vs. five semi-dwarf accession for shallow root weight (SRM), deep root weight (DRM), total root biomass (RM), shoot biomass (SM), root / shoot ratio (R/S) plant height (PH), days to anthesis (DTA), grain yield (GY) and harvest index (HI). Plants were grown in 80 cm PVC tubes in 2011 and in 2012. For each trait, means followed by the different letter are significantly different at $p \leq 0.05$ level according to the LSD test.

	SRM	DRM	RM
Parent	3.1825a	0.9815[a]	4.164[a]
Early	2.719ab	0.8248[ab]	3.5437[ab]
Late	2.3334b	0.6531[b]	2.9866[b]
P value	0.0117	0.0091	0.0013
	SM	**R / S**	**PH**
Parent	33.978[a]	0.1271[a]	99.042[a]
Early	29.395[a]	0.1144[ab]	92.188[b]
Late	31.165[a]	0.1069[b]	74.594[c]
P value	0.2717	0.0565	0.000
	DTA	**GY**	**HI**
Parent	58.333[a]	14.141[a]	0.4375[ab]
Early	57.125[a]	11.788[a]	0.4119[b]
Late	58.094[a]	14.493[a]	0.4753[a]
P value	0.8075	0.6612	0.4570

Table 3.4: Comparison of mean values per plant for three parental, two early and four late generation Green Revolution wheats for shallow root weight (SRM), deep root weight (DRM), total root biomass (RM), shoot biomass (SM), root / shoot ratio (R/S) plant height (PH), days to anthesis (DTA), grain yield (GY) and harvest index (HI). Plants were grown in 80 cm PVC tubes in 2011 and in 2012. For each trait, means followed by the different letter are significantly different at $p \leq 0.05$ level according to the LSD test.

	SRM	DRM	RM	SM	PH	DTA	GY	HI	FT	NT
DRM	**0.80**									
RM	**0.99**	**0.88**								
SM	**0.81**	**0.72**	**0.82**							
PH	**0.66**	**0.59**	**0.67**	**0.49**						
DTA	**0.76**	**0.58**	**0.74**	**0.66**	0.12					
GY	**0.41**	0.22	**0.37**	**0.70**	0.06	**0.56**				
HI	**-0.51**	**-0.69**	**-0.58**	**-0.42**	**-0.54**	-0.23	**0.30**			
FT	**0.69**	**0.65**	**0.71**	**0.88**	**0.49**	**0.56**	**0.79**	-0.13		
NT	0.14	-0.14	0.07	0.18	-0.25	0.04	0.20	0.28	0.16	
RL	0.23	**0.47**	**0.30**	**0.41**	-0.24	**0.47**	**0.40**	0.03	**0.55**	0.26

Table 3.5: Correlation coefficients of accession means (two years combined) for; shallow root weight (SRM), deep root weight (DRM), total root biomass (RM), shoot biomass (SM) plant height (PH), days to anthesis (DTA), grain yield (GY), harvest index 1 (HI 1), number of fertile tillers (FT), number of tillers (NT) and root length (RL) for early, and late generation Green Revolution wheats and parental accessions evaluated in 80 cm tubes under well-watered conditions in 2011 and 2012.

0.30 > r > 0.50 significant correlation

r>0.50 highly significant correlation

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CHAPTER 4: Dissection of Quantitative Trait Loci (QTL) for Root Characters in SynOpDH Bi-Parental Mapping Population

Abstract

Research aimed to elucidate traits contributing to drought tolerance requires an understanding of plant physiology and the complex network of stress responses. The root system has a significant role in this response. The genetics of the root system is still not dissected for crops such as wheat and this lack of knowledge prohibits the use of marker assisted selection in breeding. The Synthetic W7984*Opata M85 bi-parental double-haploid mapping population (SynOpDH) is the reconstructed version of the well-known International Triticeae Mapping Initiative (ITMI) population. 'Opata M85' is a CIMMYT bread wheat cultivar and the synthetic wheat is an amphiploid generated from a cross between 'Altar-84', a Mexican durum wheat and *Aegilops tauschii* (D genome donor of bread wheat). Both parents and the 147 DH progeny lines were evaluated for root and shoot characteristics during growth in PVC tubes. Plants were grown to maturity, harvested and their root and shoot biomass traits were measured. There was considerable variation among the progeny lines. Two standard genetic maps were used for linkage and QTL mapping. Two major QTLs for total root biomass were detected on homoeologous chromosomes 2A and 2D with LOD scores of 10.9 and 20.8, respectively. The phenotypic effects of these two QTL were 15.9%

- 17.74% and 40.04% - 49.7%, respectively. There was close linkage between these QTL and photoperiod sensitivity genes, *Ppd-D1* (2D) and *Ppd-A1* (2A). A second set of experiments to validate the interaction between root biomass QTL and the *Ppd* genes was conducted with six pairs of lines genetically as similar as possible but contrasting for markers for root biomass QTL. This effectively blocked the effect of the *Ppd* genes. Genotypes with the Synthetic W7984 allele had significantly higher mean values for root biomass when compared to lines with 'Opata M8' allele. These results indicate that indeed two major QTL are on group-2 chromosomes in this population.

Introduction

Despite the fact that roots comprise a substantial part of a plant, historically they have not been studied in as much detail as the above ground parts, which can be studied by relatively simple observations. Currently, the necessity of studying root systems is widely recognized. Also, methodological progress in various fields has improved our ability to visualize, quantify, and conceptualize root architecture along with its relationship to plant productivity.

Information on the genetic basis of root architectural and morphological traits is very limited. Thus, extensive research is necessary to address this deficiency (Manschadi, et al., 2006). Focusing on this point, the dissection of root traits using QTL analyses may provide insight into the heritability of such traits and make marker assisted selection of root traits in plant breeding possible. Such studies can be done either in stocks / populations created specifically for the purpose, or they can use existing stocks. Here, the standard mapping population in wheat, Synthetic x Opata (SynOpDH) Sorrells, et al. (2011) population was selected for this purpose.

Root systems of field crops have been studied since the pioneering works of Weaver (1926) with many root system traits being evaluated (MacKey, 1973, Richards and Passioura, 1981, Richards and Passioura, 1989, Ehdaie and Waines, 1997, Richards, 2006, Sharma, et al., 2011). More recently with the advantage of genetic mapping, several studies have been published that studied quantitative trait loci associated with root systems. These studies applied many

different screening methods, in order to understand genetics, morphology, and anatomy of the root system, including, but not limited to gel observation chambers, clear pots, 2D and 3D imaging, soil columns, PVC tubes, field core samples and shovelomics. (Weaver, 1926, Oyanagi, 1994, Bengough, et al., 2004, Ehdaie and Waines, 2006, Trachsel, et al., 2011, Topp, et al., 2013, Richard, et al., 2015). These studies pointed out important characteristics such as: increased nodal root number, xylem vessel diameter, long root hairs, long lateral roots, seedling root vigor, long and numerous seminal roots, vigorous root system, deep root weight and high hydraulic resistance (Richards and Passioura, 1981, Lynch, 2007, Meister, et al., 2014).

The original reference population of Synthetic W7984 x Opata M85 (ITMI) recombinant inbred lines (RIL) has been studied extensively since the 1990s (Mujeeb-Kazi, et al., 1996, Sorrells, et al., 2011). More recently, Mohammadi, et al. (2007) evaluated ITMI population including parents for drought tolerance and reported significant differences between the Opata M85 and ITMI progeny in terms of drought tolerance. The synthetic line showed more efficient stress response, higher relative water content (RWC), leaf water content and less wilting compared to Opata M85. Landjeva, et al. (2008) evaluated the ITMI population to elucidate the genetic control of early seedling traits. They reported 35 QTL over 10 linkage groups associated with root, coleoptile and shoot length, and root / shoot ratio. Five QTL on chromosome 1AL, one major QTL on chromosome 2BS, and three minor QTL on chromosomes 6DL and 7DL were found to be effective on root,

shoot, and coleoptile length and root / shoot ratios under stress conditions. Unfortunately, the original ITMI population became corrupted over time and it was recently replaced by a population of doubled haploids, SynOpDH as the standard mapping population in wheat (Sorrells, et al., 2011). Still, significant differences for drought tolerance between the two parents encouraged us to identify genome regions that may be responsible for these differences.

In the past, many QTL for mostly seedling root and shoot traits have been reported in wheat. The specific characters in which we were interested, or those related to our target traits, were below-ground traits; root biomass, (Sanguineti, et al., 2007, Bai, et al., 2013, Zhang, et al., 2013, Chesnokov, et al., 2014); root / shoot ratio, (Landjeva, et al., 2008, Bai, et al., 2013, Zhang, et al., 2013); the longest / maximum root length (Landjeva, et al., 2008, Sharma, et al., 2011, Liu, et al., 2013, Zhang, et al., 2013, Botwright Acuña, et al., 2014, Kabir, et al., 2015, Petrarulo, et al., 2015); total root length, (Sanguineti, et al., 2007, Li, et al., 2011, Bai, et al., 2013, Liu, et al., 2013, Canè, et al., 2014); deep root ratio (Hamada, et al., 2012); root surface area (Liu, et al., 2013, Kabir, et al., 2015); root volume, (Kabir, et al., 2015); number of root tips and seminal root number, (Christopher, et al., 2013, Liu, et al., 2013, Zhang, et al., 2013, Canè, et al., 2014, Kabir, et al., 2015, Petrarulo, et al., 2015); and above-ground traits; plant height , (Sanguineti, et al., 2007, Li, et al., 2011, Bai, et al., 2013, Zhang, et al., 2013, Kabir, et al., 2015); shoot biomass, (Sanguineti, et al., 2007, Bai, et al., 2013); and thousand grain weight, (1000gW) 3A, 4D, 5A, and 6A (Sanguineti, et al., 2007) and just about

every single chromosome in the wheat genome has been implicated with many more than once. Another interesting characteristics was the root growth angle and the number of seminal roots evaluated by Oyanagi (1994) and Manschadi, et al. (2007), who reported strong interactions between drought tolerance and narrow root angle. Other QTL were reported for seminal root angle on chromosomes 1A, 2A, 2B, 3A, 3B, 3D, 4A 4B 5B 6A, and 6B (Christopher, et al., 2013, Liu, et al., 2013, Canè, et al., 2014).

Genetic variation of root traits among 297 germplasm accession were analyzed by Narayanan, et al. (2014) in a glasshouse using plants grown for 61 days in 150 cm long PVC columns. There was significant variation in rooting depth, root biomass and the root / shoot ratio. There was a significant positive correlation between root and shoot biomass, total root surface area and the number of tillers, and total root surface area and coleoptile length. There was no correlation between root traits and plant height. Out of 297 accessions tested, those originating from Mediterranean type climates had longer roots when compared with accessions from South Asia, Latin America, Mexico and Canada. Similar results were reported by Narayanan and Vara Prasad (2014) with an association mapping panel consisting of 250 spring wheat accessions.

The purpose of this study was to determine genotypic variation for root, shoot and grain yield traits in the SynOpDH population and to identify genome regions associated with total root biomass (RM) and several other characteristics of the

root system, and possible associations of such characteristics with parameters of the above-ground plant parts.

Materials and Methods

Seed samples of a set of doubled haploid lines from the SynOpDH mapping population were provided by Dr. Mark Sorrels, Department of Plant Breeding and Genetics, Cornell University, Ithaca, NY. This population consists of 215 lines of double haploids generated from the F1 hybrid of “Synthetic W7984 with cv. Opata M85. ‘Synthetic W7984' is a manmade amphiploid derived from the durum wheat line, 'Altar 84' (Triticum turgidum L.), crossed with accession (219) 'CIGM86.940' of *Aegilops tauschii* Coss. (Nelson, et al., 1995). Cv. Opata M85 is a spring wheat from the CIMMYT breeding program.

This set of DH lines is a replacement for the original mapping population ITMI that consisted of recombinant inbred lines. As a reference population it was widely used around the world as a resource for genetic markers, genes, and QTL mapping as listed in Sorrells, et al. (2011). From the entire set of DH lines in the SynOpDH population 147 lines were selected for the experiments. The same set of 147 lines genotyped by Poland, et al. (2012). A total of 147 lines and the two parents were used in QTL mapping.

Seedlings with vernalization requirement were planted in flats with sand and vernalized for 10 weeks at (2-5 ^{0}C). Other seeds without a vernalization requirement were germinated five days before transplanting into tubes. Seedlings

of similar size were transplanted into PVC tubes of 1 m long and 10 cm diameter filled with 10.5 kg #30 grade silica sand in plastic bags (Ehdaie and Waines, 2006, Sharma, et al., 2009) on February 20 2013 and January 15 2014. Two small holes were made at the bottom of each plastic bag for proper drainage. Seedlings were planted using randomized complete block design (RCB) with three replications. Sand filled tubes were brought to water holding capacity of (24%) by generous watering for two consecutive days prior to planting (Maheepala, et al., 2015). Plants were grown until maturity, spikes and shoots were harvested separately and dried in a hot air oven at 65º C for 72 hours. The plastic sleeves were taken out of the PVC tubes and cut lengthwise. Roots were washed out of the sand carefully and their total length was measured. Deep roots and shallow roots were separated at 30 cm depth from the soil level, packed separately for air drying in the glasshouse followed by a hot air oven for 72 hours at 65º C. Days from transplanting into tubes to booting (DTB), to heading (DTH), to anthesis (DTA), and to maturity (DTM) were recorded for each plant, as well as plant height (PH), the number of tillers (NT), number of fertile tillers (FT), number of spikes (NS). Flag leaf length (FLL) and width (FLW) were measured prior to harvest. Shoot biomass (SM), shallow root weight (SRM) and deep root weight (DRM), total root biomass (RM), grain yield (GY), 1000 grain weight (1000gW), number of seeds per spike (SS), number of seeds (NS), harvest index 1 (HI 1), and root to shoot ratio (R / S) per plant were collected after harvest.

QTL validation experiment

Six pairs of lines were selected based on the maximum marker similarity but with contrasting markers for the associated region on chromosomes 2D, by using the Flapjack software package (Milne, et al., 2010). As a result, one line in each pair had the marker from one parent and *Ppd-D1b* gene and other line had neither. The validation experiment was conducted in a glasshouse until heading date, with RCB design and 5 replications as blocks. Plants were grown in pots filled with 5 kg silica sand (#30), and 18 hours of light was provided in order to block the effect of *Ppd-D1b* and *Ppd-A1b* genes. Plants were harvested when >50 % of the lines in a replication reached heading.

Statistical Analysis:

Statistical analyses were performed using the Statistix software (Analytical Software; Tallahassee, FL, USA). Normality of data distribution was tested by normal probability plots. Combined analysis of variance (ANOVA) (Steel, et al., 1997) was performed to evaluate main phenotypic effect of genotype, year and genotype x year interactions for all traits. Genotypes were replicated three times each year, and averages of three replicates were calculated in order to obtain means for each genotype.

Genetic Mapping

SynOpDH population is a standard population, so it was mapped multiple times. Two of the latest maps by Poland, et al. (2012) and Saintenac, et al. (2013) were SNP based and had higher marker densities when compared with previous ones. Poland et al. (2012) mapped 147 out of 215 DH lines of the SynOpDH population using Genotyping by Sequencing (GBS) approach (Elshire, et al., 2011) with approximately 20,000 SNP markers and 367,000 tags. A total of 1485 GBS SNP markers were placed on 21 linkage groups with a total of 3243.53 cM map length. A similar approach of GBS was used by Saintenac et al. (2013). They generated a high density genetic map of the same population with 196 lines, by using a three-enzyme GBS protocol. They mapped a total of 2740 gene associated SNP markers from the 9K iSelect SNP assay (Cavanagh et al. 2013), and a total of 416,856 GBS markers were developed. The genetic linkage map of Poland, et al. (2012) was used for QTL mapping and marker data from Saintenac, et al. (2013) was used to create a second linkage map for validation.

Linkage mapping was done using the software package JoinMAP (Van Ooijen, 2006). The mean phenotypic value of three replications in each year was used to detect QTLs by the software package IciMapping by composite interval mapping (Li, et al., 2008). Means for each trait from 2014 data were used to validate the QTLs found in 2013. Simple correlation analyses were performed to evaluate interactions between all traits.

Results

Analysis of variance was performed to determine the genotypic variation for each trait measured in the experiment. Histograms for all traits were prepared and most distributions were normal (Figure 4.1). Parental lines Synthetic W7984 and 'Opata M85' were significantly different for total root biomass, shallow root weight, and deep root weight, number of seeds per plant, seed per spike, plant height, root length, and days to heading in both years. There were no consistent differences between parents for shoot biomass, grain yield, number of fertile tillers, number of tillers, days to booting, to heading and to anthesis, thousand grain weight, harvest index 1 and harvest index 2, root to shoot ratio and total plant biomass over two years. However, strong transgressive segregation and highly significant differences were observed within the progeny. For most traits the range of variation among progeny was well outside of the range of parents (Table 4.2).

Significant main effects for genotype and for year were observed for all traits ($p<0.01$), except the number of fertile tillers. The magnitude of the genotype x year interaction was significant across all traits except deep root weight, plant height, 1000gW and R / S. Therefore, the data for experiments conducted in 2013 and 2014 were analyzed separately and compared afterwards for ratings of genotypes and histogram distributions.

The mean values for biomass accumulated in 2014 was higher than in 2013. Mean values of total root biomass ranged from 0.61 g to 8.57 g plant^{-1} in 2013 and 1.59 g to 13.1 g plant^{-1} in 2014, respectively. Means of shoot biomass ranged from

8.8 g to 62.5 g plant^{-1} in 2013 and 25.7 g to 98.8 g plant^{-1} in 2014, respectively. Shallow root weights ranged between 0.61 g to 7.51 g in 2013 and between 1.59 to 11.57 g plant^{-1} in 2014. Means for deep root weight were between 0 g and 2.10 g in 2013 and between 0 g and 1.95 g plant^{-1} in 2014. The phenotypic range of plant height was between 60.66 cm and 99 cm in 2013 and between 51.1 cm and 137.9 cm in 2014. Mean values for grain yield ranged from 1.6 g to 23.4 g plant^{-1} in 2013 and 8.9 g to 39.5 g per plant in 2014 (Table 4.2).

Significant positive correlations (r >0.3 significant, and r >0.5 highly significant) was observed when total root biomass on one hand and shoot biomass, number of tillers, root length, days to anthesis, deep root weight, shallow root weight on the other. Shoot biomass was positively correlated with; grain yield, deep root weight, number of tillers, root length and days to anthesis. Grain yield was positively correlated with; root length and total plant biomass, whereas harvest index 1 was moderately negatively correlated with; total root biomass, shallow root weight and number of tillers. (Table 4.1).

The QTL tests showed statistically significant associations between the expression of a character and the presence of a specific segment of chromatin from one of the parents. Strong statistical associations were detected over fifteen linkage groups for fourteen different traits. A total of 41 phenotype – marker associations in 2013, and 27 in 2014 were detected, and 14 of those were co-located in both years (Table 4.3). A total of 27 associations were only detected in

2013 and 13 associations were only detected in 2014 (Table 4.4). These results suggest strong genotype x environment interactions for those traits.

The same segment on chromosome 2D was associated with deep root weight, shallow root weight, total root biomass, shoot biomass, days to anthesis, root length, flag leaf width and grain yield in both years. A apparently homoeologous locus on chromosome 2A was associated with deep root weight, shallow root weight, total root biomass, shoot biomass and days to anthesis, and it was detected in both years. Finally, a locus on chromosome 4D affected the number of fertile tillers and was detected in both years (Table 4.3). The region on chromosome 2D observed here to affect deep root weight, shallow root weight, total root biomass, shoot biomass, days to anthesis, root length, flag leaf width and grain yield is in the general area of the *Ppd-D1* locus affecting day length insensitivity. Similarly, the region on chromosomes 2A observed here to affect deep root weight, shallow root weight, total root biomass, shoot biomass and days to anthesis is in the general area of *Ppd-A1* locus. To test if the *Ppd-D1b* (day length sensitive) locus had an effect on the above phenotypic values of the tested lines, a validation experiment was conducted in 2014. Four out of six pairs evaluated reached heading within 4 days of each other. The other two pairs were 14 days apart. In the previous year the same 5 pairs of genotypes had a minimum 11 and maximum 40 days difference between pairs of lines and one pair of lines did not differ. The average difference in heading was 26 days between day length sensitive and insensitive lines for the 6 pairs of lines. Under 18 h day light treatment

this was reduced to 5 days. Five lines with the phenotype associated region and *Ppd-D1b*-sensitivity gene had higher mean root biomass when compared with lines without markers for that allele. The differences for individual pairs ranged from 8 % to 128% total root biomass increase for lines with the previously detected markers present vs. those with the markers absent.

Discussion

The present study aimed to dissect QTL affecting the below ground traits of wheat plants at maturity in a standard mapping population, and to look for the associations of these traits with the above-ground characteristics. The below ground traits, or the characteristics of the root system, can be measured / expressed in a number of ways. Perhaps the most accurate, but technically the most challenging, would be to count the numbers of nodal and / or seminal roots, to determine their weights and lengths individually, and as classes. Such approaches are common at the seedling stage. However, at maturity the root system of wheat is very dense and it would be extremely difficult to separate individual roots, or even classes of roots. For this reason, in this study, the total root biomass and the weights of deep and shallow roots were measured.

SynOpDH population was selected for its unique characteristics. Even if the parents ‘Synthetic W7984’ and ‘Opata M85’ does not differ for some of the traits, the wide genetic background of the population almost guarantees segregation in progeny. Additionally, the grandparent, accession (219) 'CIGM86.940' of *Aegilops*

tauschii was specifically selected for genetic improvement in bread wheat by Mujeeb-Kazi, et al. (1996) after evaluating hundreds of accessions. Since it is a standard reference population for a number of publications, phenotypic and genotypic data were numerous. Since 1996 many genetic maps have been published with the ITMI population and three new maps were published with the SynOpDH population. Those three latest maps by Sorrells, et al. (2011) with 1446 DArT and SSR markers, Poland, et al. (2012) with 1485 SNP markers, and Saintenac, et al. (2013) with 2740 SNP markers are publicly available.

Phenotypic differences between parents are not a pre-requisite for segregation in progeny. As we observed here, both parents are spring type, but there are more than 30 lines that segregate for vernalization genes in the progeny. And this vernalization process added more complication to the experimental system. Since the study was planned to run until maturity, we had to vernalize some lines but not others. We do not know if vernalization itself, and all the manual handling associated with it, has any impact on root characteristics, but we are unable to design and execute an experiment to test it. Nevertheless, all the above factors and the unique genetic structure made SynOpDH population a well-defined set of lines for our study of root system traits and QTL mapping.

Any given study evaluating crops in controlled conditions needs field trials as a validation. However, field evaluation of the root system of 147 lines in a replicated trial, was practically impossible for us. Therefore, experiments were conducted in glasshouse in PVC tubes. Data were collected, genotypic variation

was tested using ANOVA and the associations of various root and shoot characteristics with specific genome regions were done using the standard linkage maps available for the test population (Poland, et al., 2012). Additional linkage maps were generated in order to improve map resolution using the genetic map by Saintenac, et al. (2013). The IciMapping software package (Li, et al., 2008) was used for QTL mapping using both linkage maps.

Phenotypic values for most above and below ground traits were in a significant positive correlation with days to anthesis. Results of an additional experiment with contrasting markers validated the presence of genetic loci on chromosomes 2A and 2D associated with most above and below ground biomass traits. The two loci on homoeologous chromosomes 2A and 2D were responsible for 24.28 % and 24.7% of the phenotypic variation for **deep root weight**. This effect was statistically significant given that the trait is multigenic. There are no previous reports of QTLs for deep root weight at maturity, but results for other root traits have been reported, such as the narrow root angle, an indicator of deep rooting, and drought tolerance (Oyanagi, 1994, Manschadi, et al., 2008). A QTL for the narrow root angle on chromosome 2A also reported by Christopher, et al. (2013) and Canè, et al. (2014), as well as many other QTL on other chromosomes were reported by (Sanguineti, et al., 2007, Christopher, et al., 2013, Liu, et al., 2013, Canè, et al., 2014), and deep rooting QTL at seedling stage in pots, were reported on 1B and 5D by Hamada, et al. (2012). Most of the above studies focus on roots measured in very early stages of development, whereas we studied the

root system at maturity. Because of a major difference in the stage at which measurements were made we did not expect many shared results between our study and previous studies. Many environmental factors affect the growth rate of the above-ground plant parts, and it would be unreasonable to assume they do not effect root development. Especially so if the root system is capable of wide plastic responses to environmental cues. Therefore, the results collected at the seedling stage, necessarily in experiments of short duration, would not necessarily follow the same trends in the later stages of development. There is still no clear picture for the entire life cycle of the root system and factors affecting it. However, to understand the progress of the entire root development we need to evaluate it at every stage and all the way to maturity. We do know that during the grain filling period major changes take place in a wheat plant with respect to resource allocation and remobilization, and it is sensible to assume that this also involves the root system. In this sense, our measurements taken at maturity complement the picture created at the seedling stage. However, the same SynOpDH population now must be studied at earlier stages of development to establish firmly any correlations between seedling and mature plant root systems.

Plants with deep and dense root systems, which have the potential for higher soil exploration to access stored water in deeper soil zones have better stress tolerance (Bengough, et al., 2011, de Dorlodot, et al., 2007, Ehdaie, et al., 2003, Hurd, 1964, Hurd, 1974, Passioura, 1983). Additional water extracted during

grain filling improves yield directly (Manschadi, et al., 2006). Plants with 25 % more deep roots may be valuable for rain-fed farming conditions.

One major effect QTL on 2D was responsible for 24.7 % and 40.02 % of the phenotypic variation of the **shallow root weight** in 2013 and 2014, respectively. The same loci on 2D co-located with deep and total root weights as well as shoot biomass. These results indicate that lines with larger total root biomass had well distributed deep and shallow root weight through the soil profile. We are not aware of shallow root weight QTL at maturity. Shallow root weight becomes important in certain nutrient deficiencies since the number of nodal roots increases total soil volume exploration. Long nodal roots and root hairs are indicators of a large shallow root system. Shallow root weight can be used as an easy-to-measure parameter to detect genotypic variation in germplasm to prevent nutrient deficiencies (Manske and Vlek, 2002). Breeding large shallow rooted cultivars for nutrient deficient conditions and for better uptake of non-mobile nutrients may help save yield losses. Lynch (2013) suggested narrow root diameters, long haired and wide angled roots for better nutrient uptake. Therefore knowing the limitations of target fields is important to make the right decisions in breeding. Do we need a relatively large shallow root system in order to prevent nutrient deficiencies, or a deep and dense seminal root system to prevent water stress / drought? A major part of the carbon consumed by root systems goes to nodal root development. It is suggested that reduced shallow - nodal root size and increased deep – seminal root size may reduce the carbon cost of roots and increase grain yield significantly

(Watt, et al., 2013). This approach may be applicable in high-fertilizer input conditions, or soils without any mineral deficiency.

Two QTL on chromosomes 2A and 2D explained 52 % and 58% of the total phenotypic variations for the **total root biomass** (Figure 4.4 A - B). Our findings were in agreement with Bai, et al. (2013) who reported QTL affecting seminal root biomass on chromosome 2D, and Sanguineti, et al. (2007) who reported a QTL on chromosome 2A. Other studies reported root biomass (root dry weight) QTL mostly for the seedling stage (Sanguineti, et al., 2007, Sharma, et al., 2011, Bai, et al., 2013, Zhang, et al., 2013, Chesnokov, et al., 2014). Plants with deep and dense root systems, which have the potential for higher soil acquisition to access water stored in deeper soil zones have better stress tolerance and less nitrogen leaching (Bengough, et al., 2011, de Dorlodot, et al., 2007, Ehdaie, et al., 2003, Hurd, 1964, Hurd, 1974, Passioura, 1983). One of the major characteristics of Green Revolution wheats was day length insensitivity and semi-dwarfness. This provided wide adaptation of cultivars. Our study implies that the two characteristics might have also had an indirect effect on root vigor. First, semi-dwarfing genes reduced plant height and tillering in order to increase grain yield and harvest index, but also limit the carbon allocated to the above and / or below ground biomass accumulation (Youssefian, et al., 1992). Second, day length insensitivity reduced the stay green period of plants by allowing intermediate and spring types to grow in any part of the world with a shorter vegetative period (days to anthesis), allowing less time for root and shoot growth. Even though these efforts helped to increase

grain yield and harvest index under optimum growth conditions, reduced carbon allocation to roots and shoots reduced total biomass thus resulting in shallow, small rooted cultivars with limited drought tolerance.

The detected loci for root characteristics on chromosomes 2D was located in the general vicinity of the known major locus for the photoperiod response, *Ppd-D1b* on chromosome 2D. The question asked here is: are the changes observed in root characteristics a pleiotropic effect of the photoperiod response, or is there a separate locus / loci controlling root characters in the vicinity of *Ppd-D1* locus? To test this problem a different higher resolution linkage map was used, based on data by Saintenac, et al. (2013). In the new map there was one additional marker between *Ppd-D1* gene and the most likely location of the root biomass QTL. When we analyzed lines with newly located markers we observed crossing-over in the area between *Ppd-D1* gene and root biomass QTL in at least three different lines (Table 4.5). Observation of cross-over events suggest that, location of the QTL is in the general vicinity of *Ppd-D1* gene, but it is a separate locus. Moreover, with the new map, linkage block was 9 cM instead of 16 cM of the previous linkage map. On the new linkage map, *Ppd* gene is located just outside of this linkage block (Figure 4.5). As a result, the location of QTL was more accurate with the new map. Much higher resolution genetic maps and larger populations are needed to fine map the QTL and to validate the phenotypic effect of the QTL. In addition to crossing-over, a glasshouse validation study performed under conditions neutralizing the *Ppd-D1* locus (18-hour day) also confirmed that a root-specific

locus must be present in the vicinity of *Ppd-D1*. In comparisons of pairs of lines genotypically as similar as possible (within the existing population), but contrasting for the root characters, significant genotypic variation was observed for each pair of lines and line ranking from year to year was essentially the same.

There may be an interaction between *Ppd* genes and root biomass but to evaluate it properly, sets of isogenic lines, developed specifically for this purpose are needed. Unfortunately this was not feasible for us, within our time limitations.

Day length sensitivity genes (*Ppd*) have major effect on the phenological growth periods. Here we observed significant effects of *Ppd-D1b* and *Ppd-A1* alleles. Opata M85 is a spring wheat with day length insensitivity alleles *Ppd-D1a* and *Ppd-A1* and Synthetic W7984 carries *Ppd-D1b* on chromosome 2D and an allele of *Ppd-A1* on chromosome 2A. Segregation of these alleles were responsible from the 58 % and 69 % of the 62 and 54.66 days range in days to heading in 2013 and 2014, respectively. And the *Ppd* alleles on chromosome 2D of Synthetic W7984 had a positive additive (causing longer vegetative stage) effect while the *Ppd* allele on chromosome 2A from the 'Opata M85' had a negative additive effect (causing earliness) on days to heading. The *Ppd-A1* allele on chromosomes 2A caused significant but lower variation than *Ppd-D1b* on 2D in day length. However, we were not able to identify the specific allele responsible from this effect on chromosome 2A. *Ppd* genes on 2A and 2D were responsible for 58% and 69% of the total phenotypic variation in 2013 and 2014, respectively. Our results were in agreement with Li, et al. (2002).

We located the same locus on chromosome 2D with 16 % and 14 % phenotypic effect on **maximum root length** in 2013 and 2014, respectively. We did not find any previous study reporting QTL for root length on 2D, not surprising given that most previous studies reported root length at the seedling stage. However, three other associated regions that we located in only one year were on chromosomes 3B, 2D and 7D and they were in agreement with Kabir, et al. (2015), Bai, et al. (2013), Li, et al. (2011), Liu, et al. (2013), and Petrarulo, et al. (2015). Since we found significant effects of the above loci only in one year with relatively low LOD scores, we are not comfortable calling them QTL.

A moderate correlation was observed between root length and grain yield (0.26), verifying the importance of seminal root length to increased grain yield. One of the limitations of the tube system was their length. One meter long tubes were clearly too short to adequately measure the maximum root length. There are reports of wheat root lengths up to 2 meters (Gregory, et al., 1978). Even though our results were similar to previous reports, it was a limited observation of actual potential of maximum root length.

Similar to root-biomass traits, **shoot biomass** was associated with the same loci on 2A and 2D with 65.65 % and 59.75 % phenotypic effects in 2013 and 2014, respectively. Unfortunately there is little information on which we can relate our results. Chesnokov, et al. (2014) reported QTL for shoot biomass at certain stages of development on chromosomes 2B and 4A. Li, et al. (2010) reported QTL for shoot biomass on 2D and 5D. Other shoot biomass QTL were reported on

seedling shoot biomass 1D, 2A, 2B, 4A, 4B, 5D, 6B, 6D (Zhang, et al., 2013), 4D, 5A (Bai, et al., 2013) and 1B (Sanguineti, et al., 2007, Petrarulo, et al., 2015).

In each of the two years we located a total of four different chromosome regions on 2A, 2D, 4D, and 7B between 2.7 to 9.7 LOD scores, associated with the **number of tillers**, but none of them were located repeatedly over two years. Therefore we are not sure if it was just a genotype x year interaction, a statistical aberration generated by a relatively small population size, or some minor effect QTL with a significant environment interaction. Huang, et al. (2003) reported QTL for the number of tillers on 2A, 2D and 4D. Li, et al. (2002) reported QTL for number of tillers on chromosome 2D for the ITMI population, which was closely linked with *Ppd-D1* gene.

We detected six loci effecting **plant height** in 2013, but none of them was reproduced in 2014. However, the observed phenotypic range suggests an additive dosage effect for *Rht-B1b* and *rht8a* genes on plant height. Accessions with *rht8a* genes were on average 8 cm taller than accessions without them (data not shown; the presence / absence of specific alleles was inferred from the parental origin of specific genome regions in which the two loci reside). Most of the previous reports for plant height were from seedling studies and we are not convinced of a clear association between plant height at the seedling stage and at maturity, especially for relatively small effects. There is a need to evaluate plant height from seedling to maturity with the same genotypes to identify trends and changes throughout the season. Previous QTL reported for plant height were

mostly for semi-dwarfing genes (Sanguineti, et al., 2007, Li, et al., 2011, Heidari, et al., 2012, Bai, et al., 2013, Zhang, et al., 2013, Chesnokov, et al., 2014, Hill, et al., 2015, Kabir, et al., 2015).

A major QTL on chromosome 2D was responsible from 22.7 % and 19.2 % of the phenotypic variation in **grain yield** in 2013 and 2014, respectively. Additionally, grain yield and most biomass traits were positively correlated (0.27 to 0.83). Vigorous growth of the above and / or below ground biomass seems associated with increased grain yield, at least in the conditions of our experiments. This is quite opposite to the popular belief, which strongly relies on increasing harvest index.

The range of variation for total biomass was much wider for roots than for shoots. The difference between the smallest and largest shoot biomass was seven fold, whereas it was fourteen fold in root biomass. Even though a good proportion of tested lines showed a strong positive correlation between above and below ground traits (Table 4.2), there were lines with contrasting results where lines with low shoot biomass had large roots and vice versa (Figure 4.2). Similar relationships were seen between grain yield and root biomass; the set contains lines with small or large root sizes and high grain yield. This implies independent control of these two characters, and is in agreement with findings by Ehdaie, et al. (2001). However, the highest grain yield was observed in lines with a moderate root size. The peak point for grain yield was with at least four grams of total root biomass, and one gram of the deep root weight (Figure 4.2). Lines with root

biomass below four grams had lower grain yield. It is important to mention that some lines with extensive root and shoot biomasses also had high grain yield. As biomass increased harvest index would get lower, but it did not limit grain yield in these lines. Of course, these lines with contrasting root biomass, shoot biomass and grain yield proportions must be tested under field conditions with drought treatment to evaluate their potential under stress.

A detailed wheat root ideotype may have deep and dense seminal roots in order to capture water for high plant water status, narrow xylem vessel diameters for high hydraulic resistance, and long root hairs for maximum soil nutrient acquisition in order to survive an entire season without a major stress limitation (Passioura, 1983, Blum, 1996). Therefore screening germplasm accessions for genotypic variation in total root biomass is necessary to identify and perhaps breed wheat lines with the above mentioned root characters.

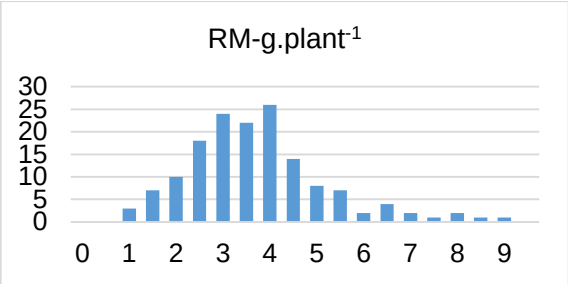
Conclusions

In the present study we evaluated root and shoot biomass traits as well as grain yield related traits at maturity for two parental lines and their 147 DH progeny in the standard wheat mapping population. Two major QTL were detected on chromosomes 2A and 2D explaining major phenotypic variation for deep and shallow root weight, total root biomass, shoot biomass and root length, flag leaf width, grain yield and days to anthesis. These QTL were closely located with the day length sensitivity alleles of the *Ppd-D1* and possibly the *Ppd-A1* loci. A

validation experiment where the effect of photoperiod was neutralized still demonstrated that lines with the QTLs did have significantly larger root biomasses than lines without a QTL.

The QTL reported here require further validation. Given the limitations of studying root systems on a large scale we plan to continue validation of the QTL on chromosomes 2A and 2D with the advantage of high throughput phenotyping and genotyping technologies in glasshouse and field conditions under different irrigation regimes. Deep rooting, seedling root vigor and dense root systems that cover a maximum soil surface early in the season were associated with grain yield increase based on a simulation conducted by Lilley and Kirkegaard (2011). Finding QTL that increase root biomass, root density, and root length may provide useful candidate lines for marker assisted breeding. There were up to 14 fold differences for root biomass among the progeny of the SynOpDH population. Targeting root ideotypes including deep rooting for water deficit conditions and a large shallow root system for nutrient deficiencies, which are needed for target environmental conditions may be more feasible and easier especially with the benefit of marker assisted selection.

2013 **2014**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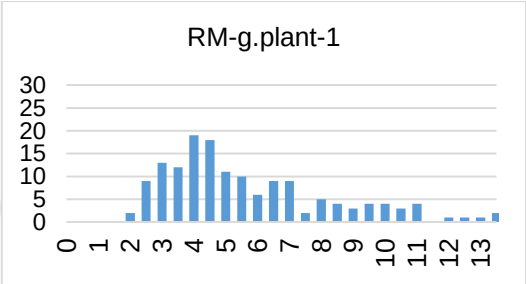
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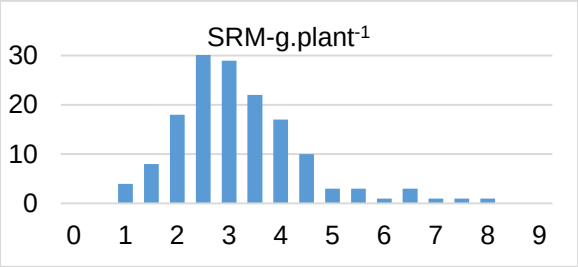
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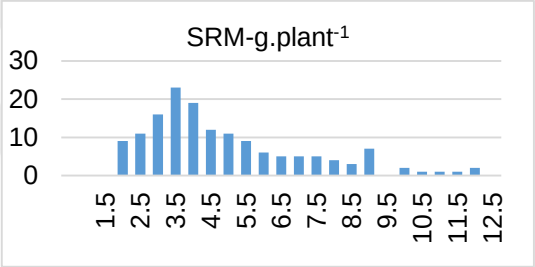
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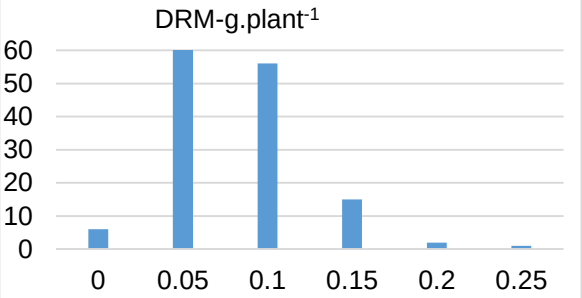
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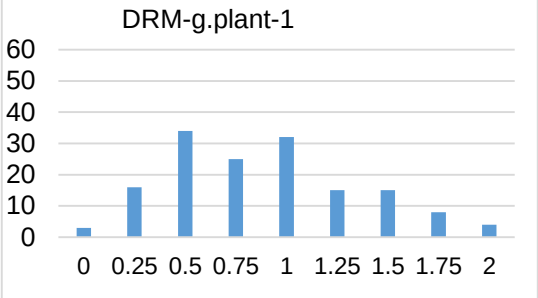
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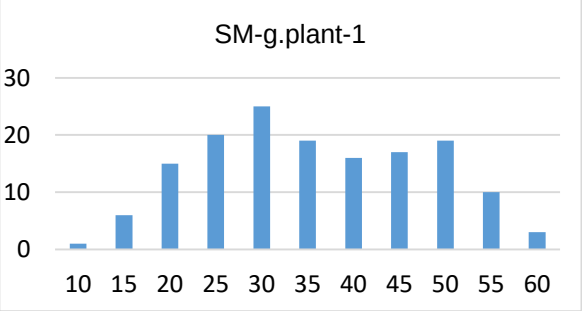
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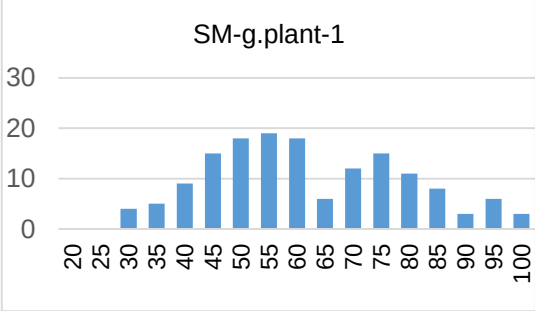
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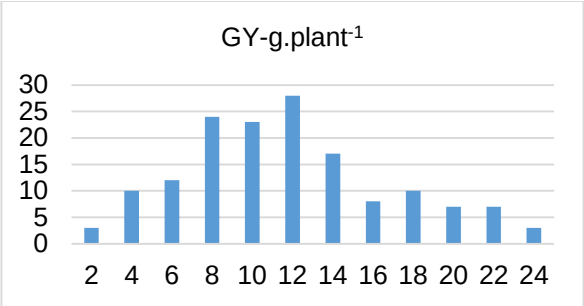
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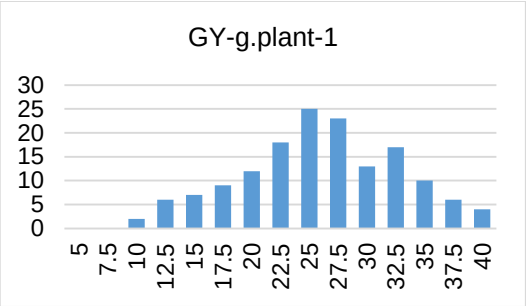
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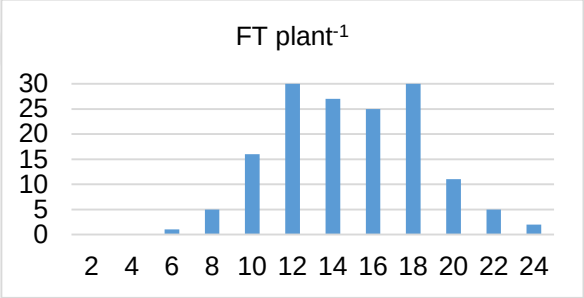
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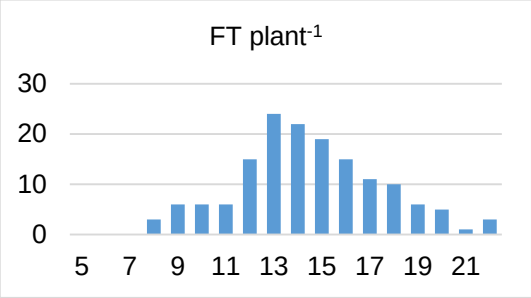
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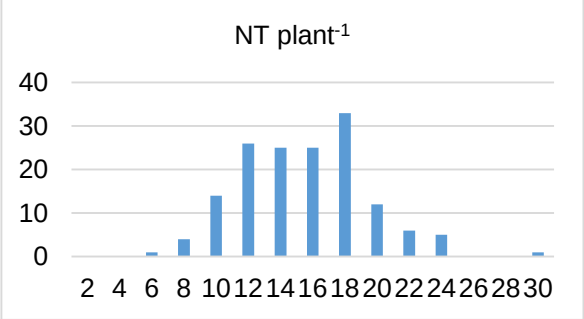
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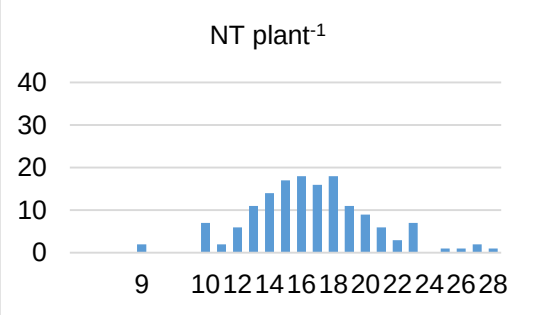
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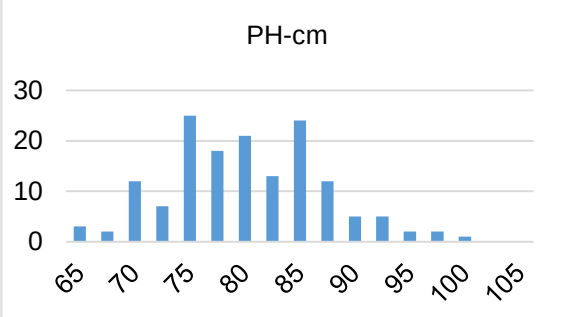
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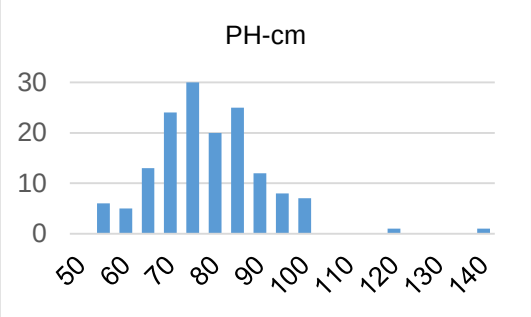
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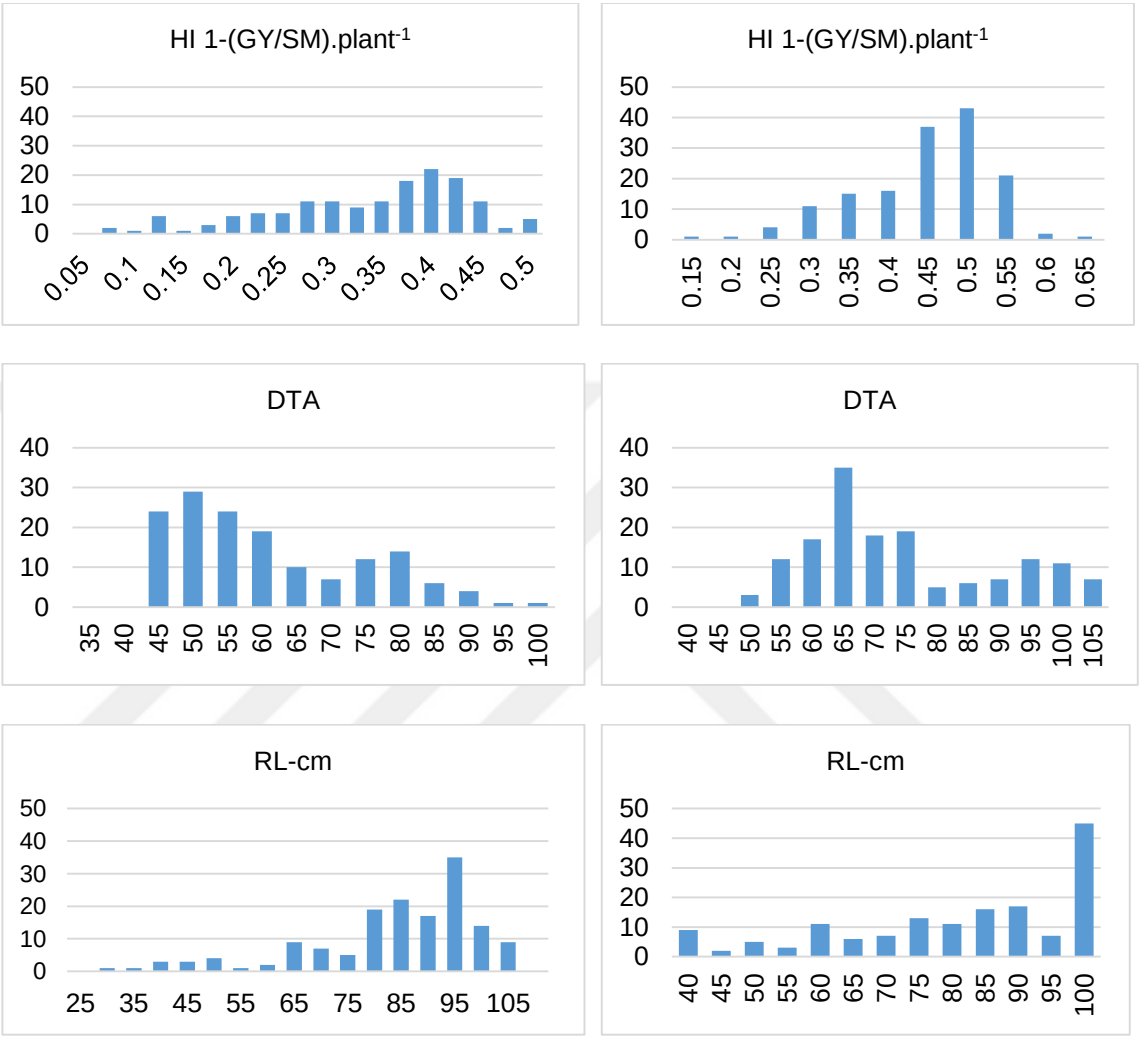


Figure 4.1: Histograms of the phenotypic values in bread wheat for total root biomass, shallow root weight, deep root weight, shoot biomass, grain yield, number of fertile tillers, number of tillers, harvest index, plant height, days to anthesis and root length for 2013 and 2014 experiments. O; 'Opata M85', S; 'Synthetic W7984'. Refer to Table 4.2 for population and parent mean values.

	GY	SM	RM	SRM	DRM	FT	NT	RL	PH	DTA	DTM
SM	**0.77**										
RM	**0.41**	**0.83**									
SRM	**0.40**	**0.81**	**0.99**								
DRM	**0.34**	**0.69**	**0.78**	**0.68**							
FT	**0.34**	**0.44**	**0.34**	**0.31**	**0.34**						
NT	0.28	**0.55**	**0.53**	**0.52**	**0.46**	**0.91**					
RL	**0.53**	**0.65**	**0.57**	**0.51**	**0.72**	**0.43**	**0.44**				
PH	**0.37**	**0.55**	**0.40**	**0.38**	**0.36**	0.12	0.20	**0.32**			
DTA	**0.43**	**0.81**	**0.78**	**0.80**	**0.50**	0.20	**0.40**	**0.31**	**0.46**		
DTM	0.27	**0.65**	**0.73**	**0.74**	**0.50**	0.10	**0.33**	**0.34**	**0.36**	**0.80**	
HI 1	**0.41**	-0.20	**-0.46**	**-0.47**	**-0.32**	-0.17	**-0.36**	-0.08	-0.24	**-0.41**	**-0.40**

Table 4.1: Correlation coefficients (r values) between line means for; grain yield (GY), shoot biomass (SM), total root biomass (RM), shallow root weight (SRM), deep root weight (DRM), number of fertile tillers (FT), number of tillers (NT), root length (RL), plant height (PH), days to anthesis (DTA), days to maturity (DTM) and harvest index 1 (HI 1) for SynOpDH bread wheat population evaluated in 1 m tubes under well-watered conditions in 2013 and 2014 . Overall means for each line from 2013 and 2014 was used for correlation analysis.
0.30 > r > 0.50 Significant correlation
r>0.50 Highly significant correlation

Trait	Synthetic W7984	Opata M85	Progeny lines-Range	Progeny lines-Mean	St. Dev.	F test	P values
RM*	2.59667	3.29667	0.610-8.57333	3.47393	1.78	4.72	0.000
SRM*	2.84333	4.22667	0.610-7.51667	2.94419	1.45	5.42	0.000
DRM*	0.28000	0.58333	0.00-2.10667	0.52974	0.52	2.00	0.000
SM	23.91000	24.62000	8.81667-62.530	33.48388	13.41	9.49	0.000
GY	6.79667	6.10333	1.62667-23.48333	10.72871	5.89	6.24	0.000
NS*	185.33333	127.33333	42.0-704.333	268.70121	164.68	8.25	0.000
S / S*	20.57698	11.35556	3.81692-46.93846	19.17759	10.16	6.72	0.000
FT	11.00000	11.33333	6.0-23.66667	14.10088	4.32	4.47	0.000
NT	12.33333	11.33333	6.0-29.333	14.72588	4.74	4.57	0.000
PH*	65.00000	87.00000	60.66667-99.0	79.02412	8.46	4.61	0.000
RL*	62.66667	94.66667	28.333-105.0	81.39408	22.26	2.28	0.000
DTB	90.00000	34.00000	27.333-90.0	46.94079	15.40	30.38	0.000
DTH	97.00000	42.66667	35.0-97.0	52.55263	14.70	3.17	0.000
DTA	103.66667	46.33333	40.3-103.66667	58.99342	14.69	27.75	0.000
1000gW	35.72048	48.17219	0.00000-58.63515	40.18029	8.31	1.02	0.448
HI 1	0.29753	0.25358	0.05369-0.49772	0.32480	0.12	3.26	0.000
HI2	0.26361	0.21260	0.04708-0.45555	0.29149	0.11	3.91	0.000
R / S	0.10860	0.13390	0.02538-0.35858	0.11700	4.67	1.00	0.498
TM	27.03333	29.43000	10.12667-66.84667	36.95781	14.56	9.70	0.000

Table 4.2 A: Mean phenotypic values for parents and 147 progeny of the SynOpDH bread wheat population; minimum and maximum values and standard deviations, F test and P values for; total root biomass (RM), shallow root weight (SRM), deep root weight (DRM), shoot biomass (SM), grain yield (GY), number of seeds (NS), seed / spike (SS), number of fertile tillers (FT), number of tillers (NT), plant height (PH), root length (RL), days to booting (DTB), days to heading (DTH), days to anthesis (DTA), thousand grain weight (1000gW), harvest index 1 (HI 1), harvest index2 (HI2) (grain yield / total plant biomass), root / shoot ratio (R / S) and total plant biomass (TM) in 1 m tubes under well-watered conditions in 2013.

Trait	Synthetic	Opata M85	Progeny lines-Range	Progeny lines-Mean	St. Dev.	F test	P values
RM*	5.16000	4.89333	1.5966-13.12667	5.43535	3.06	5.09	0.000
SRM*	4.86667	3.94000	1.5966-11.97667	4.66261	2.64	5.11	0.000
DRM*	0.29333	0.95333	0.0-1.98333	0.77314	0.71	2.74	0.000
SM	63.43667	51.25667	25.7566-98.84667	59.50664	19.14	4.01	0.000
GY	20.01333	29.09000	8.96333-39.52667	24.66689	8.76	1.99	0.000
NS*	579.00000	762.66667	159.66-922.666	540.15570	196.83	3.34	0.000
S / S*	42.06217	44.80556	10.71763-84.320	40.53071	18.12	2.15	0.000
FT	14.66667	17.00000	7.666-21.6666	14.12719	3.93	4.55	0.000
NT	18.66667	17.66667	8.333-27.333	16.32237	4.91	4.91	0.000
PH*	74.33333	53.93333	51.10-137.90	76.03377	17.63	3.28	0.000
RL*	56.66667	73.00000	27.00-100.0	78.79386	25.07	4.01	0.000
DTB	68.33333	48.00000	35.333-90.0	58.80263	15.42	16.58	0.000
DTH	75.33333	56.00000	42.00-96.66667	65.84868	14.98	6.17	0.000
DTA	82.00000	61.33333	48.66667-103.0	71.80921	15.38	14.23	0.000
1000gW	37.82514	38.00917	31.645-93.57290	46.30555	9.30	1.26	0.029
HI 1	0.32256	0.57102	0.14359-0.62846	0.42320	0.10	3.02	0.000
HI2	0.29848	0.52133	0.12714-0.58489	0.39052	0.10	3.20	0.000
R / S	0.08134	0.09547	0.04070-0.16508	0.09100	0.034	0.97	0.568
TM	68.59667	56.15000	1.5966-13.12667	64.94200	21.51	4.27	0.000

Table 4.2 B: Mean phenotypic values for parents and 147 progeny of the SynOpDH population; minimum and maximum values and standard deviations, F test and P values for; total root biomass (RM), shallow root weight (SRM), deep root weight (DRM), shoot biomass (SM), grain yield (GY), number of seeds (NS), seed / spike (SS), number of fertile tillers (FT), number of tillers (NT), plant height (PH), root length (RL), days to booting (DTB), days to heading (DTH), days to anthesis (DTA), thousand grain weight (1000gW), harvest index 1 (HI 1), harvest index 2 (HI2) (grain yield / total plant biomass), root / shoot ratio (R / S) and total plant biomass (TM) in 1 m tubes under well-watered conditions in 2014.

.

No	Trait Name	Chromosome	Year	Position (cM)	Left Marker	Right Marker	LOD	PVE (%)	Additive
1	GY	2D	2013	66.2300	synopGBS579	synopGBS1212	8.3815	22.7431	2.4269
			2014	69.2300	synopGBS1310	synopGBS745	7.6586	19.2896	3.0240
2	SM	2A	2013	44.0000	synopGBS374	synopGBS262	11.8271	15.9259	-4.8331
			2014	43.0000	synopGBS374	synopGBS262	10.5461	12.6847	-6.0381
3	SM	2D	2013	68.2300	synopGBS579	synopGBS1212	28.7604	49.7337	8.5153
			2014	68.2300	synopGBS579	synopGBS1212	29.5029	47.0760	11.6047
4	RM	2A	2013	42.0000	synopGBS1017	synopGBS374	6.2478	12.7480	-0.5337
			2014	42.0000	synopGBS1017	synopGBS374	10.9396	17.7488	-1.1143
5	RM	2D	2013	68.2300	synopGBS579	synopGBS1212	11.8589	26.6052	0.7685
			2014	68.2300	synopGBS579	synopGBS1212	20.8598	40.0480	1.6684
6	SRM	2A	2013	41.0000	synopGBS1017	synopGBS374	6.6197	12.6267	-0.4415
			2014	42.0000	synopGBS1017	synopGBS374	11.4227	18.3967	-0.9861
7	SRM	2D	2013	68.2300	synopGBS579	synopGBS1212	12.0590	24.7044	0.6161
			2014	68.2300	synopGBS579	synopGBS1212	21.0773	40.0207	1.4497
8	DRM	2A	2013	46.0000	synopGBS374	synopGBS262	3.1739	7.2611	-0.0994
			2014	43.0000	synopGBS374	synopGBS262	2.9768	6.8181	-0.1250
9	DRM	2D	2013	62.2300	synopGBS579	synopGBS1212	6.7513	17.0202	0.1518
			2014	69.2300	synopGBS1310	synopGBS745	7.7114	17.9760	0.2025
10	FT	4D	2013	0.0000	synopGBS838	synopGBS570	4.3443	8.5731	-1.0700
			2014	0.0000	synopGBS838	synopGBS570	3.4662	8.4491	-0.8708
11	RL	2D	2013	68.2300	synopGBS579	synopGBS1212	7.6124	16.6847	6.6057
			2014	63.2300	synopGBS579	synopGBS1212	5.3770	14.0827	7.3716
12	DTA	2A	2013	33.0000	synopGBS855	synopGBS1016	13.5656	18.1694	-5.9840
			2014	41.0000	synopGBS1017	synopGBS374	19.9053	27.2447	-7.7978
13	DTA	2D	2013	67.2300	synopGBS579	synopGBS1212	25.7259	40.4835	8.9389
			2014	68.2300	synopGBS579	synopGBS1212	27.8183	42.9904	9.7529
14	FLW	2D	2013	71.2300	synopGBS745	synopGBS250	7.3270	16.4576	0.0817
			2014	69.2300	synopGBS1310	synopGBS745	13.3987	29.3358	0.1702

Table 4.3: QTL associated with , grain yield (GY), shoot biomass (SM), total root biomass (RM), shallow root weight (SRM), deep root weight (DRM), fertile number of tillers (FT), plant height (PH), root length (RL), days to anthesis (DTA) and flag leaf width (FLW) in the SynOpDH population. Plants were grown in 1 m PVC tubes under well-watered conditions until maturity for two seasons. Peak positions with highest LOD score, left and right markers, logarithm of the odds (LOD) scores, percent phenotypic effects, and additive effects.

[a] LOD score of 3.0 was used for declaration of QTL.

[b] Phenotypic variation explained by QTL

[c] Additive effect of QTL

No	Year	Trait Name	Chromosome	Position (cM)	Left Marker	Right Marker	LOD	PVE (%)	Additive
1	2013	1000GW	2D	68.2300	synopGBS579	synopGBS1212	4.1908	11.3699	-2.8223
2	2013		5D	222.0000	synopGBS737	synopGBS576	3.0333	8.0156	-2.3689
3	2013	GY	2A	43.0000	synopGBS374	synopGBS262	3.0219	7.0736	-1.3578
4	2014		4A	42.8500	synopGBS1186	synopGBS1416	3.4356	8.1081	-1.9788
5	2014	HI	2A	71.0000	synopGBS1475	synopGBS916	3.1259	7.9469	0.0239
6	2014		2D	68.2300	synopGBS579	synopGBS1212	5.3637	14.0689	-0.0317
7	2013	SM	5D	113.0000	synopGBS1282	synopGBS656	3.4005	3.9694	2.4099
8	2014		4A	56.8500	synopGBS525	synopGBS325	3.9367	4.0702	-3.4387
9	2014		7D	89.4800	synopGBS251	synopGBS1449	2.7692	2.8441	2.8528
10	2013	RM	5D	144.0000	synopGBS1273	synopGBS613	3.3553	6.5519	0.3898
11	2013	SRM	5A	125.0000	synopGBS902	synopGBS147	3.3893	6.0144	-0.3039
12	2013		5D	142.0000	synopGBS1273	synopGBS613	3.8496	6.9980	0.3357
13	2013	DRM	3A	19.0000	synopGBS706	synopGBS1260	3.0382	6.7136	-0.0961
14	2013		6A	91.0000	synopGBS1239	synopGBS1401	3.5921	8.1762	-0.1062
15	2014		7D	89.4800	synopGBS251	synopGBS1449	2.6581	5.7383	0.1144
16	2013	FT	1B	82.9600	synopGBS1482	synopGBS1086	3.4165	6.8507	0.9444
17	2013		2D	68.2300	synopGBS579	synopGBS1212	10.3159	22.7059	1.7173
18	2014		5A	127.0000	synopGBS147	synopGBS429	2.9280	7.3109	0.7986
19	2014		7B	111.3700	synopGBS952	synopGBS1301	3.3965	8.5685	-0.8669
20	2013	NT	2A	44.0000	synopGBS374	synopGBS262	2.5128	5.8713	-0.9645
21	2013		2D	68.2300	synopGBS579	synopGBS1212	9.7382	23.4817	1.9231
22	2013		4A	0.0000	synopGBS838	synopGBS570	2.9509	6.3075	-1.0105
23	2014		7B	111.3700	synopGBS952	synopGBS1301	2.7123	8.3323	-1.0951
24	2013	PH	2A	42.0000	synopGBS1017	synopGBS374	3.4211	7.5067	-1.9319
25	2013		2D	66.2300	synopGBS579	synopGBS1212	2.8781	6.7032	1.8191
26	2013		4A	27.8500	synopGBS1339	synopGBS82	3.2091	7.0017	-1.8652
27	2013		5A	47.0000	synopGBS851	synopGBS826	4.5801	10.3250	2.2620
28	2013		6D	81.0000	synopGBS965	synopGBS1413	2.5778	5.5599	1.6770

29	2014		6B	86.0000	synopGBS1461	synopGBS1235	2.7812	8.3059	1.2236
30	2013	RL	3B	105.0000	synopGBS753	synopGBS1241	2.9651	6.0136	-3.9662
31	2013		6A	93.0000	synopGBS1239	synopGBS1401	5.5205	12.0558	-5.6851
32	2013		7D	32.4800	synopGBS254	synopGBS773	2.5756	5.2144	3.7107
33	2014		6B	108.0000	synopGBS900	synopGBS286	2.6452	5.8114	4.7592
34	2013	DTA	5A	69.0000	synopGBS293	synopGBS1405	2.9316	3.3273	-2.5959
35	2013		5B	66.0000	synopGBS1315	synopGBS1269	3.8163	4.3380	2.9661
36	2013		5D	134.0000	synopGBS371	synopGBS611	4.2059	4.9476	3.2116
37	2013	FLW	5A	127.0000	synopGBS147	synopGBS429	6.5515	14.8346	-0.0776
38	2014		5B	130.0000	synopGBS1330	synopGBS127	3.3704	6.2238	-0.0784
39	2013		2A	26.000	SynopGBS399	SynopGBS855	3.1872	6.6993	-0.0524
40	2014		2A	42.0000	synopGBS1017	synopGBS374	3.8694	7.2358	-0.0848

Table 4.4: Phenotype associated chromatin regions of the SynOpDH population that were detected only one year (2013 or 2014) for thousand grain weight (1000gW), grain yield (GY), harvest index 1 (HI 1), shoot biomass (SM), total root biomass (RM), shallow root weight (SRM), deep root weight (DRM), fertile number of tillers (FT), number of tillers (NT), plant height (PH), root length (RL), days to anthesis (DTA) and flag leaf width (FLW). Plants were grown in 1 m PVC tubes under well-watered conditions until maturity for two seasons. Peak positions with highest LOD score, left and right markers, logarithm of the odds (LOD) scores, percent phenotypic effects, and additive effects.

[a] LOD score of 2.5 was used for as threshold

[b] Phenotypic variation explained by associated loci

[c] Additive effect of specific loci.

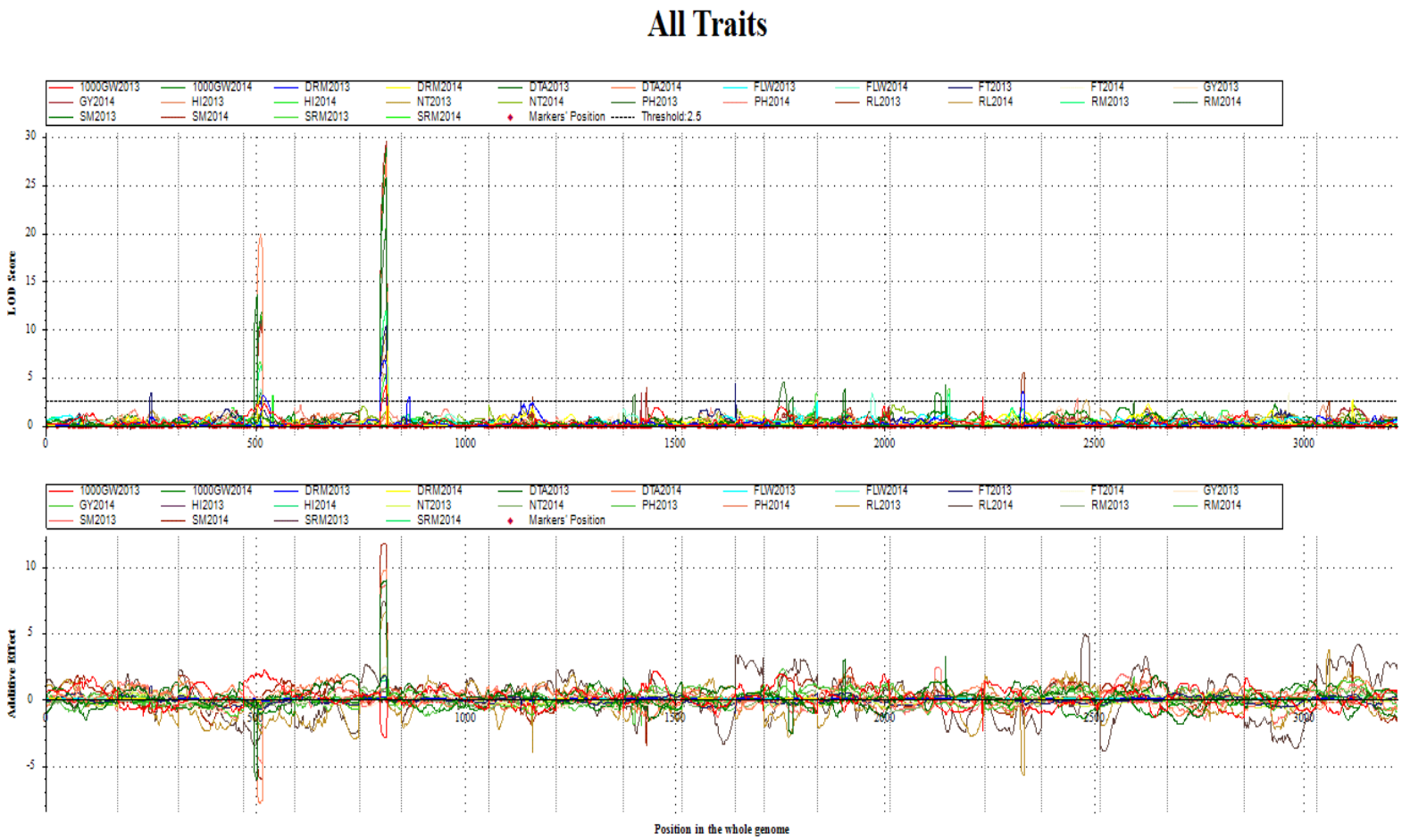
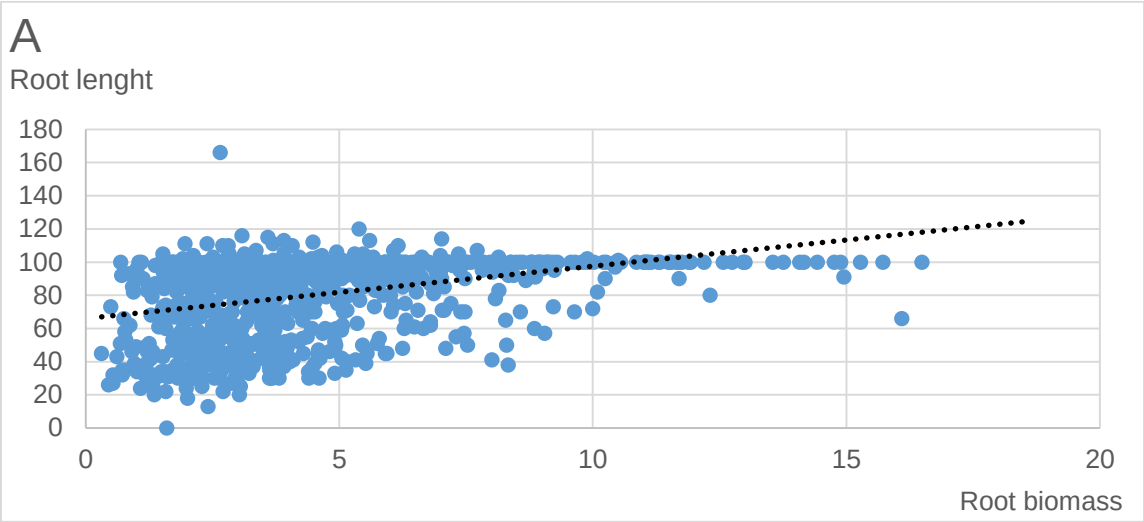
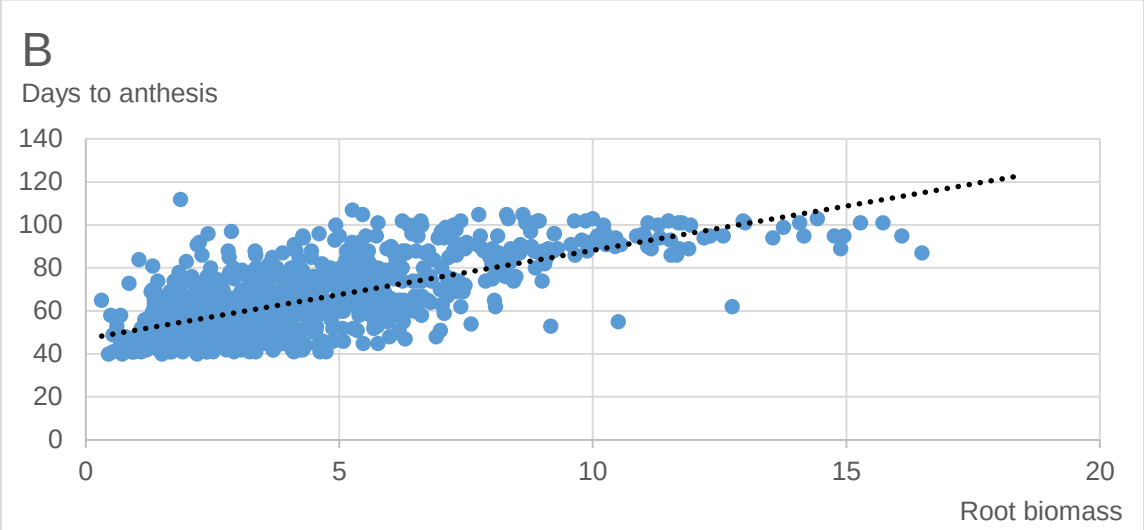
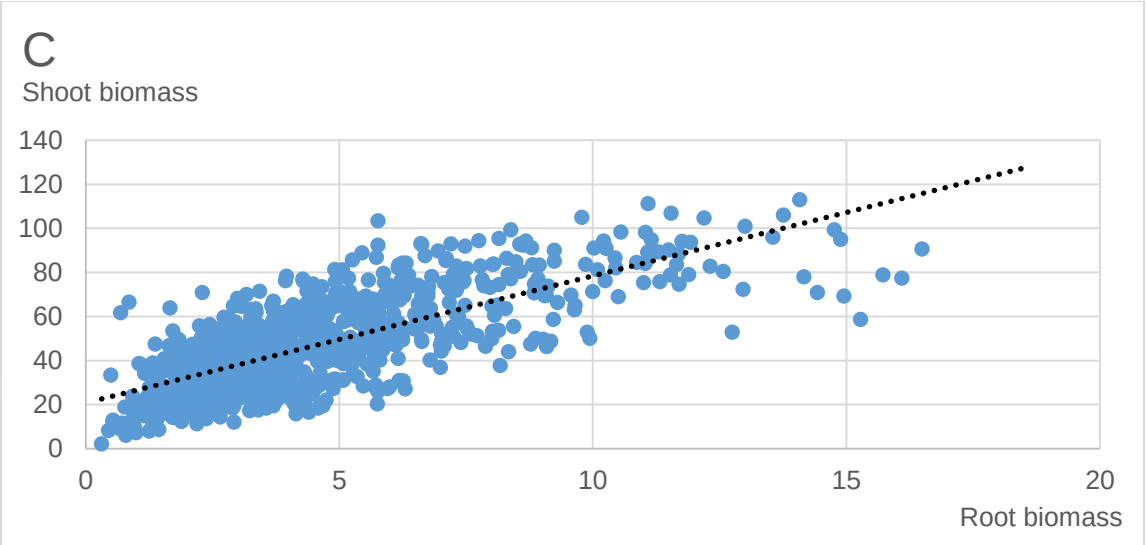
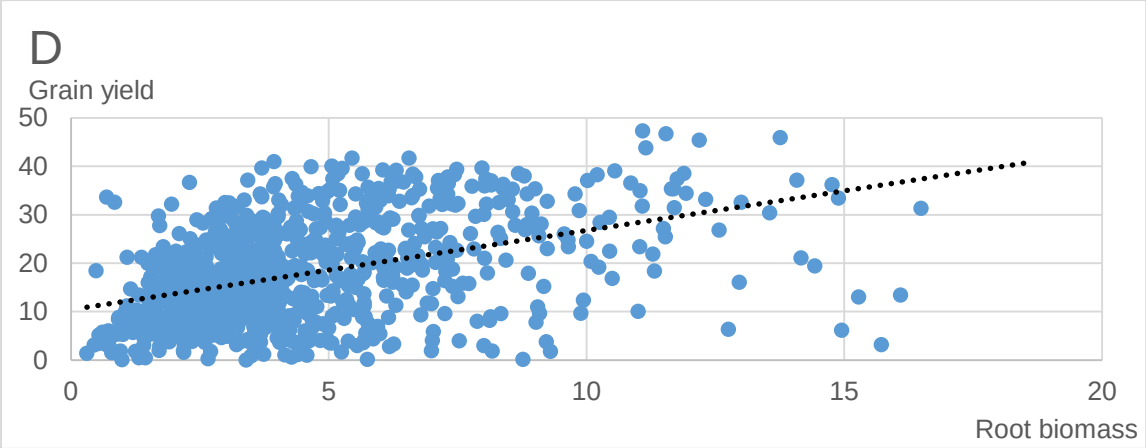


Figure 4.2: Distribution of QTL and additive effects associated with thousand grain weight (1000gW), grain yield (GY), shoot biomass (SM), total root biomass (RM), shallow root weight (SRM), deep root weight (DRM), number of fertile tillers (FT), number of tillers (NT), plant height (PH), root length (RL), days to anthesis (DTA), flag leaf width (FLW), and harvest index 1 (HI 1), over twenty one linkage groups of the bread wheat genome.


A
Root lenght
180
160
140
120
100
80
60
40
20
0
0
5
10
15
20
Root biomass


B
Days to anthesis
140
120
100
80
60
40
20
0
0
5
10
15
20
Root biomass


C
Shoot biomass
140
120
100
80
60
40
20
0
0
5
10
15
20
Root biomass


D
Grain yield
50
40
30
20
10
0
0
5
10
15
20
Root biomass

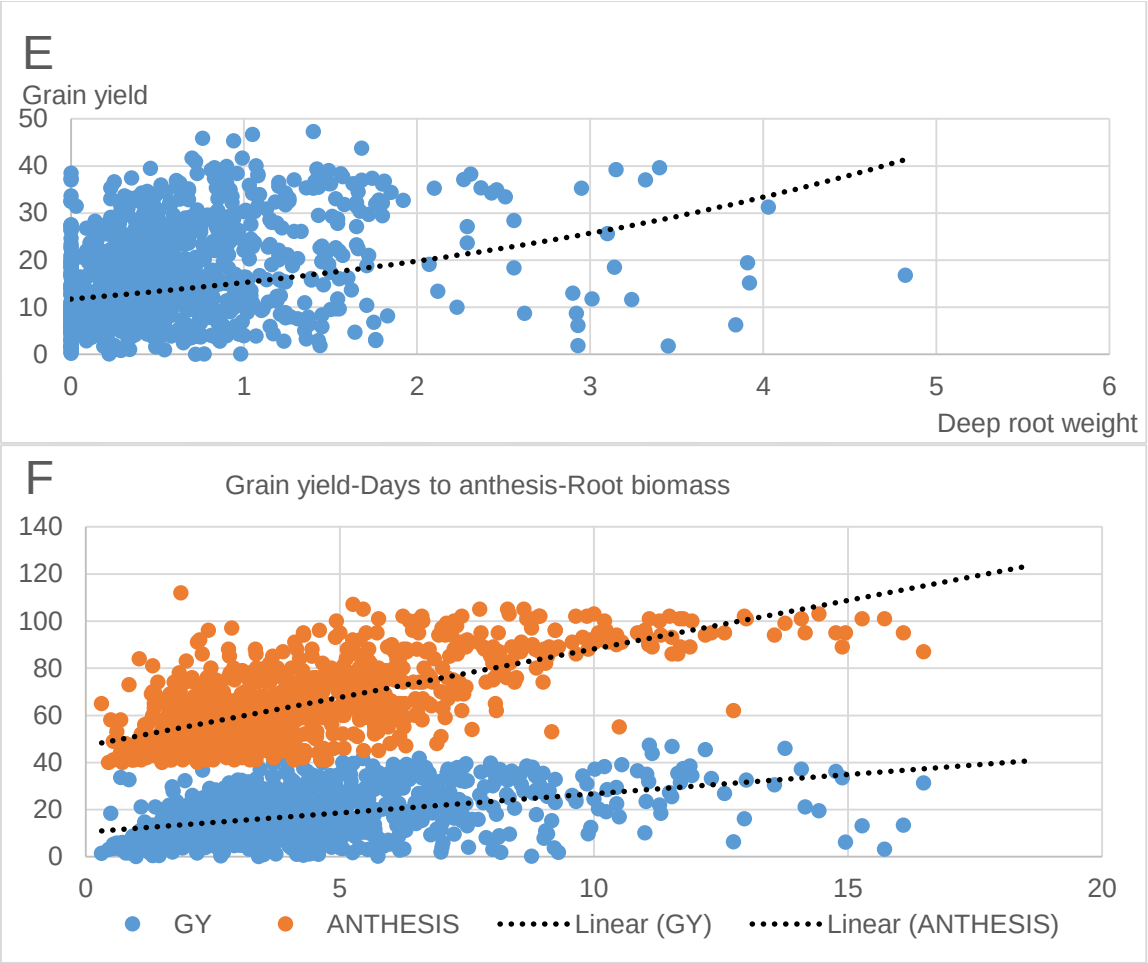


Figure 4.3: Scatter plots of interactions and trends between traits in bread wheat; **A**: Root Length (RL) vs. Root Biomass (RM), **B**: Days to Anthesis (DTA) vs. Root Biomass, **C**: Shoot Biomass (SM) vs. Root Biomass, **D**: Grain Yield (GY) vs Root Biomass, **E**: Grain Yield vs. Deep Root Weight (DRM) and **F**: Days to Anthesis vs. Grain Yield and Root Biomass. Combined data of 2013 and 2014 experiments were used for correlation analysis.

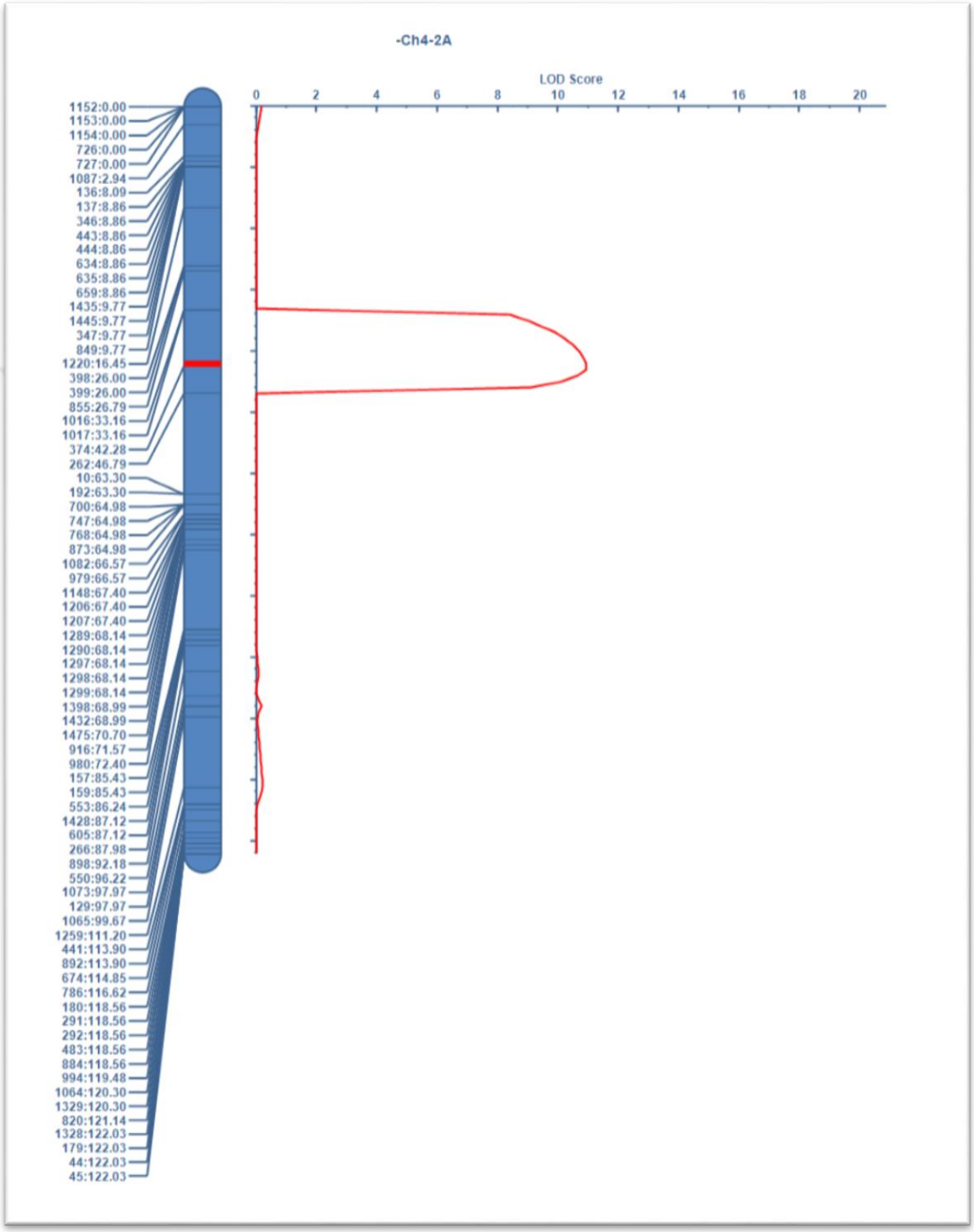


Figure 4.4 A: Location of QTL on bread wheat chromosomes 2A for deep root weight, shallow root weight, total root biomass, shoot biomass and days to anthesis with the linkage map from Poland, et al. (2012). Marker names, marker positions and LOD scores are presented on linkage map.

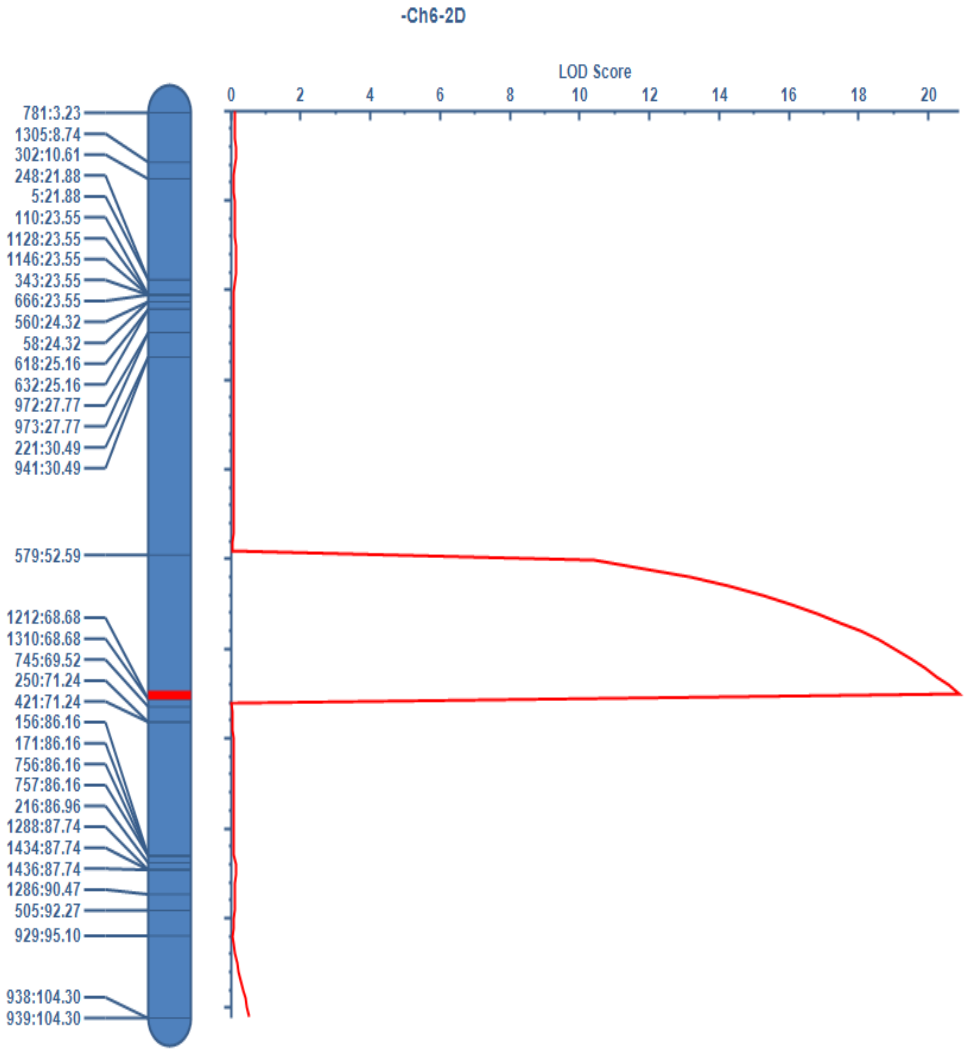


Figure 4.4 B: Location of QTL on bread wheat chromosomes 2D for deep root weight, shallow root weight, total root biomass, shoot biomass, days to anthesis, root length, flag leaf width and grain yield with the linkage map from Poland, et al. (2012). Marker names, marker positions and LOD scores are presented on linkage map.

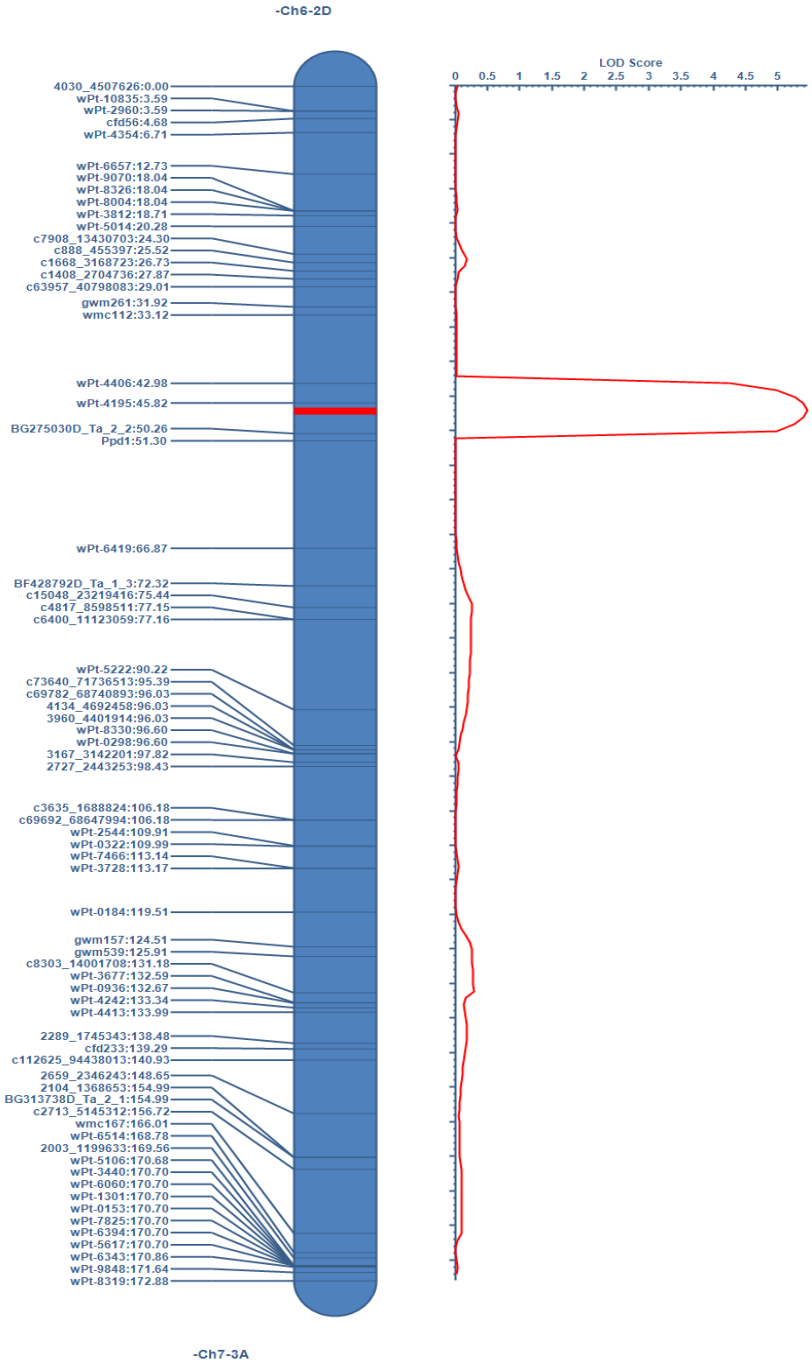


Figure 4.5: High resolution linkage map from Saintenac, et al. (2013) for the QTL on chromosome 2D of bread wheat. Marker names, locations and LOD scores are presented on the linkage map. An additional marker between the peak point of QTL and *Ppd-D1* gene is located.

Marker	Lines Position cM	3	7	14	15	17	29	34	50	77	84	96	152	193
wPt-4195	45.823	-	b	a	a	b	-	a	b	-	b	a	b	-
BG275030D_Ta_2_2	50.255	b	b	a	a	b	a	-	a	b	a	b	a	a
Ppd1	51.3	-	-	-	a	b	-	b	b	b	-	b	a	a
	DTA-2014	62.00	72.00	61.67	96.33	55.67	97.00	67.33	61.33	60.33	100.67	63.67	67.67	84.33
	RM-2014	4.01	3.62	4.50	13.13	2.26	8.37	5.27	3.77	4.06	6.37	2.50	6.39	10.32

Table 4.5: Individual lines of the SynOpDH population with markers and positions, linkage map from Saintenac, et al. (2013). Cross-over for the marker “BG275030D_Ta_2_2” that is located between day length sensitivity gene (Ppd) and QTL near the marker “wPt-4195” for total root biomass. The letter ‘a’ for Synthetic W7984 and ‘b’ for ‘Opata M85’

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General Conclusions

Three members of the family Poaceae, wheat, maize and rice provide more than 50% of our caloric intake followed by legumes (*Fabaceae*). For thousands of years farmers made selections for many favorable traits such as grain yield, leaves, roots and straw yield. Since the dawn of agriculture, man has selected, distributed and eliminated thousands of different accessions of these crops. Selection by farmers was mostly non-destructive, until the last century when genetics and modern selection techniques became a major tool. Major events in 20th century caused dramatic changes in germplasm resources. Thousands of new cultivars have been released within decades. Introduction of high yielding modern crops in many developing countries, such as India, Pakistan, Mexico, and Turkey not only doubled the grain yield of wheat, maize, and rice, it was also the cause of serious changes in farming practices and replacement of old landraces and varieties. However, it was soon realized that loss of genetic diversity was inevitable. Southern corn leaf blight on maize in early 1970s in USA, with losses reaching millions of tons of grain, illustrated the damaging effect of the excessive use of a single male sterile cytoplasm. Southern corn leaf blight was a lesson for plant researchers. Genetic variation in maize was very narrow and there was a definite need for improvement. Similar problems started occurring in other crops with any new strain of biological pathogens that caused serious yield losses. The search for allelic diversity switched to wild crop relatives, landraces, and alien translocation lines. SynOpDH bi-parental double-haploid mapping population was

developed to transfer some of the diversity from bread wheat ancestor *Ae. tauschii* into modern wheats. Similarly wide genetic diversity from other wild species was reported and some traits were successfully transferred such as disease resistance genes from wild *Aegilops* and *Triticum* taxa. In addition to wild germplasm collections, landraces were also recognized as a source for genetic variation.

The importance of genetic diversity in germplasm resources is well recognized. However, much of the research and surveys were dedicated to above-ground traits of plants. Relatively little research was published that evaluated the root system. In my dissertation I aimed to evaluate four different sets of germplasm accessions for root system traits from wheat wild ancestors, to Turkish bread wheat landraces and modern wheats, CIMMYT bread wheats, and a synthetic wheat standard mapping population in order to test the diversity for root characteristics.

In the first chapter, fifteen wild *Triticeae* accessions, including four *Ae. speltoides spp. speltoides,* two *Ae. speltoides spp. ligustica*, three *Ae. tauschii*, three *T. urartu, T. monococcum* and two *T. dicoccoides* were evaluated. Plants were grown in 1 m PVC tubes for two years under well-watered conditions until maturity. Significant differences were observed between *Aegilops* and *Triticum* taxa as well as within each of the five wild species for the shallow and deep root weights and total root biomass. Mean values for root and shoot biomass were greater in *Aegilops* spp. when compared with *Triticum* spp. Mean values for the number of tillers in *Aegilops* spp. were at least two times more than in *Triticum*

spp., which may have been a reason for greater shallow root biomass in *Aegilops spp.,* since number of tillers may increase the number of nodal roots. *T. urartu* and *T. monococcum* had smaller roots systems when compared to *Ae. speltoides, Ae. tauschii* and *T. dicoccoides*. Additionally, *Ae. speltoides* and *Ae. tauschii* had narrow root diameters and fibrous root systems, a possible indication of high hydraulic resistance. Significant novel allelic diversity for root anatomical and morphological traits were detected in the wild ancestors of bread wheat.

In the second chapter, nineteen wheat accessions from Turkish wheat germplasm were evaluated in 1 m and 1.5 m PVC tubes for two years with three separate experiments under well-watered conditions until maturity. Significant genotypic variation was observed within and between the groups of landraces and modern wheats. Landraces had significantly larger deep and shallow root weights and total root biomass when compared with modern wheats. Moreover, the number of tillers, number of fertile tillers, plant height and shoot biomass were also greater in landraces. On the other hand, harvest index, and grain yield was greater in modern wheats as expected in 2013 but not in 2014. To assess the effect of growth habit on root, shoot and grain yield traits, mean differences between eight winter, eight intermediate, and four spring wheats were compared. Intermediate wheats had higher mean values for total root biomass when compared with winter and spring types. Number of days to anthesis was 10 days longer for intermediate wheats, so that 10 extra days may have been a positive factor for additional

biomass accumulation. Winter wheats had greater average grain yield and harvest index.

The mean differences were calculated for root and shoot biomass traits between semi-dwarf (75 cm to 90 cm), mid-height (91 cm to 100 cm) and tall (>101 cm) accessions. Tall genotypes had significantly larger root and shoot biomass, maximum root length and deep root weights when compared with mid-height and semi-dwarf wheats. Since major changes in wheat breeding happened after 1970s, the Green Revolution genes or modern plant breeding may have caused a reduction on root and shoot biomass in Turkish wheat accessions.

In the third chapter, some of the parental accessions for *Rht-B1*, *Rht-D1*, and *Rht8* genes, as well as early and late generation Green Revolution wheats were evaluated up to maturity for root and shoot biomass traits in 80 cm PVC tubes under well-watered conditions for two years. Significantly larger root biomasses were observed in ‘Mentana’, ‘Nainari 60’ and landrace ‘Marroqui’ with recessive *rht-B1a and rht8a* genes when compared with accessions with dominant *Rht-B1b, Rht-D1b and Rht8b* semi-dwarfing genes. In conclusion, there was a trend for root biomass to decline from old-tall wheats to modern, semi-dwarf wheats. Given the limited number of accessions representing each group, we were not able to dissect the effect of each of the different *Rht alleles* on root and shoot traits. The study was a general survey for comparison of tall and semi-dwarf wheats for root system

traits. Therefore, results could not clearly define the role of each *Rht-B1a/b*, *Rht-D1a/b* and *Rht8a/b* alleles in tested accessions.

In the fourth chapter, 147 lines and parents from SynOpDH mapping population were evaluated until maturity in 1 m tubes under well-watered conditions for two years in 2013 and 2014. Significant genotypic variation was observed for the root and shoot biomass traits as well as grain yield. Two previously published genetic maps were used for linkage and QTL mapping. A total of 68 phenotype associated loci for fourteen different traits over fifteen linkage groups were detected. Twenty seven of these regions were only detected in 2013 and thirteen were only detected in 2014. A total of fourteen phenotype associated loci (QTL) were located in both years. Two major effect QTLs on chromosomes 2A and 2D were responsible from significant phenotypic variation for deep root weight, shallow root weight, total root biomass, shoot biomass and days to anthesis. The same QTL on chromosome 2D also explained phenotypic variation for root length, flag leaf width and grain yield. Another QTL on chromosome 4D explained some phenotypic variation for number of fertile tillers. A previously known gene *Ppd-D1* (day length sensitivity) was closely linked with the QTL on chromosome 2D, and possibly *Ppd-A1* with the QTL on chromosome 2A. Since *Ppd* genes cause day length sensitivity and longer vegetative growth, it was suspected that *Ppd* genes may cause a pleiotropic effect for biomass accumulation. In order to validate the QTL on chromosome 2D, six pairs of genotypes with maximum marker similarities,

but contrasting alleles for QTL and *Ppd-D1 gene* from parents were selected. Genotypes were grown in pots under well-watered conditions with 18 hour daylight until heading to block the effect of *Ppd* genes. Four pairs reached heading within four days of each other and other two pairs reached heading within fourteen days of each other. Mean difference for days to heading between lines with *Ppd-D1b* (day length sensitive) and *Ppd-D1a* (day length insensitive) genes was reduced to 5 days from 26 days of previous year. The mean values for root biomass for the lines with the QTL were 8% to 128% larger than lines without a QTL. Even though this validation set was small, it supports the hypothesis that chromosome 2D has a major effect QTL for root and shoot biomass traits that is in the vicinity of *Ppd-D1b* gene. Fine mapping with near isogenic lines is needed in order to validate the QTL and to evaluate any interaction between *Ppd* genes and biomass accumulation.

Overall significant genetic variation was observed within and between Turkish wheat accessions, wild wheat relatives, parents and progeny of Green Revolution wheats, and the SynOpDH standard mapping population. Important allelic diversity was observed for root characters such as deep and dense rooting, maximum root length, root thickness and large shallow and deep root weights.

Analysis of the correlation coefficient means in each chapter revealed strong positive correlation between above and below ground biomass accumulation that is consistent (0.68 to 0.83) over the entire set of accessions

tested (wild wheat relatives, landraces, modern wheats and mapping population). Four sets of accessions were significantly different for growth habits (winter, spring and intermediate), plant height (semi-dwarf, mid-height, and tall), vernalization requirements, tillering capacity, water use efficiency, evapotranspiration efficiency, and yielding capacity. Even with major genetic, phenological, morphological and physiological differences, correlations between root and shoot biomass within and between the wide ranges of accessions were similar as well as number of tillers and plant height being positively associated with root biomass. These shared correlations shed light on the change of biomass accumulation from wild ancestors to modern crops. Selection pressure for high harvest index in modern wheats is possibly the main reason for the reduction in biomass accumulation. More carbon is stored as grain in modern wheats than any other groups here. In our experiments, we observed mean root biomass of 6.29 g, 9.79 g, 5.44 g, and 3.65 g plant^{-1} for wild plants, Turkish landraces, Turkish modern wheats and CIMMYT wheats, respectively when 2013 and 2014 data were combined. These results suggest that root and shoot system size increased after domestication thru landraces and decreased again with modern genetics and plant breeding. Long term selection of landraces for efficiency and stability under various, generally low-input conditions must have resulted in larger plant sizes for maximum capacity of photosynthesis as well as water and nutrient uptakes. In conclusion, two standard characteristics of modern plant breeding; irrigation and excessive fertilizer

application, may have caused a reduction in root system size and an increase in harvest index.

Even though the above interactions clearly address positive growth pattern between above and below ground organs, we observed the opposite case, such as small root with a large shoot and vice versa. There were lines with large root biomass and low grain yield, or small root biomass with high grain yield. However these contrasting results we observed here may be just a genotype x environment interaction. Further glasshouse and field experiments are needed to test those individuals with small root - high grain yield or other lines with outlier characteristics. Overall, our observations suggest that, root and shoot development are two characteristics inherited separately, but have strong pleiotropic interactions.

A study of the root system in depth with newly developed high throughput phenotyping and genotyping technologies will help to clearly measure each part of the root system and interactions with shoot and yield components. High resolution genetic maps would make fine mapping of QTLs possible. As we progress to marker assisted selection, root traits would be part of selection procedures in order to breed cultivars with greater drought tolerance.