Quantitative and molecular analysis of water stress resistance
in wheat (*Triticum aestivum* L.)

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Statement of candidate contribution

This thesis contains published work and work ready for publication. The publications arising from this thesis are the original work undertaken by the student (Habtamu Ayalew Tamir), with guidance from his two supervisors (Prof Guijun Yan and Dr Hui Liu). This thesis was finished during the course of enrolment in a PhD degree at the University of Western Australia. This thesis or any part of it has never been submitted to any educational institution for any diploma or degree.

Publications arising from this thesis:

Chapters 3—5 from this thesis are published. These manuscripts are attached in the Appendix part of this thesis.


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Chapters 6 and 7 from this thesis are manuscripts under review. The texts in these chapters appear as in the manuscripts.


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5. Habtamu Ayalew, Hui Liu, Andreas Börner, Borislav Kobiljski, Chunji Liu, and Guijun Yan (2016) GWAS revealed that the B genome of hexaploid wheat harbours major QTLs controlling root length for water stress resistance. (to be submitted for publication) (Chapter 7).

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This thesis has been packaged as individual submitted papers. As a result, some repetition between chapters was unavoidable.
Abstract

Water stress is one of the major environmental constraints in agriculture. Wheat production has been constrained due to low moisture availability and less adapted cultivars. Adaptation to dry environments can be genetically improved through selection for longer roots. However, research to improve root traits was hampered as current root phenotyping techniques are costly and labour intensive. In this thesis, a hydroponic system was customized to enable easy access to intact roots. High molecular weight polyethylene glycol (PEG 6000) was used to induce water stress. Experiments were conducted to analyse genetic variability and inheritance of wheat root length under water stress and non-stress conditions.

The first experiment evaluated a total of 838 genotypes in an augmented complete block design with seven blocks, and six standard control varieties. Highly significant differences were observed under both stress and non-stress growing conditions. Osmotic stress caused an average reduction of 54% root length. Colotana 296-52, Compare, Santa Elena, and Tammarin Rock, were identified as the most resistant genotypes while Aus 16356, Elia, Camm, Portugal 3, and Sentinel were the most susceptible. The same phenotyping setup was used to evaluate 248 Ethiopian wheat genotypes revealing high level of genetic variability.

Following the identification of contrasting genotypes in the first experiment, four spring wheat lines were selected and crossed in a full diallel design. All of the parental lines and their F1’s were evaluated under the same level of water stress as in the first experiment. Analysis showed highly significant differences among the parental lines and their F1 progenies. Long root was found to be dominant and the gene effects were additive. Dominant and over-dominant control of root length under water stress suggests that root length can be improved through hybrid breeding approach.
Even though quantitative genetic analysis of traits based on phenotypic data is one of the most important approaches in genetics, the specific QTLs/genes responsible for the observed variation remain unknown. Therefore, associating phenotypic and molecular data is essential to identify and map QTLs. A RIL mapping population derived from a cross between Synthetic (W7984) and Opata 85 wheat lines was evaluated to identify QTLs under stress and non-stress conditions. A total of eight major QTLs; four under each water condition, were detected. QTLs $Q_{rl.uwa.1BL}$, $Q_{rl.uwa.2DS}$, $Q_{rl.uwa.5AL}$ and $Q_{rl.uwa.6AL}$ combined explaining 43% of phenotypic variation were identified under non-stress condition. Opata was the source of favourable alleles for all of the QTLs except $Q_{rl.uwa.6AL}$. QTLs $Q_{drl.uwa.1AS}$, $Q_{drl.uwa.3AL}$, $Q_{drl.uwa.7BL.1}$ and $Q_{drl.uwa.7BL.2}$ explaining a combined 47% of phenotypic variation were identified under water stress. Synthetic wheat contributed favourable alleles for $Q_{drl.uwa.1AS}$ and $Q_{drl.uwa.3AL}$ while Opata contributed positive alleles to the remaining two QTLs on chromosome 7BL ($Q_{drl.uwa.7BL.1}$ and $Q_{drl.uwa.7BL.2}$). Three validation populations were developed by crossing cultivars Lang, Yitpi, and Chara with Synthetic to transfer two of the four QTLs identified under stress condition. The $F_{2.3}$ and $F_{3.4}$ validation lines were phenotyped under the same level of water stress as RILs were evaluated to examine the effect of these QTLs. There were 13.5% and 14.5% increases in average root length due to the inheritance of $Q_{drl.uwa.1AS}$ and $Q_{drl.uwa.3AL}$, respectively. The result indicated that closely linked SSR markers $Xbarc148$ ($Q_{drl.uwa.1AL}$) and $Xgwm391$ ($Q_{drl.uwa.3AL}$) can be incorporated into marker assisted selection for water stress improvement in wheat.

The fifth experiment evaluated a core collection of 91 winter wheat genotypes for genome wide association mapping. Average LD decay was approximately 35 cM with 0.25 $r^2$ cut-off value. A total of five DArT markers were significantly associated with root length. The B genome harboured the entire major water stress resistance QTLs.
GWAS identified two QTLs one chromosome 3B one each under stress and non-stress conditions. Water stress resistance QTLs from the two mapping experiments on chromosomes 3A and 3B collocated with the *DREB1A* and *DREB1B* genes, respectively.

In conclusion, this study showed the presence of high genetic variability of root length among worldwide wheat collections which can be exploited to breed drought resistant cultivars. It has also demonstrated the potential of hybrid wheat breeding to improve drought resistance. Several QTLs associated with root length were identified indicating the potential of marker assisted breeding to improve water stress resistance. The validated SSR markers can be incorporated into marker assisted breeding, marker assisted back crossing, and for the development of near isogenic lines for further research. QTLs on the 3A and 3B homeologues of wheat need to be further analysed to precisely characterize the putative genes for water stress resistance.
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My parents, parents-in-law, sisters, and brothers, thank you for your love and encouragement. My wife Menby and our son Binyam, thank you so much for your love and patience which gave me the courage to pursue further.

I praise the Almighty God, nothing would have been done without Him.
Dedication

I dedicate this thesis to my grandpa, Mr Chanie Alemu who was able to foresee my future and did everything to convince my parents to get me to school.
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List of abbreviations

a Additive genetic effects
AFLP Amplified fragment length polymorphism
ANOVA Analysis of variance
b Dominance genetic effects
b1 Mean deviation of F₂ from the mid-parental value
b2 Variation of deviation of F₂ from their mid-parental value
b3 Part of dominance variation unique to each F₂
BIC Bayesian information criterion
cM CentiMorgan
CIM Composite interval mapping
CMLM Compressed mixed linear model
DHL Doubled haploid line
D Variance due to additive effects
DArT Diversity array technology
DNA Deoxyribonucleic acid
dNTPs Deoxyribonucleotide triphosphate
EMMA Efficient mixed model association
GAPIT Genomic Association and Prediction Integrated Tool
GCA General combining ability
GWAS Genome wide association scan
H² Broad-sense heritability
H1 Dominance variance (overall)
H2 Directional dominance variance
h² Narrow-sense heritability
H2/4H1  Proportion of genes with positive and negative effects
√(H1/D) Average degree of dominance
Hi-Di Highly deionized formamide
ICIM Inclusive composite interval mapping
ITMI International Triticeae Mapping Initiative
LD Linkage disequilibrium
LOD Logarithm of odds
MAF Minor allele frequency
MAS Marker assisted selection
PCA Principal component analysis
PCR Polymerase chain reaction
PEG Polyethylene glycol
QTL Quantitative trait locus
RAPD Random amplified polymorphic DNA
RFLP Restriction fragment length polymorphism
RFU Relative fluorescence unit
RIL Recombinant inbred line
SCA Specific combining ability
SNP Single nucleotide polymorphisms
SSR Simple sequence repeat
TASSEL Trait analysis by association, evolution and linkage
UPGMA Unweighted pair group method with arithmetic mean
Vp Variance of inbred parents
Vr Variance of all array means
Wr Covariance between parental and F2 hybrids
Chapter 1 General introduction

1.1 Background

Wheat is one of the earliest cereals domesticated. It is a staple food for more than 35% of the world population providing over 20% of global food supplies (Bushuk 1998, Ortiz et al. 2008). Wheat grows well between the latitudes 30° and 60°N, and 27° and 40°S (Briggle and Curtis 1987). According to a USDA (2016) report, a total of 728 million metric tonnes of wheat was produced globally in the 2014/2015 cropping season. The global wheat production and productivity have been on the increase in the past years (Cattivelli et al. 2008, Curtis et al. 2012). Nevertheless, the current productivity of wheat, and all other crops, needs to be further increased to feed the ever increasing population of the world (Jaradat 2011). Nearly a billion people were reported to be food-insecure worldwide, the highest prevalence being in Africa (FAO 2012). The world population is estimated to be about 8 billion by 2025, and the world wheat production has to be increased by about 200 million metric tonnes annually to meet the wheat demand (Alexandratos and Bruinsma 2012, Curtis 2002).

The effort to improve production and productivity of wheat is, however, deterred by different biotic and abiotic stresses. Drought stress is one of the most serious environmental constraints limiting crop growth and productivity in many regions. Drought has been one of the major causes of food crisis in the horn of Africa. Sporadic mega droughts have been hitting the region leaving millions to starvation and disease (Stager et al. 2011, Chuhan-Pole 2015). Australia is also one of the driest regions in the world suffering from recurrent drought. Drought has impacted wheat farmers in Australia and all over the world (Dai 2011). Wheat production was reduced by half (10 million tonnes) during the 2002/2003 drought in Australia (Balouchi 2010, Productivity Commission 2010). In the 2006/2007 cropping season, there was a loss of 9.8 million
tonnes, which was 15.5 million tonnes less than the production in 2005/2006 in Australia (ABARE 2007). Apart from those mega droughts that mostly associated with the increased El Niño episodes (Dai 2011), physiological drought can also happen in a fairly wet season causing drastic reductions in crop productivity.

Drought mitigation mechanisms range from crop management like mulching to full or supplemental irrigation. However, irrigation is not a feasible strategy due to water scarcity and the physical unavailability of fresh water globally. One of the options the best options to mitigate drought is to select genotypes that are adapted to dry environments. Identification of new sources of drought resistance genotypes genes and pyramiding these genes into a cultivar are of paramount importance for effective and better adaptation.

Drought happens at various intensities and durations across the crop development cycle. Early season drought is one of the major causes for poor crop establishment. Seedling establishment is critical for a crop in its future survival and productivity. Water stress at this early stage was reported to have adverse effect hampering further crop establishment (Blum et al. 1980, Manschadi et al. 2006, Demissie and Fujimura 2010, Govindaraj et al. 2010). However, unlike the later stage traits of wheat, research and documentation is scant on germination and seedling reactions to water stress. Screening large number of lines with different genetic backgrounds may give the opportunity to identify lines genes that enable better resistance to early stage water stress.

Screening for drought is one of the most expensive and labour intensive activities to routinely exercise over large number of genotypes. It becomes even more complicated when root is the target trait. Selection and gene pyramiding can be made more efficient
through benchmarking molecular markers that are closely associated with the genes of interest. However, it needs a lot of research before marker assisted selection is practiced. A reliable marker trait association has to be established with a sizable gene/QTL effect that can be tracked down in progenies.

1.2 Objective of the study

The overall objective of this research project was to identify drought resistant genotypes for early season drought and map QTLs for marker assisted breeding. To meet the major objective stated above, the following specific objectives were targeted.

- Evaluate a large number of wheat germplasm to identify drought tolerant genotypes.
- Identify lines with specific and general combining abilities and investigate the nature of gene actions through diallel analysis.
- Understand the inheritance of drought resistance through QTL mapping and marker validation for MAS.
- Identify and map QTLs using GWAS approach on an unstructured population.

1.3 Thesis structure

Findings in this thesis research are presented in eight chapters: general introduction, literature review, five major research chapters and general discussion and conclusion.

Chapter one gives general background of the study and the rationale behind the study.
Chapter two provides a brief background on wheat origin and diversity, production and productivity, production constraints, the adverse impacts of water stress on wheat, and wheat breeding for drought resistance.

Chapters three and four address the phenotypic evaluation and identification of drought resistant wheat genotypes for downstream genetic analysis. A large number of wheat genotypes collected from all over the world were evaluated for water stress resistance and the extremely resistant/susceptible wheats were identified for crossing.

Chapter five describes findings on the genetic control of drought resistance in bread wheat (*Triticum aestivum* L.) as revealed by $4 \times 4$ full diallel cross analyses. This study revealed that drought resistance in wheat is governed by dominantly inherited additive genes.

Chapter six describes the use of recombinant inbred lines (RILs) to identify and map QTL conferring water stress resistance in wheat. In this study four major water stress resistance QTLs were detected and the closest markers were used to validate the phenotypic effect of the identified QTLs in other cultivars and the importance of markers for MAS.

Chapter seven describes the use of unstructured populations for QTL identification using genome wide association study (GWAS). In this study two major loci for root length under water stress and three under non-stress condition were detected.

Chapter eight discusses the significant findings of this research and implication for future studies.
Chapter 2  

Literature review

2.1  

Origin and diversity of wheat

2.1.1  

Geographical origin of wheat

Cultivated wheat is believed to originate some 10,000 years back in history along the Fertile Crescent (Feldman and Kislev 2007, Shewry 2009); the present days of Israel, Lebanon, Jordan, Syria, and Iraq where both cultivated and wild progenitors of the crop are still co-found (Acquaah 2007). Emmer and einkorn wheats were among the founder crops that initiated agriculture in the old world (Zohary 1999, Feldman and Kislev 2007). The earliest einkorn (*Triticum monococcum* L.) remains were found in northern Syria while the first definite domesticated einkorn wheat was found in southern Turkey (Zohary 1999, Zohary et al. 2012).

Similarly, it is in Syrian archaeological sites where the first cultivated emmer (*Triticum turgidum sp. dicoccum*) wheat, dating back to 7,500 BC, was discovered (Zohary 1999). Dinkel wheat (hexaploid, 2n = 6x = 42) is a new wheat species that evolved under domestication (no wild forms exist) from the domesticated *T.turgidum* stock (Zohary et al. 2012).

Hexaploid wheat is the hybridization product between tetraploid *Turgidum* wheat and a wild diploid grass *Aegilops tauschii* (= *Ae. Squarrosa* L). There are no wild counterparts or wild progenitors for the hexaploid species *T. aestivum* (2n = 6x = 42, AABBDD) and *T. zhukovsky* (2n = 6x = 42, AABBGG) (Zohary et al. 2012) (Fig. 2.1).
2.1.2 Evolution and diversity of wheat

Wheat belongs to the family Poaceae and genus Triticum (Acquaah 2007). The Genus Triticum consists of a series of ploidy levels of wheat species which are generally classified into three ploidy levels, i.e. diploids ($2n = 14$), tetraploids ($2n = 28$) and hexaploids ($2n = 42$) which are often called einkorn, emmer, and dinkel, respectively (Feldman and Levy 2005). According to Goncharov et al. (2009), there are 29 species under the genus Triticum, six of which are synthetic species (Table 2.1). Around 7,000 BC, the first domestic diploid wheat, einkorn (Triticum monococcum) was brought under cultivation directly from its wild form Triticum monococcum (= ssp Triticum aegilopoides) followed by the domestication of the cultivated emmer wheat Triticum
*dicoccum* (*2n = 2x = 28, AABB*) that adapted from the wild emmer *Triticum dicoccoides* (*2n = 2x = 28, AABB*) (Feldman and Levy 2005).

Emmer wheat (*Triticum dicoccum*) is the result of an amphiploidy between *Triticum urartu* (*2n = 2x = 14, A^U&A^U*) and *Aegilops speltoides* (*2n = 2x =14, BB*) (Feldman and Levy 2005). Hexaploid wheat is believed to be the product of two successive hybridization events. In the first hybridization event the ‘A’ genome progenitor (*Triticum urartu = A^A^A^A*) combined with the ‘B’ genome progenitor to form a primitive tetraploid wheat (*2n = AABB*), although it still remains daunting to exactly indicate which species is the B genome donor (Gupta *et al.* 2005). The second event involved hybridization between the tetraploid (*AABB*) and the D genome progenitor (*Aegilops tauschii ssp squarrosa*) to produce the basic hexaploid configuration AABBDD (Kimber and Sears 1987). The first hybridization resulted in the formation of allotetraploid wheat (half a million years ago) and the second in the formation of hexaploid wheat (approximately 10,000 years ago) (Feldman and Levy 2005, Feldman *et al.* 2012). van Slageren (1994), Unlike Goncharov *et al.* (2009), adopted a classification that recognizes two species from each of the ploidy levels *i.e.* two diploid species (*Triticum monococcum* L. and *Triticum urartu* Tum. ex Gand.), two tetraploid species (*Triticum turgidum* L. and *Triticum timopheevii* (Zhuk.) Zhuk.) and two hexaploid species (*Triticum aestivum* L. and *Triticum zhukovskyi* Men. & Er).
Table 2.1 Phylogenetic classification of wheat (Goncharov, 2009).

<table>
<thead>
<tr>
<th>Section</th>
<th>Group of species</th>
<th>Species</th>
<th>2n</th>
<th>Genomes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monococcum Dum.</strong></td>
<td>Hulled</td>
<td><em>T. urartu</em> Thum. ex Gandil.</td>
<td>14</td>
<td>A&lt;sup&gt;u&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. bioticum</em> Boiss.</td>
<td>14</td>
<td>A&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. monococcum</em> L.</td>
<td>14</td>
<td>A&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Naked</td>
<td><em>T. sitkajae</em> A. Filat et Kurk.</td>
<td>14</td>
<td>A&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Dicoccoides Flaksb</strong></td>
<td>Hulled</td>
<td><em>T. dicoccoides</em> (Korn. Ex Aschers. Et Graebn.) Schweif</td>
<td>28</td>
<td>BA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. dicoccoides</em> (Schrank) Schuebl.</td>
<td>28</td>
<td>BA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. karamyschevii</em> Nevski</td>
<td>28</td>
<td>BA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. ispahanicum</em> Heslot</td>
<td>28</td>
<td>BA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Naked tetraploids</td>
<td><em>T. turgidum</em> L.</td>
<td>28</td>
<td>BA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. durum</em> Desf.</td>
<td>28</td>
<td>BA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. uranicum</em> Jakubz</td>
<td>28</td>
<td>BA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. Polonicum</em> L.</td>
<td>28</td>
<td>BA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. ethiopianicum</em> Jakubz</td>
<td>28</td>
<td>BA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. carthlicum</em> Nevski</td>
<td>28</td>
<td>BA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Triticum</strong></td>
<td>Hulled</td>
<td><em>T. macha</em> Dekapr. Et Menabde</td>
<td>42</td>
<td>BA&lt;sup&gt;a&lt;/sup&gt;D</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. spelta</em> L.</td>
<td>42</td>
<td>BA&lt;sup&gt;a&lt;/sup&gt;D</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. vavilovii</em> (Thum) Jakubz</td>
<td>42</td>
<td>BA&lt;sup&gt;a&lt;/sup&gt;D</td>
</tr>
<tr>
<td></td>
<td>Naked hexaploid</td>
<td><em>T. compactum</em> Host</td>
<td>42</td>
<td>BA&lt;sup&gt;a&lt;/sup&gt;D</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. aestivum</em> L.</td>
<td>42</td>
<td>BA&lt;sup&gt;a&lt;/sup&gt;D</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. sphaerococcum</em> Pervic.</td>
<td>42</td>
<td>BA&lt;sup&gt;a&lt;/sup&gt;D</td>
</tr>
<tr>
<td><strong>Timopheevi A. Filat.</strong></td>
<td>Hulled</td>
<td><em>T. araraticum</em> Jakubz</td>
<td>28</td>
<td>GA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. timopheevii</em> (Zhuk.) Zhuk.</td>
<td>28</td>
<td>GA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. zhukovskyi</em> Menabde et Erizjan</td>
<td>42</td>
<td>GA&lt;sup&gt;a&lt;/sup&gt;A&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Compositum N. P</strong></td>
<td>Hulled</td>
<td><em>T. palmovae</em> G. Ivanov (syn. <em>T. erubini</em> Gandil.)</td>
<td>28</td>
<td>DA&lt;sup&gt;a&lt;/sup&gt; (DA&lt;sup&gt;a&lt;/sup&gt;)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. dimonococcum</em> Schieman et Staudt.</td>
<td>42</td>
<td>BA&lt;sup&gt;a&lt;/sup&gt;A&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. kiharae</em> Dorof. Et Migusch.</td>
<td>42</td>
<td>GA&lt;sup&gt;a&lt;/sup&gt;D</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. soveticum</em> Zhebrak</td>
<td>56</td>
<td>BA&lt;sup&gt;a&lt;/sup&gt;GA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>T. borissi</em> Zhebrak</td>
<td>70</td>
<td>BA&lt;sup&gt;a&lt;/sup&gt;DGA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Naked octaploid</td>
<td><em>T. flaksbergeri</em> Navr.</td>
<td>56</td>
<td>GA&lt;sup&gt;a&lt;/sup&gt;BA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
2.2 Wheat production

Cultivated wheat is largely composed of common wheat (\textit{Triticum aestivum}) and durum wheat (\textit{Triticum durum}). Wheat accounted for about 29\% of total cereal production in the 2015 cropping season worldwide with a total production of about 733 million tonnes of grain (FAO 2016). Nearly 90\% of wheat production worldwide is covered by bread/common wheat while durum wheat accounts for 5\% of the production. China, India, Russian Federation and USA were among the top wheat producers in the 2014/15 cropping season (FAOSTAT 2015) (Table 2.2).

Table 2.2 Top wheat producing countries, area covered (million hectares), total production (million tons) and productivity (tons per hectare) in 2014/15 (FAO 2016).

<table>
<thead>
<tr>
<th>Country</th>
<th>Area harvested</th>
<th>Total production</th>
<th>Productivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>China, mainland</td>
<td>25.0</td>
<td>126.2</td>
<td>5.0</td>
</tr>
<tr>
<td>India</td>
<td>31.2</td>
<td>94.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Russian Federation</td>
<td>23.9</td>
<td>59.7</td>
<td>2.5</td>
</tr>
<tr>
<td>United States of America</td>
<td>18.8</td>
<td>55.4</td>
<td>2.9</td>
</tr>
<tr>
<td>France</td>
<td>5.3</td>
<td>39.0</td>
<td>7.4</td>
</tr>
</tbody>
</table>

2.2.1 Durum wheat production

Durum wheat is adapted to a vast array of agroecologies ranging from tropical to temperate (Jaradat 2011). It is better adapted to the dry Mediterranean climate than bread wheat (Kneipp 2008, Shewry 2009). Even though durum wheat is adapted to various agroecologies, its production coverage has remained low (Autrique \textit{et al.} 1996, Mengistu \textit{et al.} 2016). It covers only about 5\% of the total wheat production in the world. Durum wheat generally has large, solid, vitreous, and plump kernels with protein content of 13\%–22\% of their dry weight (Blanco \textit{et al.} 1996). In contrast to bread wheat, durum wheat has a better cooking quality, harder grain, an intense yellow colour and a nutty flavour (Kneipp 2008).
2.2.2 Bread wheat production

Bread wheat (*Triticum aestivum* L.) is the most variable and economically most important aggregate of domesticated wheat globally. Wheat is considered as one of the strategic food crops in Ethiopia and Africa at large (Tadesse *et al.* 2015, World Bank 2016). Ethiopia produced about 2.3 million metric tons of wheat grain on 1.4 million hectares of land in the 2015/16 cropping season (CSA 2016). The Ethiopian national average wheat productivity is low (1.6 t/ha) as compared to the global average (3 t/ha) mainly due to drought.

Wheat in Australia is widely grown both for domestic consumption and for exotic markets (Balouchi 2010). Australia was the 8th largest wheat producer in 2015 with 24.5 million tonnes of grain in the world. China, India, and the US were leading producers with production volumes of 120.6, 93.9, and 61.8 million metric tonnes of grain respectively during the same cropping season (FAOSTAT 2015). Western Australia and New South Wales are the leading wheat producing states in Australia contributing 40% and 30% of national wheat production respectively (Productivity Commission 2010). Large proportion (about 60%) of Australia’s wheat is exported to Asian countries.

2.3 Major wheat production constraints

Wheat in general is one of the widely cultivated crop species in the world owing to its adaptation to wide range of ecologies and its nutritional value. However, it suffers many production constraints which are exacerbated by the prevailing climate change (Swaminathan and Kesavan 2012). In Australia, the yield loss caused by different diseases to wheat was significant; the average annual loss of the year 2009, for example
being about a worth of 913 million AUD (Ma 2010). A paralleled damage is inflicted by the abiotic constraints like drought, heat, salinity and cold stress.

Drought is the single most serious obstacle for crop production in Australia and in the world at large. The impact of drought is dependent on the stage of crop growth, the stress severity, and the duration of the stress (Blum et al. 1980, Passioura 2012, Tuberosa 2012).

Drought can be expressed as a three dimensional entity explained by the stress severity, duration of stress and the stage of the crop. As a result, there are very diverse and sometimes conflicting conclusions in many reports on the subject. Drought results in changes in the physiological and biochemical processes (Dai et al. 1997) which control plant growth and development. One needs to use exactly the same levels of the three dimensions to come up with a comparable result (Blum 2011a).

Early growth stage evaluation for drought resistance is reported to be pivotal in distinguishing between drought resistant and susceptible genotypes (Singh et al. 1999, Demissie and Fujimura 2010, Govindaraj et al. 2010, Hoffmann et al. 2012). Drought resistance is relatively high in a germinating seed which later deteriorates as the plant advances to a seedling stage (Blum 2005). Similarly, Szira et al. (2008) reported that barley seedlings showed a relatively lower resistance index as compared to adult plants.

Soils at field capacity, -0.01 and -0.03 MPa, are generally considered to be optimum for plant growth and soil microbial respiration (Krizic et al. 2004). If water is continually taken-up by plants and no additional water is added, then the water potential of the soil will drop to about -1.5 MPa which eventually causes permanent wilting (Krizic et al. 2004).
2.4 Breeding for abiotic stress resistance

Drought, salinity and nutrient deficiency are among the various causes of abiotic stress in crop production (Acquaah 2007, Jenks et al. 2007). Nevertheless, not all crop varieties are equally affected by environmental stresses. Most crop species show considerable genetic variation in response to environmental stress factors which creates a fertile ground for successful implementation of plant breeding. Plant breeding tools enable the improvement of complex traits like drought and heat resistance through manipulation of the heritable component of genetic variation. The challenge remains on how to find those best adapted genotypes and understand their mechanism of adaptation.

2.5 Conventional breeding for drought resistance

Drought resistance, from the agricultural context can be defined as the ability of plants to survive, and give satisfactory yield under limited water supply or under periodic conditions of water deficit (Turner 1979). Breeding for drought resistant genotypes is one of the most effective and ecofriendly approaches to overcome the impact of drought now and in future (Blum 2011b). Wheat, being the most important and widely consumed crop all over the world, has enjoyed significant scientific attention and funding. As a result of breeding endeavours done so far, the productivity of wheat has been doubled and different biotic and abiotic resistance QTLs have been identified and mapped.

The conventional approach for drought resistance breeding was based on the assumption that high yielding genotypes in an optimum condition will do better under stressed conditions as well. However recent research has found that drought resistance
is dependent on both crop growth stage and the nature of stress which calls for the need to test genotypes under target environments (Tardieu 2012). Generations of selection only on one target environment can also erode the genetic base of the crop which makes genetic improvement sluggish to stagnant as a result of the narrowing down of the germplasm pool (Blum 2011a, Grassini et al. 2013).

2.5.1 Phenotyping for drought resistance

Drought resistance mechanisms can be classified as dehydration avoidance, dehydration tolerance and drought escape. Dehydration avoidance and dehydration escape mechanisms have some similarity that in both cases the plant is not exposed to the stress condition by either having an extensive root that helps in acquiring water from deep soil layers or by bearing fruit before the stress comes (Blum 2011a). Dehydration resistance on the other hand is the ability of a plant to strive in a dehydrated condition. The most effective mechanism of drought resistance is dehydration avoidance, implying the need to invest more effort in traits that contributing to this mechanism (Blum 2011a).

Drought resistance, being a quantitative trait, is highly affected by the environment and it is also stage specific requiring breeders to work on different growth stages and for unlimited stress levels, which further complicates the task (Tuberosa 2012). Development of wheat cultivars with the ability to maintain high production under drought stress has been a significant objective of wheat breeders (Balouchi 2010). Variations in agro-morphological traits under drought stress and non-stress conditions are used as drought indices to distinguish between drought resistant and susceptible genotypes (Mitra 2001). Morpho-physiological characteristics of wheat such as dry matter yield, water use efficiency and chlorophyll fluorescence are influenced by water
stress and the genetic composition of plants (Ehdaie et al. 1991), indicating the possibility of screening germplasm for these traits. Constitutive traits like deep root system, fine roots with small diameters (Blum 2011a, Comas et al. 2013), leaf rolling, leaf waxy layer, and osmotic adjustment (Blum 2011a) are among the frequently studied traits that impart dehydration avoidance to plants.

Drought is a stage specific phenomenon in wheat (Blum et al. 1980, Szira et al. 2008). As a result, any plant breeding effort to bring about improvement in resistance to drought should consider designing the right drought scenario to a particular environment (Blum 1996, Passioura 2012). Despite the fact that resistance to drought is dependent on the stage of crop growth, resistance at any stage contributes directly or indirectly for the successful growth at later stages. For instance, genotypes with early vigour can enhance water use by minimizing evaporative loss of water from the soil surface and making use of the existing moisture in time which facilitates faster root and shoot growth. The less direct importance of early stage resistance is that the possible extrapolation of results across different growth stages; however, careful analysis is advised in this regard (Hoffmann et al. 2012).

Root traits are among the major targets of phenotyping in modern plant breeding because of their obvious involvement in accessing water. Though it is a simple logic that roots are organs of prime importance for water acquisition, measuring the various root characteristics has never been easy due to their obscured nature. Root traits can be improved by screening genotypes at early stage which can also be used as an indicator for later stage resistance (Hoffmann et al. 2012, Comas et al. 2013, Blum 2014). Researchers have developed different phenotyping protocols though there is no agreement on any single approach (Blum 2014). Some suggest growing plants in the
field and measure above ground plant performance without any clear understanding of
the rooting nature, while others emphasise on understanding the genetics of root
variation and their inheritance irrespective of the growing medium. As a result,
hydroponic culture was a medium of choice for many researchers for the latter.

2.5.2 Genetic variability and inheritance

Genetic variability is the foundation for any successful plant breeding program (Allard
1999, Tadesse et al. 2012). Genetic variability can be accessed from naturally occurring
 genetic variants of a species, through importation, or through hybridization (Sleper and
Poehlman 2006). Understanding the pattern and extent of genetic diversity in a
population is important to establish a successful crop improvement programme. It
provides valuable information for line selection from the existing diversity and
introgression of desirable genes into established cultivars.

Genetic variability has been the main focus of study on various crops (Autrique et al.
 plant parts, and for root genetic variability (Davies 2007, Chloupek et al. 2010,
Atkinson et al. 2015) to mention only a small portion of accumulated literature.

Observed phenotypic variation is a cumulative effect of underlying genes and the
environment (Bos and Caligari 2007). Heritability is a portion of genetic variation that
passes to progenies and plays the role for the resemblance between relatives (Falconer
and Mackay 1996, Bos and Caligari 2007). Not the entire component of genetic
variability is passed to progenies. It is only the additive component of variation that is
reliably inherited in a population. Heritability can also refer to the extent to which a
certain trait variability is due to factors other than environment. As a result, heritability
is often classified as broad sense (H2) and narrow sense (h2). Heritability values hugely vary depending on the nature of genetic control of traits, available variability and effect of the environment.

2.5.3 Combining ability and heterosis

Existing genetic variability can be directly used as a platform to select drought resistant varieties (Moose and Mumm 2008) or selected lines can be hybridized to create more genetic variation and heterotic vigour (Hallauer et al. 2010, Whitford et al. 2013). Crosses with contrasting genotypes can produce transgressive progenies in both directions of the parents which can be used as a breeding stack for selection. The genetic variability following crosses can also be used to analyse how the traits under study are genetically controlled in the population (Hayman 1954, Mather and Jinks 1982, Fridman 2015).

During the random combination of gametes at fertilization, some parents may give some specifically good combinations of gametes that can give better performing progeny with one or a few lines than with others while some genotypes may combine fairly stably across several lines. The former is termed as specific combining ability (SCA) while the latter is general combining ability (GCA) (Hallauer et al. 2010). Analysing general combining ability helps select lines with wider genetic base and those that are able to give heterosis in combination with various lines while SCA identifies parents for hybrid seed production (Whitford et al. 2013).

Knowledge of the genetic control of a trait is essential for designing a successful breeding strategy. Even in this era of genomics, making crosses among contrasting genotypes followed by quantitative analysis is frequently used to study the different
gene actions and mechanism of their inheritance. Several previous studies employed this strategy to figure out the nature of inheritance of traits in different crops including wheat (Danehloueipour et al. 2007, Lippman and Zamir 2007, Fan et al. 2014, Song et al. 2015). Different crossing designs and data analysis methods have been developed depending on the nature of the data and mating design used (Hayman 1954, Griffing 1956, Mather and Jinks 1982). Among them, diallel mating design is the most explored and frequently used (Hallauer et al. 2010). It works by partitioning the phenotypic variance into different sources of variation. Various genetic parameters controlling testis such as additive and non-additive genetic effects, the dominance versus recessive genetic interactions, heritability (broad sense and narrow sense), and the involvement of major or minor genes in controlling traits were reported using diallel analysis in various crop species (Danehloueipour et al. 2007, Crestani et al. 2012, Laude and Carena 2014).

2.6 Molecular genetics and breeding

2.6.1 Genetic markers

Technological advancement in the area of molecular biology and genetics has brought about the use of molecular markers in plant and animal breeding to facilitate various genetic analysis activities ranging from variability analysis to gene pyramiding (Collard et al. 2005, William et al. 2007, Collard and Mackill 2008, Xu 2010).

Genetic markers can be categorised as classical markers (biochemical, cytological and morphological markers), and molecular markers or deoxyribonucleic acid (DNA) markers (Collard et al. 2005, Semagn et al. 2006). The use of morphological markers for breeding and selection has limitations due to their finite number, environmental dependence and the need to grow plants to the stage of maturity in case of some traits
The advancement of molecular biology has made various kinds of molecular markers available, suiting the diverse needs of scientists (Collard et al. 2005, Semagn et al. 2006, Miah et al. 2013, Singh and Singh 2015). Restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs) diversity array technologies (DArT) and single nucleotide polymorphisms (SNP) are among the frequently used DNA markers in order of their chronology.

DNA markers can be classified into two based on the mechanism of fragment amplification as Southern hybridisation-based (non PCR based) and polymerase chain reaction (PCR) based markers (Semagn et al. 2006, Agarwal et al. 2008). RFLPs are the most commonly used non PCR based markers (Xu 2010).

Most often used PCR-based markers include AFLPs and SSRs (Vignal et al. 2002, Semagn et al. 2006, William et al. 2007, Jiang 2013). DNA markers are especially valuable when they are able to differentiate between close relatives of individuals in the same species (Hildebrand et al. 1994). Such markers are known as polymorphic markers for genotypes while markers that cannot differentiate between genotypes are termed as monomorphic markers.

A marker can be regarded as dominant or co-dominant depending on its ability to discriminate between heterozygotes from homozygotes. A co-dominant marker suggests alterations in allele size, while dominant markers are scored based on their presence or absence. AFLP and SSR markers are among the highly polymorphic and frequently used co-dominant markers that show high levels of consistency and robustness (William et al. 2007).
The use of molecular markers in wheat breeding has been a challenging task due to the large genome size (16 x 10⁹ base pairs) of the species and low level of polymorphism among DNA markers (William et al. 2007). As a result it is imperative to have large number of markers to make any sensible analysis in wheat than is the case with maize, rice or barley (Langridge and Chalmers 2004).

A large variety of genetic analyses studies use molecular markers for genetic variability, trait mapping, genotype finger printing and positional cloning (Vignal et al. 2002, Collard et al. 2005). Mapped markers can be used as sign posts to indicate where in the genome a QTL controlling a trait lies. Once a reliable trait marker association is established, genotypes can be evaluated based on the presence or absence of identified markers that are really close to identified QTLs (Mohler and Singrün 2004). The potential of molecular markers in plant breeding, however can only be fully realized when it gets well synchronized with the concepts of conventional plant breeding theories and practices (William et al. 2007).

2.6.2 Genetic linkage mapping

A linkage map is an abstract record of all the genes within a chromosome, and is commonly based upon recombination frequencies (Hayes 2007). Having polymorphic DNA markers is a prerequisite to create genetic maps that can be used to map genes or gene regions using statistical algorithms to establish associations between markers and traits of interest (Song et al. 2005). Linkage maps give the ultimate importance in plant breeding and genetic studies. Such maps can be used to detect genome regions that encompass genes determining the inheritance of agronomic traits. Nature of mapping population, level of marker polymorphism, and linkage analysis algorithms used
determine the accuracy and final utility of linkage maps (Collard et al. 2005). Large genome species like wheat pose considerable challenge in linkage mapping due to the need for large number of polymorphic markers to cover the genome and the high ploidy level to address.

2.6.3 Quantitative traits loci (QTL) mapping

Quantitative traits like yield and drought resistance are controlled by several loci contributing for the phenotypic expression of traits (Bos and Caligari 2007, Sehgal et al. 2016). As a result, there is no clear phenotypic classification of the individuals; the variation is continuous in the bounds of the extreme individuals where phenotypes reflect only a portion of available genetic values (Sehgal et al. 2016). Biometrical genetics was coined in an effort to explain the inheritance of these classes of traits which are controlled by several loci contributing minor effects to the phenotype.

QTL mapping is a statistical procedure by which linkage maps are used to locate regions of chromosomes that contribute for the phenotype of various traits (Collard et al. 2005). The concept of QTL identification was developed by (Sax 1923). The paralleled sophistication in biometric strategies to handle large data sets of markers has contributed to the development of different QTL mapping techniques. Different mathematical models are used to establish associations between markers and phenotypes (Li et al. 2010, Zhang et al. 2010, Wang et al. 2012, Meng et al. 2015).

The conventional way of QTL mapping relies on the development of structured populations and a reasonably dense genetic linkage map (Long et al. 2008). Precise phenotypic data and appropriate computer statistical software are essential for a reliable mapping of QTLs (Kearsey and Farquhar 1998).
Creation or development of mapping population, generating marker data and linkage construction are the major activities in the workflow before phenotypic data is associated with markers (Sehgal et al. 2016).

Recombinant inbred lines (RILs), and doubled haploid lines (DHLs) are most frequently used structured population types in self-pollinated crops like wheat while segregating F\textsubscript{2}, F\textsubscript{3} and back crosses populations are commonly used for cross pollinating crop species (Collard and Mackill 2008). RILs and DHLs are perpetual populations that can help to run replicated trials across environments and laboratories enabling measurements of repeatability and reliability of mapping techniques (Semagn et al. 2010, Sehgal et al. 2016).

Despite all these improvements in the application of linkage mapping, their use in gene tagging and positional cloning is still a tedious task due to the fact that identified QTLs may be more than 10 cM apart from the flanking markers which means the target genome region subtends tens to hundreds thousands of base pairs making analysis difficult (Sehgal et al. 2016). In addition to the cost in terms of both time and resources, effects of identified QTLs and their chromosomal positions are often limited to the mapping population used and difficult to apply to other populations. Construction of structured populations can also be practically impossible for some species (Neale and Savolainen 2004).

QTL mapping can also be done using unstructured populations with distantly related individuals using genome wide association study (GWAS) approach (Abdurakhmonov et al. 2008, Xu 2010, Bac-Molenaar et al. 2015, Sehgal et al. 2016). Linkage disequilibrium mapping and conventional QTL mapping can compensate each other. A
large number of individuals and a dense linkage map give a better resolution and a more reliable result, especially for mapping some general variations in a population. GWAS can be done on a representative sample or core collection of various kinds of populations including gene bank collections, elite breeding materials and/or specialized populations. Core collections have broad genetic variability making them suitable in reducing relatedness and representing maximum variation (Pritchard et al. 2000).

Developments in molecular biology enabled the generation of large amount of molecular data which called for the need to have a more advanced data management strategy. Several QTL mapping methods have been developed ranging from the simplest single marker regression analysis to composite interval mapping (CIM) (Lander and Botstein 1989, Jansen 1993, Zeng 1994). CIM has been the standard method in mapping QTLs using structured populations.

The areas of bioinformatics and statistical genetics have developed numerous computer programs to facilitate identification and mapping of QTLs. QTL Cartographer is one of the commonly used mapping software since 1994 (Basten et al. 1994). Other commonly used QTL mapping software include MapMaker/QTL (Lincoln et al. 1993), Map Manager/QTX (Manly et al. 2001), and QGene (Nelson 1997). TASSEL and GAPIT/R are commonly used to map QTLs in case of GWAS (Bradbury et al. 2007, Lipka et al. 2012).

2.6.4 Markers assisted selection

The ultimate goal of QTL mapping is to enable genotypic evaluation of individuals at molecular level (Jiang 2013). Once a close association between a markers and trait of interest is established, the identified QTLs or genes can be tracked down generations
simply by genotyping successive individuals for the possession of the targeting markers (Song et al. 2005, Collard and Mackill 2008). The use of molecular markers to facilitate genetic manipulation of plants is called masker assisted selection (MAS) (Jiang 2013). MAS saves time and avoids the influence of environment, and genotypes can be evaluated at any growth stage, traits with low heritability can be selected effectively, and particular traits can be examined when phenotypic screening is not possible (Collard et al. 2005, Semagn et al. 2010, Sehgal et al. 2016).

For successful implementation of MAS, the target QTLs/genes and the reference markers should be close enough so that no cross-over will happen between them, and the markers should be polymorphic (Collard and Mackill 2008, Jiang 2013). To ensure usability of identified markers for MAS, QTL mapping should be followed by validation trials using other genetic backgrounds other than the mapping population. Validation of mapped QTLs can be done through independent populations or by using near isogenic lines (NILs) developed from parents having the desired QTL and marker (Collard et al. 2005, Collard and Mackill 2008).
Chapter 3 Screening wheat (*Triticum* spp.) genotypes for root length under contrasting water regimes: potential sources of variability for drought resistance breeding

3.1 Abstract

Screening for root traits has been one of the most difficult areas to practice over large number of genotypes. Hydroponic systems enable easy access to roots while high molecular weight polyethylene glycol (PEG) is commonly used to induce water stress. In this experiment, a total of 838 genotypes were evaluated for root length in a hydroponic system both under osmotic stress and non-stress growing conditions. Augmented complete block design with seven blocks (planting time), and six standard control varieties was used. Half strength Hoagland’s solution and PEG 6000 at -0.82 MPa stress level were used. Root length differences were highly significant under both stress (*P* < 0.01) and non-stress (*P* < 0.05) growing conditions. Osmotic stress has caused an average reduction of 54% on root length. The adjusted mean root length under stress ranged from 1.4 cm to 13.3 cm with an overall mean of 6.6 cm. Similarly, under non-stress condition root length ranged from 4.4 cm to 23.3 cm. The best control variety for drought resistance was significantly (*P* < 0.05) outperformed by four new entries namely Colotana 296-52, Compare, Santa Elena, and Tammarin Rock, while the shortest adjusted root length was measured on five genotypes *i.e.* genotypes Aus 16356, Elia, Camm, Portugal 3, and Sentinel. The six control varieties didn’t show any significant difference among themselves under non-stress condition. The performance of genotypes under the two water regimes was drastically different which indicates the difficulty of selecting new drought resistant varieties under optimum growing environments. Crossing among the most contrasting genotypes was carried out to analyse the inheritance of root length under water stress.
3.2 Background

Drought is amongst the most serious environmental constraints limiting crop growth and productivity in many parts of the world (Balouchi 2010, Comas et al. 2013). Breeding for drought resistant genotypes through identification of new sources of resistance and pyramiding resistance related genes into a cultivar is an efficient and cost effective means of adaptation (Fleury et al. 2010). Plants use different mechanisms to avoid damage from a dehydrating environment and produce progenies to ensure continuity of the species. Based on mechanisms of survival, plants can be classified into dehydration avoidant, dehydration tolerant and drought escaping types (Blum 2011a). Dehydration avoidance is the ability of plants to maintain hydration irrespective of the external moisture status while dehydration resistance is the ability of plants to function in a dehydrated state. Generally dehydration avoidance is a most desirable mechanism of drought resistance for crop productivity improvement.

Constitutive traits like deep root system, fine roots with small diameters, root length density (Blum 2011a, Comas et al. 2013), leaf rolling, leaf waxy layer, and osmotic adjustment (Blum 2005, Blum 2011b) are among the frequently studied traits that impart dehydration avoidance to plants. Root traits can be improved by screening genotypes at early stage which can be used as an indicator for later stage performance (Hoffmann et al. 2012, Comas et al. 2013). Improving the resistance of seedlings to water stress has a twofold benefit. The first and direct benefit is that it enables crop establishment through withstanding early season drought (Blum 1996, Abrecht et al. 2012, Passioura 2012) that happens shortly after successful germination. Water from precipitation or irrigation can be lost in the form of crop respiration, soil transpiration and percolation into deeper soil layers (Shaxson and Barber 2003). Plants can re-access
the water that has gone into deep percolation only if they have long and vigorous root
growths at early stage. The second advantage is that water stress resistance at early
stage can also be indicative of resistance at later growth stages (Comas et al. 2013,
Rehman et al. 2016), which makes root evaluation easier. However, many researchers
warned the need to be cautious in extrapolating early stage results for later stage
resistance unless it is tested and proved in the field (Passioura 2012, Comas et al. 2013).

Germplasm collections from water-deficient environments are highly likely to harbour
genes that help their adaptation and survival in such environments. Evaluating diverse
germlasm is at the very start of any breeding programme. However, evaluation of root
traits is a challenging task especially when targeting large number of genotypes. As a
result, there is no best way for root phenotyping to date. Therefore, this research was
undertaken (i) to develop an efficient root phenotyping technique, (ii) to find genotypes
which can grow deep root system under early-stage water stress and (iii) to identify
contrasting (resistant and susceptible) genotypes for genetic studies and molecular
breeding.

3.3 Materials and methods

Screening was carried out in the school of plant biology, The University of Western
Australia. A hydroponic system was developed from plastic boxes (3000 ml) with holes
of about 8 mm diameter drilled on lids that supported plant growth on the surface of a
solution (Fig. 3.1). The experiment was setup in a way that the boxes were filled with
water/solutions, and the lids were perforated and lined with filter paper to keep plants in
place and the surface moist.
A total of 838 wheat lines, including control varieties, were evaluated. Genotypes consisted of bread, and durum wheats, and some wild relatives from Africa (34), Asia (520), Australia (58), Europe (116), N/America (67) and S/America (43), accessed through the Australian winter cereals collection. Augmented complete block design with seven blocks (planting time) and six released control varieties was used. The control varieties – Drysdale (CSIRO 2008), Gladius, Young, Wyalkatchem, Guardian and Mace (Wheeler 2013) – are among the most popular wheat varieties for their adaptation to low rainfall areas in Australia.

Seeds were first germinated in Petri dishes lined with filter paper soaked with distilled water for 48 h, and then seedlings were transferred to the hydroponic system. Osmotic stress of -0.82 MPa was induced using PEG 6000 (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China). Plants were grown in water for the first seven days followed by either in half strength Hoagland’s solution alone (control) or half strength Hoagland’s solution and PEG 6000 (treatment). The pH of the solution was adjusted to 5.5–5.7 and the relative humidity was 65–70 % while the temperature was 25/22 °C day/night. Light intensity of 300 µmol m⁻²s⁻¹ was supplied using cool florescent lamps in 10/14 dark and light timing using an automatic timer. The solution was being constantly aerated using an electric air bubbler. Data were recorded on root length (longest roots) 14 days after planting (seven days after stress treatment) using a graduated ruler.
3.4 Data analysis

Analysis of variance was carried out based on augmented complete block design using CrpStat 6.1 software (International Rice Research Institute 2007) accounting for both inter- and intra-block differences. The plot numbers were considered in the residual to account for any measurement errors. The following mixed model was used: 

\[ y_{ijk} = \mu + G_i + C_j + B_k + \epsilon_{ijk}, \]

where \( y_{ijk} \) is the observed mean, \( \mu \) is the general mean, \( G_i \) is the genotype, \( C_j \) is the effect of the \( i^{th} \) control variety, \( B_k \) is the block and \( \epsilon_{ij} \) is the error effects. The test genotypes and the random error were considered random while the block and control genotypes were considered fixed. Means were adjusted for inter- and intra-block variations and were compared based on the standard errors of the differences between controls and new entries. Due to the imbalance created owing to the occurrence of new entries in a block, different standard errors were used in comparing different means. Consequently, there were four types of comparisons, which in turn gave rise to four different standard errors computed as follows:
Between two controls = $\sqrt{2\text{MSe}/b}$

Between two adjusted means in the same block = $\sqrt{2\text{MSe}}$

Between two adjusted means in different blocks = $\sqrt{2\text{MSe}(1 + \frac{1}{c})}$

Between adjusted means and control mean = $\sqrt{\text{MSe}(b + 1)(c + 1)/bc}$, where MSe is mean square of error, b is the number of blocks and c is number of control varieties.

Paired group mean comparison among continental means and the different ploidy levels was done using one tailed t-test.

3.5 Results

3.5.1 Seedling performance under stress condition

Root length reduction of up to 54% was observed as a result of the induced water stress compared to the non-stressed growing condition. Analysis of variance depicted a highly significant difference ($P < 0.01$) among both control varieties and new entries (lines other than control varieties). The adjusted mean values ranged from 1.4 cm to 13.3 cm (Fig. 3.2a) while the overall mean was 6.6 cm.

The six control varieties performed generally better than most of genotypes tested. Differences among five out of six control varieties (Gladius, Drysdale, Wyalkatchem, Guardian, and Mace) were not significant except Young which showed a significantly ($P < 0.05$) shorter root. Therefore, the numerically longest rooted variety (Mace) was used to compare with the new entries. Four genotypes namely Colotana 296-52, Compare, Santa Elena, and Tammarin Rock significantly ($P < 0.05$) outperformed
Mace, the longest rooted control variety. Aus 16356, Elia, Camm, Portugal 3, and Sentinel were among the shortest rooted genotypes.

Figure 3.2 Phenotypic distribution of 838 wheat genotypes for adjusted root length under -0.82 MPa (a), under non-stress growing conditions (b), and (c) the pictorial representation of the mean root length ± SDE of all genotypes under stress and non-stress conditions.
Table 3.1 Critical values for root length mean comparisons under stress (left) and non-stress (right) conditions.

<table>
<thead>
<tr>
<th>Comparison between</th>
<th>Stressed</th>
<th>Non-stressed</th>
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<tbody>
<tr>
<td></td>
<td>SED</td>
<td>LSD (5%)</td>
</tr>
<tr>
<td>Control means</td>
<td>0.9</td>
<td>1.8</td>
</tr>
<tr>
<td>New entries in same block</td>
<td>2.3</td>
<td>4.8</td>
</tr>
<tr>
<td>New entries in different blocks</td>
<td>2.5</td>
<td>5.2</td>
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<tr>
<td>New entries and control means</td>
<td>1.9</td>
<td>3.9</td>
</tr>
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</table>

*SED = standard error of the difference between two means, LSD = Least significant difference at 5% probability. ‘New entries’ refers to genotypes other than control/check varieties.

3.5.2 Seedling performance under non-stress condition

Analysis of variance didn’t show any significant difference among the control varieties under non-stress condition. However, the presence of replicated control varieties helped to filter out the residual and make adjustments to means enabling comparisons among the new entries. There was a very high spread of mean values ranging from 4.4 cm to 23.3 cm (Fig. 3.2b) with an overall mean value of 14.5 cm. Genotypes Persia 28, W20A, Iraq 33, SST 3, and Triticum spelta vulpinum Alef showed the longest roots ranging from 23.3-22.5 cm in that order. In the lowest end of the spread (Fig. 3.2b), genotypes Persia 6, Tincurrin, LA Florida, Datatine, and Secale africanum showed the shortest roots ranging from 4.4 cm to 6.1 cm. The mean difference between any of those genotypes from the two extremes was in the range of 16.4 to 18.9 cm which was far larger than 10.8, the LSD value at 5% level of significance (Table 3.1).

3.5.3 Genotype differences based on continents of origin

The combined mean result of genotypes from the respective continents of origin showed comparable root lengths under non-stress condition. However, the difference was much
pronounced under stress condition. The overall mean root lengths of Australian and Asian genotypes were significantly ($P < 0.05$) shorter under stress as compared to the rest of the continents. Genotypes from South America, Africa and Europe showed relatively smaller reduction in root length as a result of the stress. The highest reduction in root length was observed among Australian germplasm.

![Figure 3.3](image-url) Relative reduction in root length among continents of origin under stress and non-stress conditions.

3.5.4 Genotypic differences based on ploidy levels and level of domestication

Mean differences among the different ploidy levels and domesticated and wild forms were significant ($P < 0.05$) both under stress and non-stress conditions. Generally, the domesticated forms were the ones with longer roots under stress (Fig.3.4). Common wheat was among the most prevalent in the tested germplasm and was having significantly ($P < 0.01$) longer roots in both growing conditions. The wild tetraploid forms ($T. turgidum$ dicoccoids and $T. timopheevi$) showed the shortest roots under stress. The distant hybrids of wheat (wheat by rye and other synthetic species) showed
relatively longer roots than other wild forms of wheat. *T. monococcum* showed a comparable root length with common, and durum wheat under the stress condition.

Figure 3.4 Relative reduction in root length among different ploidy levels and different levels of domestication under stress and non-stress conditions.

3.6 Discussion

In this particular study, wheat genotypes with diverse genetic backgrounds were evaluated for root length both under stress and non-stress conditions to identify genotypes for further evaluation and genetic studies. As it is the target environment that dictates ‘what and how’ to select for water stress resistance (Blum 1996, 2005, Blum 2011a, Passioura 2012), root length was the main focus of this study keeping in mind environments having initial rainfall enough for germination but followed by a dry spell before the actual rainy season resumes. Water lost because of percolation from early showers can be re-accessed if plants have deeper roots. Results from this study showed that the wheat collections tested do vary significantly (*P* < 0.01) for root length under -
0.82 MPa osmotic stresses which is half way to permanent wilting. There was a significant variation under the non-stress condition as well. Soils at field capacity (-0.01 to -0.03 MPa), are generally considered to be optimum for plant growth and soil microbial respiration. If water is continually taken-up by plants or lost in the form of soil transpiration, then the water potential of the soil will drop to about -1.5 MPa which eventually causes permanent wilting (Krzic et al. 2004). Deep rooting is also helpful for early vigour which in turn is believed to counter balance water loss through early ground cover and transpiring the available water through the leaves than vain it in the form of soil transpiration (Burdon et al. 2012, Gupta et al. 2012). The present study is in accordance with the idea that genotypes with deep rooting ability have the potential to maintain their cellular hydration through the extra water from deeper soil profile which in turn improves productivity (Blum 2005, Blum 2011a, Burdon et al. 2012, Tanaka et al. 2013).

The short rooted genotypes already identified can also be targeted to select genotypes for irrigated areas, or for soil conditions that are having their moisture on the top few centimetres of soil depth. Deep root is important only when there is water in the lower soil profile. These short rooted plants may also economize water through suppressing the transpiration pool thereby conserving water for later crop stages. Plants that economize water can give a reasonable yield at times of severe stress but it works at the cost of some yield advantage from heavy water users at times of good seasons (Blum 2011a). Water use instead of conservation is assumed to be the most effective way of drought resistance improvement (Blum 2005, Blum 2011a).
In addition to being sources of variability for direct selection for different drought scenarios, the contrasting genotypes identified can also be used to study the genetics of drought resistance through gene/QTL mapping and gene expression profile analysis.

Root phenotyping has been the most challenging task so far, especially for large number of genotypes. The use of hydroponic systems was given sound practical backup by various researchers (Szira et al. 2008, Hoffmann et al. 2012). In this study standard commercial wheat varieties for low rainfall areas were used as benchmarks/controls both for genotype comparison and system performance control. The consistency in the performance of the control varieties under stress indicates that the hydroponic system was successful in simulating the stress and in discriminating genotypes for early stage water stress. It also indicates the importance of early stage resistance as an indicator of later stage performance; the control varieties were found good at early stage resistance in addition to their claimed resistance at later stages (Wheeler 2013). The newly optimized phenotyping hydroponic setup coupled with augmented complete block design (Federer and Crossa 2012, Mejza and Mejza 2013) enabled us to successfully and objectively compare large numbers of genotypes with relatively low difficulty to access plant roots at low cost.

Comparison based on continents of origin of genotypes did not show any significant variation under non-stress condition (Fig. 3.3). However, the stress simulated had differentiated genotypes from the six continents into extremely resistant and susceptible groups. Genotypes from S/America grew the longest roots both under stress and non-stress conditions followed by African and European genotypes. The longest rooted genotype under stress, Colotana 296-52, was from Brazil while the shortest rooted was from Australia. Colotana 296-52 is a late maturing line with very thick culm and
profuse tillers (Unpublished). The sample size across continents; however, was not the same to make valid continent to continent comparisons. Contrary to our expectations, the Australian genotypes showed the lowest average root length under stress which might be due to the adaptation of these genotypes to a Mediterranean ecology where early/dry sowing is practiced that might have conditioned the seeds to respond slowly to stress at early stage.

Differences as a function of level of domestication were in accordance with the established fact that highly selected and domesticated plants are vigorous and grow quicker as compared to wild relatives (del Blanco et al. 2000). Hexaploid wheat and other high ploidy artificial wheat hybrids showed longer roots under non-stress condition which indicates that higher ploidy level is favourable for better growth (Fig. 3.4). This corroborates with the idea that hybridization (allopolyplody) of two or more genomes increases allelic diversity which provides genetic buffer and also expression of novel phenotypes which is helpful for environmental stress resistance (Udall and Wendel 2006).

All polyploid wheat inherited their AA genome from the same progenitor, *T. urartu* (A<sup>a</sup>A<sup>u</sup>) (Goncharov et al. 2009, Goncharov 2011) which gives the same ground to compare the effect of the GG, BB, and DD genomes for rooting capacity. The wild tetraploids; *T. turgidum dicoccoids* (BBA<sup>u</sup>A<sup>u</sup>) and *T. timopheevi araraticum* (GGA<sup>u</sup>A<sup>u</sup>) showed significantly shorter roots than that of durum wheat (*T. turgidum* durum) and other wheat classes which indicates that the GG and/or BB genomes, both from *Ae. speltoides*, were not contributing for early root vigour (seedling water stress resistance). This difference may also indicate that the A<sup>a</sup>A<sup>u</sup> genome in the wild forms is different from the one in cultivated wheat (tetraploid and hexaploid). The A<sup>a</sup>A<sup>u</sup> and BB genomes...
in *T. durum* and *T. aestivum* might have undergone other forms of evolution/changes before they attain their present forms. Hexaploid wheat showed the longest root in overall performance under stress which indicates that the DD genome was positively contributing to long rooting in addition to the A\textsuperscript{u}A\textsuperscript{u} genome which was instrumental in *T. durum*. From this study we can conclude that the DD genome and the A\textsuperscript{b}A\textsuperscript{b} (*T. monococcum* showed long roots comparable with durum and common wheat roots) were significantly contributing to long rooting under early stage water stress. Unlike all other tetraploids, durum wheat showed relatively longer roots which might be due to changes in either or both the A\textsuperscript{u}A\textsuperscript{u} and BB genomes.

The differential root lengths among the different ploidy levels might also be due to competition for water and nutrients as all the lines were grown together (Fig. 3.1) in the hydroponic system which might have exposed them for fierce completion among and within themselves (Song *et al.* 2010). Competition might have also triggered a naturally short rooting genotype to grow long roots and vice-versa as access to water and nutrients is a function of root systems (Ehdaie *et al.* 2010).

In conclusion, data from this study showed the presence of ample genetic variability which can be used as a source of resistance for early stage water stress resistance breeding, and also to study the genetics of root traits. The hydroponic system was found to be a handy tool for root phenotyping for large number of genotypes. The most contrasting genotypes are being hybridized to obtain segregating populations for further genetic studies and also for drought resistance breeding.
Chapter 4  Performance of Ethiopian bread wheat (*Triticum aestivum* L.)
genotypes under contrasting water regimes: potential sources of
variability for drought resistance breeding

4.1  Abstract

Drought is a common abiotic stress in Ethiopian agriculture. Crop yield is at risk due to
drought that happens at various developmental stages of the crop. This experiment
evaluated 248 Ethiopian bread wheat genotypes under water stress and non-stress
growing conditions. Augmented complete block design with three blocks and eight
replicated entries was used. Analysis of variance showed significant diversity among
the genotypes in reaction to water stress. The average root and shoot lengths were
reduced by 33% and 29%, respectively, due to water stress. The average fresh biomass
per plant was 192 mg for non-stressed and 116 mg for stressed treatments, suffering a
41% reduction due to stress. Accessions 8314, 204463, 204454 and 204521 showed the
longest roots while accessions 222381, 222405, 222439 and 204586 showed the
shortest roots under stress conditions. Drought tolerance indices were calculated based
on root length. Geometric mean performance (GMP) index was found helpful in
identifying the relatively stable genotypes across the two water regimes. High GMP
indices were observed for genotypes 8314, 204521, 231614, and KSN81 which were
long rooting genotypes under both stress and non-stress conditions. ANOVA based on
region of collection showed that genotypes from Southern Nations Nationalities and
Peoples Region had the longest roots. Elevation of origin did not show any significant
difference for any of the traits measured. This study demonstrated the presence of large
variations for water stress response in the Ethiopian bread wheat germplasm. The
identified stress resistant genotypes can be used as potential breeding stocks to develop
drought resistant cultivars.
4.2 Background

Agriculture is the largest sector of employment and main source of livelihood in Ethiopia. Nearly 85% of the population directly depends on farming. Grain production constitutes the major share of the domestic agricultural production. Nearly 98% of cereals are produced by small holder farmers (USDA 2014). Ethiopia is the largest wheat producing country in Sub-Saharan Africa, with annual production of more than four million tons of grain on 1.6 million hectares of land which accounted for 13% of total land allotted to cereals (CSA 2014, USAID 2014). Wheat is mainly grown in the central and south eastern highlands during the main rainy season (June to September) (Hailu et al. 1991).

The Ethiopian agriculture is mainly rain-fed in that its performance is highly dependent on the timing, amount and distribution of rainfall (Cheung et al. 2008). This makes the sector vulnerable to drought and other natural calamities. Due to the changing global climate, the rain fall trend is also changing (Schlenker and Lobell 2010, Funk et al. 2012, Hellin et al. 2012, Stroosnijder et al. 2012). The rains are becoming more erratic with a trend of starting late and ceasing early in the season. This has posed an eminent danger for crop production. The production loss due to both biotic and abiotic factors coupled with the increasing population has made it difficult to attain food security in the country.

Improving the adaptability of crop varieties to a changing environment supported by appropriate crop management strategies is the working principle worldwide in ensuring crop productivity (Blum 2011a, Stroosnijder et al. 2012, Wasson et al. 2012, Farooq et al. 2015). However, crop improvement for water stress is a much complicated task as
drought damage is manifested in various forms at various crop growing stages making breeding for drought resistance uneasy (Blum 2005, Szira et al. 2008, Fischer et al. 2012, Tuberosa 2012). Therefore, breeding for drought resistance needs to integrate all methodologies that help in genotype evaluation and selection at all stages of the crop instead of one final stage (Qu et al. 2008). Seedling or early vigour, and deep root system are believed to contribute for better drought resistance (Al-Karaki 1998, Lilley and Kirkegaard 2011). Some genes that contribute to seedling drought resistance may also contribute to later stage resistance (Chloupek et al. 2010, Hoffmann et al. 2012, Comas et al. 2013). According to Sarker et al. (2005) long roots are positively correlated with high biomass yield in lentil. Initial root parameters and above-ground biomass were also reported to be positively correlated in wheat (Atkinson et al. 2015).

Genetic variability for any trait of interest is the first and foremost requirement for the success of any breeding program (El-Beltagy and Madkour 2012, Tadesse et al. 2012). The Ethiopian wheat germplasm was extensively studied for its variability in agromorphological and molecular traits (Tesfaye et al. 1991, Belay et al. 1993, Pecetti and Damania 1996, Alamerew et al. 2004, Hailu et al. 2006). However, most of the previous studies were focussed on the final crop growth stage such as yield and yield related traits, which had overlooked the importance of seedling evaluation for water stress resistance. It was hypothesised that Ethiopia might harbour valuable genetic resources for water stress resistance as a result of the sporadic dry spells that have stricken the country for many decades and long history of wheat production in the country (Hailu et al. 1991, Conway and Schipper 2011, Kassie et al. 2014). Therefore, the present research was undertaken to evaluate the phenotypic variability among Ethiopian bread wheat genotypes and to identify the most tolerant genotypes for early-
stage water stress, and to assess the relationship between underground and aboveground plant biomass in response to water stress.

4.3 Martials and methods

Germplasm evaluation for seedling water stress resistance was conducted at Debre Markos University in a laboratory of the Department of Horticulture. A hydroponic system was developed from plastic boxes (3,000 ml of volume each) with 8mm diameter holes drilled on lids that supported plant growth on the surface of the solution, following the same methodology as described in Ayalew et al. (2015). The experiment was set up in a way that the boxes were filled with water/solutions and the lids were perforated and lined with filter paper to keep plants in place and the surface moist.

A total of 248 bread wheat genotypes were evaluated, including 160 landrace collections from the Biodiversity Institute of Ethiopia and 88 breeding lines from Kulumsa Agricultural Research Centre. The landraces were collected from four administrative regions in Ethiopia (Amhara, Oromia, Tigray, and Southern Nations Nationalities and Peoples Region (SNNPR) (Fig. 4.1) while the breeding lines were under observation and characterization nursery at Kulumsa Agricultural Research Centre during the 2012 cropping season (Represented as KSN for ‘Kulumsa screening Nursery’).
An augmented complete block design was setup with three blocks (planting time) and eight randomly selected genotypes as repeated checks/controls. Seeds were first germinated in Petri dishes lined with filter paper soaked with tap water for 48 hours and seedlings were transferred to the hydroponic system. Osmotic stress of -0.82 MPa was induced using PEG 6000 (Sinopharm Chemical Reagent Co. Ltd, China). Plants were grown in water for the first seven days followed by either in half strength Hoagland’s solution alone (control) or half strength Hoagland’s solution with PEG 6000 (treatment). Natural light was used and the solution was being aerated using manual agitation. Data were recorded on root length and shoot length using a scaled ruler and fresh biomass using a sensitive balance 14 days after planting.
4.4 Statistical analysis

Analysis of variance (ANOVA) was carried out based on augmented complete block design using CrpStat 7.2 statistical (International Rice Research Institute 2007) accounting for both inter- and intra- block differences. The following linear model was used: \( y_{ij} = \mu + a_i + b_j + \varepsilon_{ij} \), where \( y_{ij} \) is the observed phenotype, \( \mu \) is the population mean, \( a_i \) is the genotype effect, \( b_j \) is the block effect and \( \varepsilon_{ij} \) is the random error. The plot numbers were considered in the residual to account for any measurement errors. Pearson’s simple correlation was also calculated among the traits measured. Means were adjusted for inter- and intra- block variations and were compared based on the standard errors of the differences between two means with controls and with new entries. Due to the imbalance created owing to the occurrence of new entries in a block, four different standard errors (Federer and Raghavarao 1975) were computed as follows:

Between two controls = \( \sqrt{\frac{2\text{MSe}}{b}} \)

Between two adjusted means in the same block = \( \sqrt{2\text{MSe}} \)

Between two adjusted means in different blocks = \( \sqrt{2\text{MSe}(1 + \frac{1}{c})} \)

Between adjusted means and control mean = \( \sqrt{\text{MSe}(b + 1)(c + 1)/bc} \), where MSe is mean square of error, \( b \) is the number of blocks and \( c \) is number of control varieties.

One-way ANOVA was used to compare differences among the four regions of landrace collection and four altitude groups (< 2,000 masl, 2,000 - 2,500 masl, 2,500 - 3,000 masl and > 3,000 masl).

The following drought indices were calculated based on the respective authors:
1) Stress susceptibility index (SSI) = \( (1 - \frac{Y_s}{Y_p}) / (1 - \frac{\bar{Y}_s}{\bar{Y}_p}) \) (Fischer and Maurer 1978)

2) Stress tolerance index (STI) = \( \frac{Y_s + Y_p}{\bar{Y}^2} \) (Fernandez 1992)

3) Relative drought resistance index (DRI) = \( \frac{Y_s}{Y_p} / \frac{\bar{Y}_s}{\bar{Y}_p} \) (Fischer and Wood 1979) and

4) Geometric mean performance index (GMP) = \( \sqrt{Y_s * Y_p} \) (Fernandez 1992), where in all the above equations \( Y_s \) is yield of cultivar under stress, \( Y_p \) is yield of cultivar under non-stress condition, \( \bar{Y}_s \) and \( \bar{Y}_p \) are the mean yields of all cultivars under stress and non-stress conditions, respectively.

4.5   Results

4.5.1   Analysis of variance

Analysis of variance for the two growing conditions was done separately after checking the error heterogeneity between the two treatments.

Table 4.1. SEDs and LSD values for root length (RL) shoot length (SHL) and fresh biomass (FBM) data at 5% probability under stress and non-stress conditions.

<table>
<thead>
<tr>
<th>Comparison between</th>
<th>Non-stress</th>
<th>Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RL</td>
<td>SHL</td>
</tr>
<tr>
<td></td>
<td>sed</td>
<td>lsd</td>
</tr>
<tr>
<td>Control means</td>
<td>2.2</td>
<td>4.6</td>
</tr>
<tr>
<td>New entries in the same block</td>
<td>3.8</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>3.3</td>
<td>7.0</td>
</tr>
<tr>
<td>New entries in different blocks</td>
<td>4.0</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>3.3</td>
<td>7.0</td>
</tr>
<tr>
<td>New entries and control means</td>
<td>4.0</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>3.3</td>
<td>7.0</td>
</tr>
</tbody>
</table>

* SED = standard error of differences and LSD = least significant difference at 5% probability, RL = root length (cm), SHL = shoot length (cm), FBM = fresh biomass yield (mg), New entries refers to genotypes other than control/check varieties.

ANOVA did not show any statistical difference among the replicated entries in both growing conditions. However, the non-replicated entries varied significantly in both stress and non-stress growing conditions based on the calculated LSD values (Fig. 4a-c, Table 4.1).
4.5.1.1 Under non-stress condition

Root length variation ranged from 3.6cm to 21.1cm while shoot length was from 6.7cm to 22.7cm. The longest roots were found in genotypes KSN 85, KSN 6, KSN 15 and KSN 34 (20 – 21 cm) while the shortest roots were found in accessions KSN55, 204585, 226939, and 231553 (3.6 - 7.7 cm) (Fig. 4.2a). The average biomass yield was 192 mg per plant with the highest biomass yields measured on genotypes KSN 51 and KSN 78, 226931, and 243714 (353.7 - 392.5 mg per plant) and the lowest biomass yield was measured on genotypes KSN 38, KSN 55, KSN 56, KSN 8 and 226236 (22.5 - 53.8 mg per plant) (Fig. 4.2c).

Figure 4.2. Phenotypic distribution of 248 wheat genotypes for root length (a) shoot length (b) and fresh biomass yield (c) under water stress (right) and non-stress (left) growing conditions.
4.5.1.2 Under stress condition

The induced stress caused reduction in the performance of genotypes for all the three traits. The average root and shoot lengths were reduced by 33% and 29%, respectively, while fresh biomass yield was reduced by 41% (Fig. 4.3a&b). Root length ranged from 2.0 cm to 19.6 cm while the range for shoot length was from 2.6 cm to 20.6 cm (Fig. 4.2b). The longest root length was recorded in accessions 8314, 204463, 204454 and 204521 while the shortest was in 222381, 222405, 222439 and 204586 (Fig. 4.2a). Biomass yield ranged from 33 mg to 273 mg with an average yield of 115.6 mg per plant. The highest biomass yield was measured in accessions 226941 and 226261 (273 mg per plant) (Fig. 4.2c).

Figure 4.3. The mean performance of 248 bread wheat genotypes for root length; shoot length (a) and fresh biomass weight (b) under stress and non-stress growing conditions.

4.5.2 Genetic variation based on geographic locations

Out of the total 248 tested bread wheat genotypes, only 160 had geographic data of collection. One-way ANOVA based on regions of collection showed a significant ($P < 0.05$) difference for root length among genotypes (Fig. 4.4a). Shoot length and fresh biomass yield did not show any significant difference among regions of collection (Fig. 4.4b).
4.4 b&c). Genotypes from SNNP region showed significantly longer ($P < 0.05$) roots as compared to the root lengths of accessions from other regions.  

The collection sites were arbitrarily grouped into four elevation/altitude categories viz. < 2,000 masl, 2,000 - 2,500 masl, 2,500 - 3,000 masl and > 3,000 masl. One-way ANOVA was conducted based on this grouping, but no significant difference was found for any of the traits.

Figure 4.4. The relative difference in the performance of wheat genotypes for root length (a), shoot length (b) and fresh biomass yield (c) based on regions of landrace collection.
4.5.3 Association of traits

All the traits were significantly ($P < 0.01$) and positively correlated in both stress and non-stress conditions. The magnitude of correlation between fresh biomass yield and root length was higher under stress condition while correlation between shoot length and fresh biomass was higher under non-stress condition (Table 4.2).

Table 4.2. Simple correlation of traits under non-stress (below diagonal) and stress (above diagonal) conditions.

<table>
<thead>
<tr>
<th></th>
<th>Root Length</th>
<th>Shoot length</th>
<th>Fresh biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root length</td>
<td>1</td>
<td>0.60**</td>
<td>0.36**</td>
</tr>
<tr>
<td>Shoot length</td>
<td>0.39**</td>
<td>1</td>
<td>0.49**</td>
</tr>
<tr>
<td>Fresh biomass</td>
<td>0.37**</td>
<td>0.62**</td>
<td>1</td>
</tr>
</tbody>
</table>

** indicates significant correlation at $P < 0.01$

4.5.4 Drought resistance indices

The relative importance of the calculated indices was weighed in their ability to differentiate genotypes that perform better in both stress and non-stress growing conditions. SSI (stress susceptibility index) index was good in differentiating genotypes that are very sensitive to water stress while GMP was higher in identifying the most stable genotypes across the two water regimes. Higher GMP values were associated with genotypes that were long rooted and at the same time less affected by the stress and a small GMP value indicated genotypes that were short rooted but not much affected due to stress. Based on the ISS index genotypes KSN55, 204454, 204463, 221735 and 243696 showed the lowest susceptibility index while accessions 226235, 16352, 222405, 222439, and 204586 were with the highest susceptibility indices. High GMP index was observed for genotypes 8314, 204521, 231614, and KSN81 while genotypes 204585, 222405, 204586, 231553 and 226939 were with the smallest GMP
values. No meaningful association of genotypic performance was possible with the rest of the indices.

4.6 Discussion

Root phenotyping is among the most marginalised area of crop improvement research mainly because of the difficulty of root traits measurement (Passioura 2012, Tuberosa 2012). Hydroponic systems were reported to be handy tools for root phenotyping (Atkinson et al. 2015, Ayalew et al. 2015). The present study employed hydroponic culture to get easy access to intact roots. Seedling is one of the vital stages in plants which determines the level of crop establishment and crop stand performance in dry seasons. However, this succulent stage of crop plants was less emphasised in research literature partly because phenotyping for seedling resistance is presumed unattainable.

Results from this study indicated that the Ethiopian bread wheat genotypes are highly diverse in terms of root length, shoot length and fresh biomass yield. Previous studies have also found significant variability in the Ethiopian wheat germplasm for several agro-morphological traits such as days to heading and maturity, plant height, grain yield, and harvest index (Tesfaye et al. 1991, Belay et al. 1993, Hailu et al. 2006). Depending on a target drought scenario, the identified genotypes can be further evaluated to develop varieties through line selection or hybridization to pyramid different favourable genes into a cultivar. Genotypes with long roots at early stage can be valuable assets for breeding drought tolerant lines in environments with early-growing-season rainfall and with soil types that can retain water at deeper layers. The less vigorous genotypes can be targeted for environments where farming is dependent
on ruminant moisture that requires water saving for later stage crop growth (Blum 2005).

The regions of collection did not show any significant difference which might be due to the fact that bread wheat is an exotic cereal to Ethiopia and did not differentiate into diverse ecotypes except for root length (Engels et al. 1991). Genotypes from Southern Nations, Nationalities and Peoples Region were significantly long rooted than genotypes from the other regions. This might be due to the many years of exposure of genotypes to low precipitation and the thick top soil that can hold water in its deeper layers (Funk et al. 2012, Kassie et al. 2014).

All the three traits were highly and positively correlated which enables simultaneous genetic improvement. Biomass yield can be used as a good indicator of long roots under stress condition as the level of magnitude and significance of correlation between these traits were higher as compared to the case with the rest of traits. This finding is in agreement with (Abdel-Ghani et al. 2014).

Among the drought resistance indices geometric mean performance index (GMP) was helpful in identifying the most stable genotypes in this study. The use of drought tolerance indices is dependent on the selection strategy one follows to improve drought resistance (Sio-Se Mardeh et al. 2006). For environments generally getting enough precipitation for most of the years/seasons but are impacted by sporadic drought, selection for genotypes that yield highest at optimum moisture and again able to give reasonable yield under stress are favourable genotypes (Ud-Din et al. 1992, Blum 2011a). However, if any two environments are characterized by a marked differences in terms of moisture availability, selection and breeding needs to be done separately.
(Ceccarelli and Grando 1991). Stress susceptibility index (SSI) enabled identifying resistant genotypes under stress conditions, however; it was not helpful in the non-stressed situation.

In conclusion, the present study has found the presence of genetic variation among Ethiopian bread wheat genotypes both under severe water stress and non-stress conditions. There was a change in the ranking of genotypes under the two water regimes which calls for a separate breeding strategy for stress and non-stress conditions. The contrasting genotypes can be used as parental lines for further genetic study and as breeding lines based on different drought scenarios.
Chapter 5  Quantitative analysis of gene actions controlling root length under water stress in spring wheat (*Triticum aestivum* L.) genotypes

5.1 Abstract

Understanding the genetic control of agronomic traits is important in designing genetic improvement programs. The present study was conducted to analyse the genetic control of root length under water stress. A full diallel cross of four spring wheat lines was conducted, and their progenies, together with parental lines, were evaluated under -0.82 MPa level of water stress in a hydroponic culture system. Analysis of variance showed highly significant (*P* < 0.01) difference among the parental lines and their F₁ progenies. Genotypes Sanata Elena, Colotana 296-52 and Pato showed comparable longer roots while Tincurrin was with a significantly (*P* < 0.05) shorter root. Genotypes with long roots were found to have more dominant genes than those with shorter roots. Both additive and dominant gene effects were found important in the control of root length under water stress. Sanata Elena was the best to pass its long root trait to its progenies among the four parental lines used while the hybrids of a specific cross (Santa Elena x Pato) showed the longest mean root length under water stress. The predominantly over-dominant gene action observed seemed to be a result of complementarity of additive dominant genes in the outstanding hybrids. The moderate narrow sense heritability (38%) indicated the possibility of improving root length under water stress through recombination and inbreeding line evaluation. Dominant and over-dominant control of root length under water stress suggests that genotypes with more dominant genes should be selected as parents in hybridization programmes and the hybrid wheat approach might be helpful in improving root length under water stress.
5.2 Background

Water stress is one of the most pressing environmental problems in dry land agriculture. As water stress is manifested in various forms at various plant developmental stages, it is apparently logical to work at various crop growth stages (Qu et al. 2008, Dai 2011, Passioura 2012, Tuberosa 2012, Ayalew et al. 2015). Several researchers have reported that different crop growth phases have different stress resistance mechanisms, which showed the need to work on different stages of crop development. The drought scenario is the dictating factor in designing any breeding and selection program for drought prone environments (Sio-Se Mardeh et al. 2006, Blum 2011a, Palta et al. 2011). In areas where the nature of rainfall is more erratic at the start of the season, drought resistance at seedling stage rather than reproductive stage is what research should target (Blum 1996, Palta et al. 2011, Passioura 2012). Genotypes with good seedling vigour can better resist water stress through more effective use of the available moisture and better vegetative ground cover to avoid soil transpiration losses.

Previous studies on seedling water stress resistance showed the presence of huge genetic diversity in wheat genotypes (Balouchi 2010, Ayalew et al. 2015, Ayalew et al. 2016a). One of the approaches to make use of this genetic variability is to directly select drought resistant varieties from the existing genetic resources (Moose and Mumm 2008). The other approach is to make crosses with the superior or contrasting genotypes and select transgressive progenies, which is also an important way to analyse how the traits are genetically controlled in the population (Fridman 2015). Knowledge of the genetic control of a trait is essential for designing a successful breeding strategy. Even in this era of genomics, making crosses among contrasting genotypes followed by quantitative analysis is frequently used to study the different gene actions and
mechanism of their inheritance, especially for wheat which is a hexaploid with a large genome size (Danehloueipour et al. 2007, Lippman and Zamir 2007, Fan et al. 2014, Song et al. 2015).

Researchers have developed different crossing designs and data analysis methods depending on the nature of the data and mating design used (Hayman 1954, Griffing 1956, Mather and Jinks 1982). Among them, diallel mating design is the most explored and frequently used (Hallauer et al. 2010). It works by partitioning the phenotypic variance into different sources of variation.

Having done genotype evaluation for water stress resistance on 838 wheat genotypes (Ayalew et al. 2015), we targeted making crosses among the divergent lines and study the nature of gene action and inheritance of root traits under the same nature of stress that we used for the screening experiment. The present research, therefore, was conducted to figure out the nature of genetic control of root length under early stage water stress in contrasting genotypes.

5.3 Materials and methods

The parents for this study were identified following the wide screening of 838 wheat genotypes for water stress resistance (Ayalew et al. 2015). Based on the previous screening experiment (Chapter 3), two of the parents, namely Colotana 296-52 and Santa Elena, were among the top performing genotypes under water stress. Genotype Pato performed moderately while Tincurrin was among the shortest rooted genotypes.
A four by four full diallel cross was conducted following a standard crossing procedure (Simmonds 1986) and seeds from individual crosses were kept separately. Both the reciprocals of F₁’s and their parental lines were used for the diallel analysis.

Randomized complete block design with three replications was used. Seeds were first germinated in Petri dishes lined with filter paper soaked with tap water for 48 hours and seedlings were transferred to the hydroponic system. Osmotic stress of -0.82 MPa was induced using PEG 6000 (Sinopharm Chemical Reagent Co. Ltd, China). Plants were grown in water for the first seven days followed by either in half strength Hoagland’s solution alone (control) or half strength Hoagland’s solution with PEG 6000 (treatment). The pH of the solution was adjusted between 5.5 and 5.7. The relative humidity of the room was between 65-70% and the temperature was 25°C day/22°C night. Light intensity of 300 µmol m⁻² s⁻¹ was supplied using cool florescent lamps with a daily photoperiodic cycle of 14 h light/10 h dark. The solution was being constantly aerated using an electric air bubbler.

Data were recorded on root length of the longest roots using a graduated ruler on 17 days old seedlings. Three to five individual plants were used to calculate the mean of an entry in each block.

5.4 Statistical analysis

Analysis of variance was conducted on the parental lines and their F₁ progenies including reciprocal crosses. Analysis of variance and estimation of genetic components were conducted based on Hayman and Jinks fixed model (Jinks 1954, Blum 1996). The pooled error variance across blocks was used to test for the significance of each source of variation. All statistical analyses were conducted using GenStat statistical software.
17th edition (VSN International 2014). The covariance (Wr)/variance (Vr) regression graph was used to study the nature of gene actions and their distribution (dominant and recessive) among the parental lines. Analysis of variance over (Wr + Vr) values for each parent across blocks was used to assess the nature of recessive-dominance gene distribution among parents while the ANOVA on (Wr – Vr) value for each parent and the three blocks was performed to test adequacy of the additive-dominance model. Further test for validity of diallel assumptions was done using parent-offspring Wr/Vr regression statistics. In the absence of epistasis and with independent distribution of genes among the parents, the linear regression of Wr/Vr has a slope of one and the Wr, Vr array points would remain along the regression line, and within an area delimited by the parabola, \( Wr^2 = Vp \times Vr \) where \( Vp \) is the variance of the parental means (Povilatis 1966, Blum 1996, Moose and Mumm 2008). Regression line between the parental mean values and the recessive-dominance indicator (Wr + Vr) values was used to differentiate parental lines with high proportions of dominant and recessive genes and to determine the genetic control of long root under early stage water stress.

5.5 Results

5.5.1 Analysis of variance

The analysis of variance showed highly significant \( (P < 0.01) \) differences among the parental lines and their first filial generation. Genotype Tincurrin showed significantly \( (P < 0.05) \) shorter roots as compared to the rest of the parents (Table 5.1, Fig. 5.1).
Table 5.1 Mean performances of parental lines (diagonal) and their F₁ hybrids (off diagonal) tested for root length under water stress in a 4x4 diallel cross.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Colotana 296-52</th>
<th>Tincurrin</th>
<th>Santa Elena</th>
<th>Pato</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colotana 296-52</td>
<td>17</td>
<td>22.8</td>
<td>20.0</td>
<td>21.2</td>
</tr>
<tr>
<td>Tincurrin</td>
<td>24.7</td>
<td>9</td>
<td>20.8</td>
<td>18.5</td>
</tr>
<tr>
<td>Santa Elena</td>
<td>19.3</td>
<td>23.5</td>
<td>19.5</td>
<td>19.8</td>
</tr>
<tr>
<td>Pato</td>
<td>16.8</td>
<td>20.0</td>
<td>29.3</td>
<td>19.0</td>
</tr>
</tbody>
</table>

SE = 1.86, LSD at 5% = 5.37, *a,b* values designated by the same letter are not statistically different.

All of the progenies from all the crosses showed longer roots as compared to the short-rooted common parent. The specific cross Pato × Santa Elena was the most outstanding cross with significantly longer roots.

Figure 5.1 Root length (Mean ± SE) of the four pure line parents tested under water stress in a 4x4 full diallel cross.

All of the genetic components, except the average maternal effect, showed highly (*P* < 0.01) significant variation (Table 5.2). Both additive (a) and dominance (b) gene effects were instrumental in the control of root length under early stage water stress. There was a significant directional dominance effect (b₁) between the parental and hybrid means. The distribution of dominant genes across the four parents was significantly variable. Parents Colotana 296-52, Santa Elena and Pato showed more dominant genes than Tincurrin. Some of the progenies showed a highly significant dominance effect which has led to a significant discrepancy between the non-maternal components of reciprocal variation (Table 5.2).
Table 5.2 Mean squares of genetic components calculated on parental, F₁ and reciprocal progenies from a 4x4 full diallel cross in spring wheat genotypes grown under water stress.

<table>
<thead>
<tr>
<th>Genetic component</th>
<th>Representation</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total additive genetic effect</td>
<td>a</td>
<td>58.54**</td>
</tr>
<tr>
<td>Dominance genetic effect</td>
<td>b</td>
<td>115.86**</td>
</tr>
<tr>
<td>Mean dominance deviation (Difference between parental and progeny mean)</td>
<td>b1</td>
<td>335.84**</td>
</tr>
<tr>
<td>Asymmetry of the gene distribution at the loci exhibiting dominance</td>
<td>b2</td>
<td>71.95**</td>
</tr>
<tr>
<td>Discrepancy in reciprocals due to dominance</td>
<td>b3</td>
<td>71.73**</td>
</tr>
<tr>
<td>Average maternal effects of each parental line</td>
<td>c</td>
<td>14.36ns</td>
</tr>
<tr>
<td>Reciprocal differences not ascribable to maternal effect</td>
<td>d</td>
<td>47.13**</td>
</tr>
<tr>
<td>Total</td>
<td>t</td>
<td>70.35**</td>
</tr>
<tr>
<td>Residual (non-genetic) effect</td>
<td>Block.t</td>
<td>10.36</td>
</tr>
</tbody>
</table>

**' denote significance at 1% confidence levels, respectively 'ns' denotes non-significant difference; MS is mean of squares.

5.5.2 Graphical analysis - model adequacy test

Analysis of variance on the (Wᵣ – Vᵣ) values over arrays and blocks did not show any significant difference which is consistent with the adequacy of the additive-dominance model (Table 5.3). Both the slope and intercept of the Wᵣ/Vᵣ regression line were significantly (P < 0.05) different from zero and the slope was close to unity. This indicated that epistasis was not a significant player in the control of root length under water stress.
Table 5.3 ANOVA on (Wr - Vr) and (Wr + Vr) values to test model validity and the presence of dominance, respectively, on root length under water stress from a 4x4 full diallel cross in spring wheat genotypes

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>MS (Wr - Vr)</th>
<th>MS (Wr + Vr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>2</td>
<td>232.1</td>
<td>216</td>
</tr>
<tr>
<td>Array</td>
<td>3</td>
<td>216.2*</td>
<td>961*</td>
</tr>
<tr>
<td>Residual</td>
<td>6</td>
<td>146.4</td>
<td>136</td>
</tr>
</tbody>
</table>

*, denotes significant at 5% confidence levels, respectively, and 'ns' denotes non-significant difference, MS = mean square

5.5.3 Graphical analysis - Wr/Vr plot analysis

The relative order of the parents along the Wr/Vr regression line indicated the distribution of dominant and recessive genes among the parents. Genotypes Colotana 296-52, Pato and Sanata Elena were close to the origin showing excess of dominant genes while genotype Tincurrin was furthest away from the origin which showed that it had an excess of recessive genes (Fig. 5.2).
Figure 5.2 Parent-offspring covariance (Wr) plotted against the variance of all F₁ hybrids in each parental array (Vr) for root length in a 4x4 full diallel cross of spring wheat genotypes tested under water stress. The right opening parabola \( Wr^2 = VrVp \) limits all the data points.

The intercept of the Wr/Vr regression line passed through the Wr axis below the origin (Fig. 5.2) indicating over dominance gene action. This was also in conformity with the mean degree of dominance value (Table 5.4) which was greater than unity. Genotype Tincurin showed the lowest mean root length and gave the largest (Wr + Vr) value (Fig. 5.3) which is characteristic of genotypes having excess of recessive genes. There was a negative correlation between (Wr + Vr) values and mid parents. Negative correlation values indicate that the increase in recessiveness is associated with lower performance of the parents.
Chapter 5

5.5.4 Genetic components and heritability

Additive genetic variance (D) and the two components of dominance variance (H1 and H2) were highly significant, indicating the importance of both additive and non-additive gene actions in the control of root length under water stress. Variation due to dominance effects (H1) and the dominance value indicating asymmetry of positive and negative gene effects (H2) were different from each other, showing the difference in the frequencies of the genes of interest among parental lines. The value of relative frequency and magnitude of dominant and recessive alleles (F) was a significantly large positive number (56.2) which showed the frequency of the dominant alleles was higher than that of recessive alleles (Table 5.4).

Figure 5.3 Relationship between the sum of parent-offspring covariance (Wr) and all F1 progeny variance (Vr) with the parental means measured on root length in a 4x4 full diallel cross.
Table 5.4 Estimates of genetic variance components for root length under water stress from a 4x4 full diallel cross in spring wheat genotypes.

<table>
<thead>
<tr>
<th>Genetic components of variance</th>
<th>Representation</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variance due to additive effects</td>
<td>D</td>
<td>39.03**</td>
</tr>
<tr>
<td>Variation due to dominance effects</td>
<td>H1</td>
<td>79.34**</td>
</tr>
<tr>
<td>Dominance indicating asymmetry of gene effects</td>
<td>H2</td>
<td>60.82**</td>
</tr>
<tr>
<td>Relative frequency of recessive and dominance</td>
<td>F</td>
<td>56.24**</td>
</tr>
<tr>
<td>Mean degree of dominance</td>
<td>$\sqrt{\frac{H1}{D}}$</td>
<td>1.43</td>
</tr>
<tr>
<td>Average gene frequency over all loci, max 0.25</td>
<td>$\overline{uv}$</td>
<td>0.19</td>
</tr>
<tr>
<td>Heritability</td>
<td>Narrow-sense</td>
<td>38%</td>
</tr>
<tr>
<td>Heritability</td>
<td>Broad-sense</td>
<td>60%</td>
</tr>
</tbody>
</table>

** denotes significant at 1% confidence levels, respectively.

The average gene frequency value ($\overline{uv}$) was different from 0.25 (value expected in equal frequency of the two gene effects) indicating the unequal frequency of dominant and recessive alleles. The gene asymmetry was also significantly different among parental lines. The value of the average degree of dominance $(H1/D)^{1/2}$ was greater than unity which indicated the presence of over dominant gene action.

The proportion of additive genes was moderate as it was reflected in the moderate narrow sense heritability (38%) value while broad sense heritability was 60% (Table 5.4).

5.6 Discussion

Analysis of variance indicated the diversity of both parental and progeny lines in their reaction to water stress. The performance of parental lines was in agreement with Ayalew et al. (2015) showing repeatability of the screening technique developed. Genotype Tincurrin showed significantly ($P < 0.05$) shorter roots while the rest three parental lines were not statistically different (Table 5.1). Santa Elena showed the
highest general combining ability while Santa Elena and Pato were the best specific combiners which gave rise to the highest hybrid vigour (Table 5.1).

Both additive and dominant gene effects showed significant role in the control of root length under water stress. Genetic asymmetry (b1) was significant at loci exhibiting dominance which implied that the general combining ability (a) was not purely additive variation (Povilatis 1966). This was reflected in the moderate level of narrow sense heritability (Table 4.3). Reciprocal differences other than the maternal effect were significant. This difference was purely of environmental which might have been caused by block interactions, or any other systematic error in the run of the experiment (Blum 1996).

The prominent gene action in the control of this trait was found to be over dominance. Over dominance may be true or pseudo depending on what has brought about it (Lippman and Zamir 2007). Complementarity of additive genes may inflate complete dominance into over dominance which increases variance (Vr) in relation to parent-offspring co-variance (Wr), particularly for more recessive parents (Allard 1956, Blum 1996). Most of the hybrids in this study showed heterosis greater than the better parent which can be best explained by the additive complementarity of dominant genes from the parent lines. Over dominance can also be caused by tightly linked genes in repulsion phase which gives uninheritable genetic component (Fasoula and Fasoula 2002, Lippman and Zamir 2007). However, the latter did not seem to be the case in this study as the genetic model assumptions were met during the analysis. Over dominance was reported to be important in the inheritance of yield and other yield related traits in rice and other crops (Sio-Se Mardeh et al. 2006, Li et al. 2008).
Correlation between parental arrays and the recessiveness-dominance indicator (Wr + Vr) were negative which indicated the association of lower performance to increasing recessive genes and vice versa (Povilatis 1966). This showed that long root under water stress was controlled by dominant genes.

The relative position of parental lines along the regression line between parental mean and (Wr + Vr) graph shows the proportion of recessive and dominant genes in each parent. Genotypes Tincurrin, Pato and Santa Elena lied above the horizontal (parental performance) line which indicated the presence of high proportion of recessive genes while genotype Pato with high proportion of dominant genes was below this line (Fig. 4.3). Based on this graphical analysis, recessiveness was more pronounced for Tincurrin than for the rest of the genotypes while dominance genes were more pronounced for Colotana 296-52. The remaining two parental lines were above the x-axis but still close to the y = 0 line which again showed large proportion of dominant genes (Fig. 4.3).

Heritability was moderate showing that selection among pure lines coming out of these hybrids may not be rapid in bringing genetic improvement in the progeny. With the broad sense heritability too, there seemed to be a large proportion of non-genetic variation that had rendered only 60% of heritability value. This is usually the case for quantitatively inherited traits that are influenced by both the frequency of favourable genes and interaction with the environment.

From the present study it can be conclude that seedling root length is under dominant genetic control. Root length under water stress can be improved through hybridization and recurrent selection of genotypes concentrating desirable dominant genes in a cultivar thereby increasing the chance of extracting water from deeper soil profiles (Sio-
Se Mardeh et al. 2006, Blum 2011a). The most promising hybrids Santa Elena × Pato, Santa Elena × Tincurrin and Colotana 296-52 × Pato can be advanced into pure lines with high chance of getting an inbred vigour that have fixed the hybrid vigour from the hybridization. Genotypes with high proportion of recessive genes may be suitable for environments having shallow soil profile and for environments that use residual moisture for crop production. Developing structured mapping population from these contrasting genotypes may help to effectively map QTLs for root length and other traits.
Drought is one of the major challenges in agriculture. Deep rooting has a potential to avoid water stress in the presence of water in deeper soil layers. Development of cultivars adapted to dry environments is highly dependent on a thorough understanding of the genetic mechanisms governing adaptive traits. This study was conducted to identify chromosome regions harbouring QTLs contributing for water stress resistance in wheat. A RIL mapping population derived from a cross between W7984 (Synthetic) and Opata 85 was phenotyped for root length under water stress and non-stress growing conditions. ANOVA showed highly significant ($p \leq 0.01$) variation among the RILs. Broad sense heritability was 86% and 81% under stress and non-stress growing conditions, respectively. A total of eight major QTLs; four under each water condition, were detected. Four QTLs $Qrl.uwa.1BL$, $Qrl.uwa.2DS$, $Qrl.uwa.5AL$ and $Qrl.uwa.6AL$ combined explaining 43% of phenotypic variation were identified under non-stress condition. Opata was the source of favourable alleles for all of the QTLs under non-stress condition except for $Qrl.uwa.6AL$. Under stress condition, QTLs $Qdrl.uwa.1AS$, $Qdrl.uwa.3AL$, $Qdrl.uwa.7BL.1$ and $Qdrl.uwa.7BL.2$ were identified contributing 47% of phenotypic variation. Synthetic wheat contributed favourable alleles for $Qdrl.uwa.1AS$ and $Qdrl.uwa.3AL$ whiles Opata contributed favourable alleles for $Qdrl.uwa.7BL.1$ and $Qdrl.uwa.7BL.2$. Three validation populations were developed by crossing cultivars Lang, Yitpi, and Chara with Synthetic to transfer two of the four QTLs identified under stress condition. The $F_{2,3}$ and $F_{3,4}$ validation lines were phenotyped under the same level of water stress as RILs were evaluated to examine the effect of these QTLs. There were 13.5% and 14.5% increases in average root length due
to the inheritance of \textit{Qdrl.uwa.1AS} and \textit{Qdrl.uwa.3AL}, respectively. The result indicated that closely linked SSR markers \textit{Xbarc148} (\textit{Qdrl.uwa.1AS}) and \textit{Xgwm391} (\textit{Qdrl.uwa.3AL}) can be incorporated into MAS for water stress improvement in wheat.

6.2 Background

Water stress is one of the most pressing environmental problems in dry land agriculture which can manifest in various forms at various plant developmental stages (Blum 2011b, a, Passioura 2012, Tardieu 2012, Vaughn and Nguyen 2013). The multitude drought scenarios necessitate understanding the target environment and developing the right crop ideotype for successful drought resistance improvement (Blum 2014, Meister \textit{et al.} 2014). The nature of prevailing drought dictates the breeding and selection strategy making breeding for drought resistance to be one of the most expensive and labour intensive processes. The global climate change has made rains more erratic and less predictable with expansion of aridity globally (Funk et al., 2012). Early season drought is becoming more prevalent as rains are not starting early in the season and if they do so, interruptions happening for a few weeks after sowing (Ortiz \textit{et al.} 2008, Schlenker and Lobell 2010). This has a drastic effect on crop establishment and productivity as a result (Blum \textit{et al.} 1980, Meister \textit{et al.} 2014, Maccaferri \textit{et al.} 2016). If sowing is delayed till the actual rains start, the crop cycle will be pushed to terminal drought as rains tend to cease early in the season. Enhancing the genetic potential of crops in relation to their capacity to access more water from deep soils will result in increase in water use and crop productivity (Manschadi \textit{et al.} 2006, Blum 2011a, Wasson \textit{et al.} 2012, Uga \textit{et al.} 2013).
Selection for drought resistance gets even more difficult when the target is to improve root traits because root phenotyping has been one of the most arduous tasks in crop improvement (Tardieu 2012, Tuberosa 2012). Several studies reported novel root phenotyping techniques (Hoffmann et al. 2012, Cane et al. 2014) and the identification of QTL for various traits and crops (Specht et al. 2001, Zheng et al. 2008, Maccaferri et al. 2016). Much of previous research, however, was mainly concentrated on later stage stress resistance which has overlooked the importance of seedling resistance especially of root traits. Root system that suits the prevailing edaphic and hydrologic nature is essential for crop establishment and effective water use adaptation to dry environments (Palta et al. 2011, Comas et al. 2013).

As drought resistance is a highly quantitative trait, genetic improvement through the sole use of morphological markers and classical quantitative genetics is less likely to make a major leap in improving crop productivity. Breeding for water stress can be made more precise and agile if the appropriate molecular tools are incorporated into conventional plant breeding techniques (Song et al. 2006, Collard and Mackill 2008, Fleury et al. 2010, Budak et al. 2015). Marker assisted selection (MAS) through the application of molecular markers and some statistical genetic tools is reported to be effective an effective strategy (Borner et al. 2002, Semagn et al. 2006). Therefore this research was undertaken to (1) study the genetic control of root length under water stress and non-stress conditions using QTL mapping and (2) confirm effect of identified QTLs on other genetic backgrounds other than the mapping population (3) validate the usability of the closest markers for MAS.
6.3 Materials and methods

6.3.1 Plant materials

One hundred and four recombinant inbred lines from the international *Triticeae* mapping initiative (ITMI) mapping population derived from a cross between the Synthetic hexaploid wheat W7984 (*T. turgidum* cv. Altar 84 - *Aegilops tauschii* Coss. line WPI 219) and the spring wheat cultivar Opata 85 (Song *et al.* 2005) were used for the identification of QTLs under non-stress and water stress conditions. Following the identification and mapping of QTLs, crosses were made between Synthetic and three other genotypes (Chara, Yitpi, and Lang) to validate the phenotypic effect of these QTLs on other genetic backgrounds. The closely linked SSR markers to the major QTLs were used to genotype segregating lines from the crosses mentioned above. All of the hybrids were F$_{3.4}$ except the hybrids from Synthetic x Yitpi cross which were F$_{2.3}$.

6.3.2 Phenotypic evaluation

The RILs were evaluated for root length variation under osmotic stress and non-stress conditions in a hydroponic system. The same experimental setup as Ayalew *et al.* (2015) was used. A total of 104 recombinant inbred lines in three replicates (repeats of the experiment) were used in a completely randomized design. Osmotic stress of -0.5 MPa was induced using PEG 6000 (Sinopharm Chemical Reagent Co. Ltd, China). The final stress level at data collection reached -0.6 ± 0.1 MPa measured using MP4 dewpoint potentiometer (Decagon Devices Inc. 2003) creating a progressive stress scenario. Plants were let to grow with their endosperm reserve food for the first seven days after which half strength Hoagland’s solution and PEG 6000 solution for the treatment and Hoagland’s solution alone for the control respectively we added. The pH
of the solution was adjusted between 5.5 and 5.7 and the relative humidity was between 65-70% while the temperature was 25/22°C day/night. Light intensity of 300 µmol.m⁻².s⁻¹ was supplied using cool florescent lamps in 10/14 dark and light timing using automatic timer. The solution was being constantly aerated by bubbling air in to the solution using electric bubbler. Data were scored on root length (longest roots were measured) 14 days after planting (seven days stress period) using a graduated ruler. The same experimental setup and level of stress as above was used to evaluate the performance of the three validation populations.

6.3.3 Molecular markers and linkage map

Molecular marker data and linkage map of the Synthetic x Opata 85 RIL mapping population was accessed from the GrainGenes database (http://wheat.pw.usda.gov/cgi-bin/graingenes). This map had a total of 1,475 simple sequence repeats (SSR) and restriction fragment length polymorphisms (RFLP) markers distributed across the 21 linkage groups with an average marker density of 3 cM. Of the total available markers 1,420 were used for this study after filtering for 40% missing values.

6.3.4 Genotyping populations for marker validation

Genomic DNA was extracted from validation lines using Nucleon PhytoPure DNA extraction Kit (GE Healthcare) following the manufacturers’ instructions. Young leaves (about 2 grams) were ground using tissuelyser (Qiagen) and their total DNA was isolated. DNA was treated with 1 mg/ml RNase (Qiagen) for three hours at 37°C to eliminate RNA contamination. The quantity and quality of the total DNA samples were measured using a NanoDrop ND-1000 (ThermoFisher Scientific). Polymorphisms of the closest SSR markers were checked between Synthetic and the three parental lines.
(Chara, Lang and Yitipi) using ordinary primes and agarose gel electrophoresis. Polymerase chain reaction (PCR) was performed in 15 µL volume. The reaction mixture contained 50 ng/µL of genomic DNA, 0.2 µM of each forward and reverse primer, 2 mM of MgCl₂, 1× PCR buffer (Fisher Biotic), 200 µM dNTPs (Fisher Biotic) and 0.1 unit/µL Taq DNA polymerase (Fisher Biotic).

PCR thermal cycling was performed as follows: denaturing for five minutes at 95°C followed by annealing for 30 seconds each at 95°C in 35 cycles, and extension for 45 seconds at 72°C. Then the reaction was hold at 72 for 4 minutes.

Genotyping on the validation populations was done using capillary electrophoresis. Following the suitability check on markers Xbarc148 and Xgwm391, their forward primers were labelled by ‘6FAM and VIC fluorescent dyes, respectively. PCRs were run for each marker separately and the products were poolplexed for capillary electrophoresis using DNA analyser (Applied Biosystems 3730xl DNA Analyser). The PCR products were mixed into a 12 µL sample volume in a 96 well AB-C PCR plate consisting of 0.5 µL of each PCR product, 10 µL of highly deionized formamide (Hi-Di) (Applied Biosystems), and 1µL of LIZ600 size standard. Allele peaks and fragment sizes were analysed using GeneMarker software version 2.6.7 (SoftGenetics, LLC® State College, PA-16803, USA). Five hundred RFU was used as a minimum relative fluorescence for allele peak detection.
6.4 Statistical analysis

6.4.1 Variance components and heritability analysis

All phenotypic data analyses were performed using GenStat statistical software 17th edition (VSN International 2014). Analysis of variance was conducted based on the following fixed effects model: \( Y_{ij} = \mu + g_j + \epsilon_{ij} \), where \( Y_{ij} \) is observed mean, \( \mu \) is general/population mean, \( g_j \) is effect due to the \( j^{th} \) genotype, and \( \epsilon_{ij} \) is random error. Heritability was estimated using the formula: \( h^2 = \frac{\delta^2_g}{\delta^2_g + \delta^2_e} \) where \( \delta^2_g \) and \( \delta^2_e \) are the estimated genotypic and error variances, respectively (Nyquist 1991). The estimated genotypic and error variances were calculated as: \( \delta^2_g = \frac{MS_g - MSe}{r} \) while \( \delta^2_e = \frac{MSe}{r} \), where \( MS_g \) is mean square of the RILs, \( MSe \) is the residual error and \( r \) the number of replicates. The mean values of each of the trials across the RILs were used for QTL analysis. The effects of the acquired QTLs from the female parent (Synthetic) were assessed as percentage of mean differences between homozygous lines based on the genotype of linked markers to the respective QTLs.

6.4.2 QTL analysis

QTL analysis was carried out using composite interval mapping (CIM) based on Kosambi’s mapping function. Windows QTL Cartographer Version 2.5 was used for the QTL analyses (Wang et al. 2012). The standard QTL analysis model (model 6) with control marker number of 5 and window size of 10 cM was used. Backward marker selection method for background marker inclusion in the regression model was used with a false discovery rate of 0.01 and default value of marker inclusion probabilities of 0.01. The whole genome was scanned in every 1 cM interval. LOD value of 3 and above was used to declare a QTL for both stress and non-stress growing conditions. The
The graphical representation of linkage groups and QTL positions were constructed using the MapChart 2.30 software (Voorrips 2002).

6.5 Results

6.5.1 Phenotypic variation

The phenotypic data analysis revealed highly ($P < 0.01$) significant differences among the RILs. Root length ranged from 5 cm to 25 cm under stress and from 7 cm to 30.3 cm under non-stress growing conditions.

Table 6.1. Mean squares, genotypic and phenotypic coefficients of variation, and broad sense heritability of root length under stress and non-stress growing conditions.

<table>
<thead>
<tr>
<th>Osmotic condition</th>
<th>MSG</th>
<th>MSe</th>
<th>$\delta^2_e$</th>
<th>$\delta^2_g$</th>
<th>$\delta^2_p$</th>
<th>$H^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress</td>
<td>58.62**</td>
<td>7.92</td>
<td>2.64</td>
<td>16.90</td>
<td>19.54</td>
<td>0.86</td>
</tr>
<tr>
<td>Non-stress</td>
<td>52.15**</td>
<td>9.71</td>
<td>14.15</td>
<td>3.24</td>
<td>17.38</td>
<td>0.81</td>
</tr>
</tbody>
</table>

** indicates significant difference at $P < 0.01$. MSG is mean square of genotype, MSe is means square of random error, $\delta^2_e$ is estimated genetic variance, $\delta^2_p$ is estimated phenotypic variance, $\delta^2_e$ is estimated error variance, and $H^2$ is heritability in broad sense.

![Figure 6.1](image)

Figure 6.1. Distribution of root length (cm) among the 104 recombinant inbred lines tested under -0.6 ± 0.1 MPa water stress. Frequency (the number of genotypes).

Mean root length distributions (Fig. 6.1) indicated presence of transgressive segregations on both directions of the parents, suggesting polygenic inheritance of root
length under water stress. Synthetic (W-7984) was the longer rooting parent as compared to Opata 85. High heritability values (81% and 86%) were recorded under the two water conditions.

6.5.2 QTL identification

6.5.2.1 Under non-stress condition

Four major QTLs explaining 43% of phenotypic variation were identified on chromosomes 1BL, 2DS, 5AL, and 6AL (Table 6.2, Fig 6.2). The D genome of Synthetic wheat was the source of favourable allele for seedling root length (root early vigour). Favourable alleles for QTLs Qrl.uwa.1BL, Qrl.uwa.5AL and Qrl.uwa.6AL were contributed by the A and B genomes of Opata 85 (Table 6.2).
Figure 6.2. Chromosomal location of the root length QTLs on 1B, 2D, 5A, and 6A under non-stress condition. QTL confidence intervals with LOD scores ≥3 are indicated by vertical bars. Qrl stands for QTL for root length followed by institution name, and chromosome arm.
Table 6.2. Chromosomal position, marker interval, and effect of QTLs identified on the ITMI Synthetic x Opata 85 RILs grown under -0.6 ± 0.1 MPa and non-stress conditions.

<table>
<thead>
<tr>
<th>Water status</th>
<th>Chromosome</th>
<th>Flanking markers</th>
<th>Position (cM)</th>
<th>LOD</th>
<th>Additive</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Stress</td>
<td>1A</td>
<td>Xbarc148/Xbarc162</td>
<td>36.6</td>
<td>3.1</td>
<td>1.3</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>3A</td>
<td>Xgwm391/Xbcd1431</td>
<td>92</td>
<td>4.0</td>
<td>1.6</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>7B</td>
<td>Xbarc90/Xbarc176</td>
<td>38.9</td>
<td>4.4</td>
<td>-1.5</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>7B</td>
<td>Xfba301/Xbarc278</td>
<td>44.7</td>
<td>4.9</td>
<td>-1.5</td>
<td>13</td>
</tr>
<tr>
<td>b) Non-stress</td>
<td>1B</td>
<td>Xbarc81/Xbcd1562</td>
<td>72</td>
<td>3.2</td>
<td>-1.5</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2D</td>
<td>Xgwm210/Xbcd611</td>
<td>19.4</td>
<td>3.8</td>
<td>1.6</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>5A</td>
<td>Xbarc330/Xbcd235</td>
<td>57.5</td>
<td>3.0</td>
<td>-1.5</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>6A</td>
<td>Xgwm169/Xfba221</td>
<td>100.4</td>
<td>3.4</td>
<td>-1.6</td>
<td>11</td>
</tr>
</tbody>
</table>

6.5.2.2 Under stress condition

The induced water stress has caused an average root length reduction of 41%. A total of four QTLs were identified on chromosome regions of 1AS, 3AL and two QTLs on chromosome 7BL with total phenotypic expression of 47%. All of the positive alleles of QTLs from the A genome were contributed by Synthetic while Opata was source of the favourable alleles for the other two QTLs on chromosome 7BL (Table 6.2). Two equally significant QTLs with a phenotypic expression of 13% each were located on chromosomes 3AL and 7BL gaining their favourable alleles from Synthetic and Opata, respectively (Table 6.2). The two significant QTLs on the long arm of chromosome 7B were only 5.8 cM apart. None of the QTLs identified under non-stress growing condition collocated with QTLs under stress condition suggesting qualitative QTL by environment interaction.
Figure 6.3. Chromosomal location of water stress resistance QTLs on chromosome arms of 1AS, 3AL, and 7BL under stress condition. QTL confidence intervals with LOD scores ≥ 3 are indicated by vertical bars. \textit{Qdrl} stands for QTL for drought resistance in terms of root length followed by institution name, and chromosome arm.
6.5.3 QTL effect confirmation and marker validation

Three validation populations inheriting \textit{Qdrl.uwa.1AS} and \textit{Qdrl.uwa.3AL} from Synthetic wheat were evaluated under the same level of stress as the RIL population to examine the effect of these QTLs in other genetic backgrounds. Individuals in each validation population were classified into three based on the combination of allele peaks from the parental lines (Fig. 6.5 a & b). Parental lines Synthetic and Lang were not polymorphic for marker \textit{Xgwm391}. Neither were Synthetic and Yitpi for marker \textit{Xbarc148}. Mean performance of genotypes based on the three types of allele combinations (AA, Aa, and aa) were used to calculate the phenotypic effect of the identified QTLs as percentage increase relative to the homozygous recessive (aa) allele. Synthetic x Chara progenies showed an average increase of 11\% in root length as a result of the presence of \textit{Qdrl.uwa.1AS} and \textit{Qdrl.uwa.3AL}.

![Graph showing average root length ±SE of validation populations classified based on allele combinations of \textit{Xbarc148} and \textit{Xgwm391}. Root length (cm) was positively correlated with the number of dominant alleles in a genotype.]

Generally, heterozygous (Aa) genotypes showed longer in average root length than their homozygous recessive (aa) parents suggesting that long root is dominant over short root.
(Fig 6.4). However, the average phenotypic performance of the homozygous dominant types (AA) was not significantly different from the heterozygous (Aa) types. The highest increase in root length was found among Synthetic x Yitpi progenies as a result of \textit{Qdrl.uwa.3AL}. Homozygous dominant progenies (AA) from Synthetic x Yitpi were the longest rooting genotypes among all validation lines.

Table 6.3. Mean root lengths (cm) of genotypes based on marker allele combination on the validation populations and their corresponding phenotypic effect (%) to root length under -0.6 ± 0.1 MPa water stress.

<table>
<thead>
<tr>
<th>Positive QTLs</th>
<th>Validation Population</th>
<th>Marker</th>
<th>Mean root length in genotypes (cm)</th>
<th>Phenotypic effect (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Qdrl.uwa.3AL}</td>
<td>Synthetic/Chara</td>
<td>Xgwm391</td>
<td>AA: 18.3, Aa: 18.3, aa: 16.4</td>
<td>10.4</td>
</tr>
<tr>
<td>\textit{Qdrl.uwa.3AL}</td>
<td>Synthetic/Yitpi</td>
<td>Xgwm391</td>
<td>AA: 21.8, Aa: 21.3, aa: 18.5</td>
<td>15.1</td>
</tr>
</tbody>
</table>

Figure 6.5. Sample electropherograms from SSR markers \textit{Xbarc148} (a), and \textit{Xgwm391} (b), showing polymorphism on parental lines Synthetic (upper pictures), Chara (middle pictures) and one hybrid line (bottom pictures).
6.6 Discussion

Identification and mapping of QTLs that contribute to drought resistance enable a focussed and well informed breeding and selection for efficient gene pyramiding, back crossing, and positional cloning with the help of molecular markers (Collard and Mackill 2008, Semagn et al. 2010). The present study was conducted aiming to identify and map QTLs for root length for improved water stress resistance. The ITMI mapping population derived from Synthetic x Opata was phenotyped for the present mapping study based on results from our previous screening study (Ayalew et al. 2015) and also other study reports on the potential of Synthetic wheat and its diploid progenitor, *Ae. tauschii* for drought resistance (Reynolds et al. 2007, Sohail et al. 2011).

Variation in bi-parental populations is essential for successful identification QTL contributing for the genetic control of a trait under study. In the present study, phenotypic variation and the corresponding broad sense heritability ($H^2$) values were high (81-86%) in both growing conditions. This high level of genetic variability enabled the identification of eight major root length QTLs under both growing conditions. The average shift in a population mean towards a desired direction is dependent on the proportion of additive genes in the observed genetic variation (Falconer and Mackay 1996).

6.6.1 Root length QTLs under non-stress condition were distributed across the three genomes of wheat.

Deep rooting and early vigour are among the most desirable traits for water use efficiency and ground cover to hinder soil evaporation loss and smother weeds (Kipp et al. 2014, Zhang et al. 2014). The present study identified four major root length QTLs combined explaining 43% of phenotypic variation under non-stress growing condition.
These early vigour QTLs were distributed across all the three genomes of wheat (Fig. 6.2). The findings in the present study were in agreement with Bai et al. (2013) who reported root length QTLs on chromosomes 2D, 5A and 6A in wheat. Similarly Parent et al. (2015) reported growth related QTLs on 1B, 2D, and 5A. Deep rooting at early crop growth stage is positively correlated with early vigour which enables better crop establishment for improved photosynthetic capacity and better biomass production (Landjeva et al. 2008, Ryan et al. 2015, Wilson et al. 2015). Breeding programs need to target improving early vigour to better utilize available water, one of the scare resources.

6.6.2 Synthetic wheat contributed positive alleles for long roots under water stress.

Four major root length QTLs were identified under stress condition. Two of them gained positive alleles from Synthetic. This finding was in agreement with some previous studies that reported the adaptive potential of Synthetic hexaploid wheat to drought environments (Reynolds et al. 2007, Sohail et al. 2011). Genotype Opata contributed the positive alleles to the two QTLs (Qdrl.uwa.7BL.1 and Qdrl.uwa.7BL.2) on chromosome 7B (Table 6.2). Several previous studies reported drought resistance related QTLs in chromosomes arms of 1A, 3A and 7B which corroborate with the present study (Rebetzke et al. 2008, Peng et al. 2011, Parent et al. 2015, Maccaferri et al. 2016). Previous research reported that chromosome 3A was one of the three homologues to have DREB1A (dehydration responsive element binding) transcription factors (TFs) further validating the present finding (Wei et al. 2009, Edae et al. 2013). The DREB1A TFs are correlated with heading date, vegetation index and biomass yield (Budak et al. 2015) which was instrumental in development of long roots and therefore
high biomass in this study. Similarly the two QTLs on chromosome 7B were mapped with ABA responsive chromosome regions which slow plant growth (Barakat et al. 2015). This justifies the negative additive value by the parent Synthetic in this study. The phenotypic effect of the two positive alleles from the A genome of Synthetic were equally counterbalanced by its negative alleles from the B genome (Table 6.2) pointing to the need of using markers of these QTLs simultaneously for MAS to select for long roots and against short roots, respectively for optimum improvement. None of the QTLs identified under non-stress growing condition did collocate with QTLs under stress condition suggesting the presence of qualitative QTL by environment interaction. In situations when the nature of QTL by environment interaction is significantly and qualitatively different, selection activities for the two environments shall be dealt separately (Qu et al. 2008, Yan and Holland 2010, Ayalew et al. 2014). The varying patterns of QTLs based on water regimes in this study conformed to prior studies explaining the significance of QTL x environment interaction and the complexity of root length and drought resistance genetics (Kamoshita et al. 2002, Li et al. 2005, Qu et al. 2008, Sharma et al. 2011, Parent et al. 2015).

6.6.3 Inheritance of the identified QTLs improved water stress resistance in other genetic backgrounds.

Confirming the phenotypic effect of identified QTLs on genetic backgrounds other than the mapping population itself, and validating the usability of linked markers for marker assisted selection is a major step towards the utilization of identified QTL genotypes in plant breeding (Zhou et al. 2003, Collard et al. 2006, Palomeque et al. 2010). In the present study three hybrid populations, which involve Synthetic (source parent of QTLs) as one of the parents, were developed to validate the effect of the identified QTLs in their
progenies. SSR markers closely linked to these QTLs, *Xbarc148, Xgwm391*, were used to track down the acquired QTLs in F$_{2.3}$ (Synthetic x Yitpi) and F$_{3.4}$ (Synthetic x Chara, and Synthetic x Lang) progenies. Synthetic x Chara progenies showed an average increase of 10% in root length as a result of *Qdrl.uwa.1AS* and *Qdrl.uwa.3AL*. The hybrid (Aa) genotypes were generally higher in average root length than their homozygous recessive (aa) parents suggesting that long root is dominant over short root. This is in agreement with our previous study on the genetics of gene actions controlling root length under water stress (Ayalew *et al*. 2016b). The highest increase in root length was found among Synthetic x Yitpi progenies as a result of *Qdrl.uwa.3AL*. This discrepancy in the level of gain from the positive alleles explains the variation in the proportion of additive dominant genes in the parental lines. Yitpi seems to have the highest proportion of additive genes as compared to the rest of the parental lines as its progenies were the highest in root length.

In conclusion, the SSR markers validated in this study can be used to identify genotypes with the desired root phenotype. Interestingly, *Qdrl.uwa.3AL* was collocated with *DREB1A* genes from Synthetic wheat which can be assayed using the close SSR marker (*Xgwm391*) validated without the need to develop functional markers for early stage water stress resistance.
Chapter 7  GWAS revealed that B genome of hexaploid wheat harbours major QTLs controlling root length for water stress resistance.

7.1 Abstract

Drought is one of the major environmental constraints limiting crop productivity in dry areas. The present study was undertaken to assess a core collection of winter wheat genotypes under early stage water stress and to identify genomic regions controlling root growth. Ninety one genotypes collected from different parts of the world were evaluated under water stress and non-stress conditions in a randomized block design with three replicates. Genome wide association analysis was performed using compressed mixed linear model (CMLM) with efficient mixed model association (EMMAX) approach. A total of 533 diversity array technology (DArT) markers with known linkage positions were used to compute marker-trait linkage disequilibrium (LD). Analysis of phenotypic data indicated a highly significant ($P < 0.01$) variation. Broad sense heritability of 59% and 73% with corresponding genetic gain of 7.6 and 9.7 at 5% selection intensity under non-stress and stress conditions was computed, respectively based on the phenotypic data. Out of the total marker pairs, 15% showed significant LD ($P < 0.05$). Nearly 16% of the significant LDs were found among inter chromosomal marker pairs which were caused by factors other than linkage. Average LD decay for all chromosomes was estimated at approximately 35 cM, when the $r^2$ cut-off value was set at 0.25. A total of five DArT markers were found significantly ($P < 0.05$) associated with root length. The B genome harboured all of the major QTLs under the stress condition, where marker loci $wPt6278$ (2B) and $wPt1159$ (3B) were significantly associated with root length explaining 17% and 14% of phenotypic variation, respectively. Two out of three major QTLs showing significant association with root length under the non-stress condition, were on the B genome associated with
markers \textit{wPt0021} (3B), \textit{wPt4487} (4A) and \textit{wPt8890} (5B). Genome wide association mapping approach was helpful in identifying genomic regions associated with water stress resistance in hexaploid wheat. The B genome played an important role in water stress resistance as it harboured all the major QTLs for root length under stress. The identified markers closely linked to the QTLs can be used for marker assisted selection in hexaploid wheat.

7.2 Background

Common wheat (\textit{Triticum aestivum} L.) is one of the earliest cereals ever domesticated and it is currently one of the largest cereal crops in terms of production in the world. According to FAO report for the 2015/2016 cropping season FAO (2016), the global wheat production has reached to 733 million metric tonnes per annum. The current productivity of wheat, and all other crops, needs to be further increased to feed the ever increasing global population. The effort to improve production and productivity of wheat is, however, deterred by different biotic and abiotic stresses. Water stress is amongst the most serious environmental constraints limiting crop growth and productivity in various parts of the world (Zhao and Dai 2015). Genetic improvement of crops is among the most plausible intervention mechanisms for better adaptation to climate change. There are various genetic analysis techniques to look into the genetic architecture of traits at various crop developmental stages that are affected by stress factors (Zheng \textit{et al.} 2008, Qie \textit{et al.} 2014, Song \textit{et al.} 2016). Traits related to stress resistance including deep rooting, thick wax layer, spiny and acute leaf angle are largely investigated (Wasson \textit{et al.} 2012, Comas \textit{et al.} 2013). However, root phenotyping is expensive and labour intensive as a routine over large number of lines. The use of closely linked molecular markers with the genes of interest holds a promise in
minimizing the labour to access roots and improving accuracy of selection (Semagn et al. 2006, Beyene et al. 2016). For successful application of molecular markers in selection, however, a significant association between traits of interest and molecular markers is essential. Quantitative traits loci can be identified and mapped in two general approaches: linkage analysis between markers and traits in segregating populations and linkage disequilibrium analysis in unstructured populations (Meuwissen and Goddard 2004).

Quantitative trait loci (QTL) mapping using segregating populations derived from parental lines is the most frequently used method (Waktola 1999, Ramya et al. 2010, Qie et al. 2014). The non-random association of alleles (linkage disequilibrium, LD) at different loci is also exploited to identify QTLs using collections of germplasm cultivars and all available genetic and breeding materials (Neumann et al. 2011, Tadesse et al. 2014, Jighly et al. 2015, Lopes et al. 2015, Tadesse et al. 2015). LD association helps the molecular dissection of complex traits providing broader genomic coverage with high resolution without the need to develop structured mapping populations. However, LD association analysis also has its own defects unless handled with care (Gaut and Long 2003). Population structure and relatedness can cause spurious LD which finally leads to false positive associations (Bradbury et al. 2007, Zhang et al. 2010). Analysis methods that account for these spurious associations have been developed thereby improving the applicability of genome-wide association studies (GWAS) (Kang et al. 2008, Zhang et al. 2010, Lipka et al. 2012). GWAS has been carried out in many crops and QTLs controlling traits of interest have been identified and mapped. QTLs associated to early vigour in foxtail millet (Qie et al. 2014), milling quality and grain yield (Tadesse et al. 2015), and resistance to various diseases (Allard 1956, Jighly et al. 2015).
Chapter 7

2015, Vuong et al. 2015) in wheat, have been reported using GWAS approach. The present study is one of the similar efforts in the search for QTLs controlling root length under water stress and non-stress conditions. The main objectives of this study were 1) to characterize the genotypic and phenotypic diversity of a core winter wheat collection, and 2) to identify genomic regions significantly associated with root length variations.

7.3 Materials and methods

7.3.1 Plant materials and phenotypic evaluation

Ninety one genotypes of a winter wheat core collection originated from the Institute of Field and Vegetable Crops (Novi Sad, Serbia) were evaluated for root length at early plant growth stage both under water stress and non-stress conditions (Table 7.1). The core collection consists of genotypes with contrasting phenotypes for various breeding traits which were collected from 21 countries across five continents (Neumann et al. 2011).

Phenotypic evaluation for osmotic stress was carried out in a constant temperature growing room in a hydroponic system at the school of plant biology, The University of Western Australia. A hydroponic culture system optimised for a similar research by Ayalew et al. (2015) was used. Plastic box of three litres was used with holes on lids drilled using an 8 mm diameter driller. The top of the lids were lined with filter paper to keep plants in place and the surface moist. Seeds were first germinated in Petri dishes lined with filter paper for 48 hours and then healthy and vigorous seedlings were transferred to the water system organized in a randomized complete block design with three replicates. Osmotic stress of -0.5 MPa was induced using PEG 6000 (Sinopharm Chemical Reagent Co. Ltd, Shangi China). The final stress level during data collection
was measured using MP4 dewpoint potentiometer (Decagon Devices Inc. 2003) and the stress was progressive which reached -0.6 ± 0.1 MPa at the time of data collection.

Plants were let to grow with their endosperm reserve food for the first seven days followed by the addition of half strength Hoagland’s solution and PEG 6000 solution for the treatment and Hoagland’s solution alone for the control respectively. The pH of the solution was adjusted to 5.5 - 5.7 with relative humidity between 65-70% while the temperature was 25/22°C day/night. Light intensity of 300 μmol.m⁻².s⁻¹ was supplied using cool florescent lamps in 10/14 h dark and light timing using an automatic timer. The solution was being constantly aerated by bubbling air in to the solution using an electric bubbler. Data were scored on root length 14 days after planting (seven days stress). Longest roots of samples were measured by using a graduated ruler.
Table 7.1 Genotypes used in this experiment and their respective countries of origin

<table>
<thead>
<tr>
<th>Accession name</th>
<th>Origin</th>
<th>Accession name</th>
<th>Origin</th>
<th>Accession name</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnif 41</td>
<td>Argentina</td>
<td>Acciaio</td>
<td>Italy</td>
<td>PKB Krupna</td>
<td>Serbia</td>
</tr>
<tr>
<td>Gala</td>
<td>Argentina</td>
<td>Ai-bian</td>
<td>Japan</td>
<td>NS 46/90</td>
<td>Serbia</td>
</tr>
<tr>
<td>Kite</td>
<td>Australia</td>
<td>Norin 10</td>
<td>Japan</td>
<td>Mina</td>
<td>Serbia</td>
</tr>
<tr>
<td>Minister Dwarf</td>
<td>Australia</td>
<td>Saitama - 27</td>
<td>Japan</td>
<td>NS 63-24</td>
<td>Serbia</td>
</tr>
<tr>
<td>Mexico 120</td>
<td>Australia</td>
<td>Tr. Compactum</td>
<td>Latvia</td>
<td>NS 74/95</td>
<td>Serbia</td>
</tr>
<tr>
<td>Timson</td>
<td>Australia</td>
<td>Vireo &quot;S&quot;</td>
<td>Mexico</td>
<td>NS 79/90</td>
<td>Serbia</td>
</tr>
<tr>
<td>Triple dirk &quot;S&quot;</td>
<td>Australia</td>
<td>Mex. 3</td>
<td>Mexico</td>
<td>Avalon</td>
<td>UK</td>
</tr>
<tr>
<td>Tr. dirk &quot;B&quot;(GK 775)</td>
<td>Australia</td>
<td>Cajeme 71</td>
<td>Mexico</td>
<td>Brigand</td>
<td>UK</td>
</tr>
<tr>
<td>Cook</td>
<td>Australia</td>
<td>Siete Cerros</td>
<td>Mexico</td>
<td>TJB 990-15</td>
<td>UK</td>
</tr>
<tr>
<td>Tr. dirk &quot;B&quot;(GK 12)</td>
<td>Australia</td>
<td>Inia 66</td>
<td>Mexico</td>
<td>Highbury</td>
<td>UK</td>
</tr>
<tr>
<td>Rusalka</td>
<td>Bulgaria</td>
<td>Mex. 17 bb</td>
<td>Mexico</td>
<td>Mironovska 808</td>
<td>Ukrain</td>
</tr>
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<td>Lambriego Inia</td>
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<td>BCD 1302/83</td>
<td>Moldova</td>
<td>HAYS 2</td>
<td>USA</td>
</tr>
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<td>Ching-Chang 6</td>
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<td>F 4 4687</td>
<td>Romania</td>
<td>WWMCB 2</td>
<td>USA</td>
</tr>
<tr>
<td>Al Kan Tzao</td>
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<td>Donska polup.</td>
<td>Russia</td>
<td>INTRO 615</td>
<td>USA</td>
</tr>
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<td>Peking 11</td>
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<td>Russia</td>
<td>UC 65680</td>
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<td>Ana</td>
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<td>NS 602</td>
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<td>Vel - USA</td>
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<tr>
<td>ZG 987/3</td>
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<td>NS 559</td>
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<td>USA</td>
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<td>ZGK 238/82</td>
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<td>L 1A/91</td>
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<td>Holly E</td>
<td>USA</td>
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<td>L 1/91</td>
<td>Serbia</td>
<td>Centurk</td>
<td>USA</td>
</tr>
<tr>
<td>Tibet Dwarf</td>
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<td>Serbia</td>
<td>Helios</td>
<td>USA</td>
</tr>
<tr>
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<td>China</td>
<td>Sofija</td>
<td>Serbia</td>
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<td>USA</td>
</tr>
<tr>
<td>Durin</td>
<td>France</td>
<td>Nizija</td>
<td>Serbia</td>
<td>Tr.Sphaerococcum</td>
<td>USA</td>
</tr>
<tr>
<td>Capelle Desprez</td>
<td>France</td>
<td>Sava</td>
<td>Serbia</td>
<td>Benni multifloret</td>
<td>USA</td>
</tr>
<tr>
<td>L-1</td>
<td>Hungary</td>
<td>NS 55-25</td>
<td>Serbia</td>
<td>Hope</td>
<td>USA</td>
</tr>
<tr>
<td>Szegedi 768</td>
<td>Hungary</td>
<td>Slavija</td>
<td>Serbia</td>
<td>Norin10/Brevor14</td>
<td>USA</td>
</tr>
<tr>
<td>Bankuty 1205</td>
<td>Hungary</td>
<td>Nov. Crvena</td>
<td>Serbia</td>
<td>Phoenix</td>
<td>USA</td>
</tr>
<tr>
<td>Hira</td>
<td>India</td>
<td>Pobeda</td>
<td>Serbia</td>
<td>Lr 10</td>
<td>USA</td>
</tr>
<tr>
<td>UPI-301</td>
<td>India</td>
<td>Renesansa</td>
<td>Serbia</td>
<td>Purd./Loras</td>
<td>USA</td>
</tr>
<tr>
<td>Sonalika</td>
<td>India</td>
<td>Ivanka</td>
<td>Serbia</td>
<td>Red Coat</td>
<td>USA</td>
</tr>
<tr>
<td>Suwwon 92</td>
<td>India</td>
<td>NS 22/92</td>
<td>Serbia</td>
<td>Purdue 39120</td>
<td>USA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Purdue 5392</td>
<td>USA</td>
</tr>
</tbody>
</table>
7.3.2 Molecular marker data

The test genotypes were assayed using diversity array technology (DArT) markers distributed along the whole wheat genome by Triticarte Pty. Ltd (Canberra, Australia; http://www.triticarte.com.au/), a whole-genome profiling service laboratory, as described by Neumann et al. (Neumann et al. 2011). Five hundred and thirty three DArT markers with known linkage position based on CIMMYT integrated map (Crossa et al. 2007) were used. Average \( P \)-value, call rate and polymorphism information content (PIC) of all of the markers were 86, 0.35 and 95, respectively.

7.4 Data analysis

7.4.1 Phenotypic data analysis

Phenotypic data were subjected to statistical analysis using CropStat 2007.3 (International Rice Research Institute 2007) software accounting for measurement and block effects based on the following fixed model: \( y_{ij} = \mu + g_i + b_j + e_{ij} \), where \( y_{ij} \) is the observed mean, \( \mu \) is the general mean, \( g_i \) is the genotype, \( b_j \) is the block and \( e_{ij} \) is the error effects. Variance components were estimated and broad-sense heritability were estimated using the following expression: \( H^2 = \frac{\delta_g^2}{(\delta_g^2 + \delta_e^2)} \), where \( \delta_g^2 \) and \( \delta_e^2 \) are the estimated genotypic and error variances, respectively. Genetic gain was calculated using the formula: \( Gs = K*H^2*(\frac{\delta_P^2}{\delta_P^2})^{1/2} \), where \( K \) is the selection intensity at 5% (\( k = 2.056 \)), \( H^2 \) is heritability in broad sense and, \( (\frac{\delta_P^2}{\delta_P^2})^{1/2} \) is phenotypic standard deviation.

7.4.2 Population structure and linkage disequilibrium

Linkage disequilibrium values (\( r^2 \) and \( P \)-value) between DArT markers were calculated using TASSEL software version 5.2.18 (Bradbury et al. 2007). Minor allele loci with <
0.05 frequency were filtered out to reduce biased LD estimations between pairs of loci (Gaut and Long 2003). The $r^2$ values for pairs of loci were plotted as a function of map distances, and LD decay ($r^2 < 0.19$) was estimated using the average distances of marker pairs showing LD values lower than 0.19 (Whitt and Buckler 2003).

Principal components and a kinship matrix were calculated using GAPIT statistical package in R software (Kang et al. 2008, Lipka et al. 2012). The kinship matrix was calculated based on VanRaden’s (VanRaden 2008) method. Unweighted pair group method with arithmetic mean (UPGMA) was used to cluster the wheat genotypes based on polymorphism of the 533 DArT markers with known chromosomal positions. The distribution of correlation coefficients ($r^2$) between DArT markers located at different physical distances of the whole wheat genome was calculated to establish LD relationship among loci.

7.4.3 Genome-wide association scan

GWAS analyses were performed using the Genomic Association and Prediction Integrated Tool (GAPIT) package in R (Lipka et al. 2012). Compressed mixed linear model (CMLM) approach with population parameters previously determined and efficient mixed model association (P3D/EMMAX) approaches were used (Zhang et al. 2010). The optimum number of principal components (PCs) to be included in the final model for GWAS was determined by a forward model selection using the Bayesian information criterion (BIC). The following mixed model was used to account for genetic relatedness among lines in the genome-wide association mapping (Zhang et al. 2010): $Y = X\beta + Zu + e$, where $Y$ is the vector of observed phenotypes; $\beta$ is an unknown vector containing fixed effects, including the genetic marker, population structure (Q),
and the intercept; \( u \) is an unknown vector of random additive genetic effects from multiple background QTL for individuals/lines; \( X \) and \( Z \) are the known design matrices; and \( e \) is the unobserved vector of residuals. The quantile–quantile (QQ) plots were constructed by plotting the observed \(-\log (P\text{-value})\) of the markers against the expected \(-\log_{10} (P\text{-value})\), under the null hypothesis that there is no association between marker and phenotype (Wilk and Gnanadesikan 1968). The QQ plot is a useful tool for assessing how well the model used in GWAS accounts for population structure and familial relatedness. The \( P \)-values from CMLM analysis were adjusted based on Benjamini-Hochberg false discovery rate controlling procedure (Benjamini and Hochberg 1995).

7.5 Results

7.5.1 Phenotypic variation

Mean root lengths ranged from 5 cm to 25 cm under stress condition and from 13 cm to 32 cm under non-stress condition (Fig. 7.1a & b). The induced stress reduced average root length by 51\% (Fig. 7.1c). Genotypes NS 63-24 and Tr. dirk "B" (GK 12) showed the longest roots under stress condition while genotypes Suwwon 92 and Holly E were longest rooted genotypes under non-stress condition.
Figure 7.1. Distribution of genotypes for root length under -0.6 ± 0.1 stress (a) and under non-stress (b) growing conditions, and the mean root length (cm) ± SE of all genotypes under stress and non-stress conditions (c). Frequency is number of genotypes.

Analysis of variance showed a significant variation for root length under the two growing conditions (Table 7.2). Heritability was moderate (59%) to high (73%) under non-stress and stress conditions, respectively. Genetic gain at 5% selection intensity ranged from 7.6 to 9.6 under non-stress and stress conditions, respectively (Table 7.2).

Table 7.2. Variance, heritability and genetic gain (at 5% selection intensity) of root length among 91 diverse wheat genotypes grown under non-stress and stress conditions.

<table>
<thead>
<tr>
<th>Growing condition</th>
<th>Genotypic variance</th>
<th>Error variance</th>
<th>Heritability (H^2) (%)</th>
<th>Genetic gain (GA) (5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-stress</td>
<td>39.0*</td>
<td>27.0</td>
<td>59</td>
<td>7.6</td>
</tr>
<tr>
<td>Stress</td>
<td>41.7**</td>
<td>15.5</td>
<td>73</td>
<td>9.7</td>
</tr>
</tbody>
</table>

**, * indicate significant variation at $P \leq 0.01$ and $P \leq 0.05$, respectively.

7.5.2 Genotypic diversity and linkage disequilibrium

The ninety one wheat accessions were grouped into three major clusters based on unweighted pair group method with arithmetic mean (UPGMA) clustering algorism based on maximum Euclidian distance calculated on the 533 DArT markers (Fig 7.2a). Principal component analysis (PCA) also showed dispersed genotypes along different
principal components suggesting very diverse genetic background of the test genotypes (Fig. 7.2b). The first four principal components (PCs) were found optimum for the GWAS in this study. Nearly 15% (3,877 out of 25,125 marker pairs) of the marker pairs showed significant LD ($P < 0.05$). A total of 196 marker pairs were in complete linkage spanning a total length of 10.45 cM. Nearly 16% (2,285 of 14,436 significant marker pairs) of the significant LDs were found among inter chromosomal marker pairs which were caused by factors other than linkage. Generally, LD value declined as the physical distance between the loci increased. The average LD value for inter chromosomal markers was 0.019 while the same was 0.069 for intra chromosomal markers. The overall average LD for all of the marker pairs showed $r^2$ value of 0.039. The significant ($P < 0.05$) marker pairs identified in the intra chromosomal linkage had average $r^2$ values greater than 0.19. Out of the significant LDs, nearly 7% of them had $r^2$ values greater than 0.1. LD decreased rapidly at genetic distances of less than 10 - 15 cM (Fig. 7.2c & d). The average genetic distance between markers with $P < 0.05$ was 25.39 cM. The average LD decay for all chromosomes was estimated at approximately 35 cM, when the cut-off value for $r^2$ was set to 0.25 (Fig.7.2d).
Figure 7.2. Population structure and linkage disequilibrium of 91 winter wheat genotypes using 533 DArT markers (a), major clusters of the core wheat collection based on unweighted pair group method with arithmetic mean (UPGMA) algorithm; (b), multidimensional scatter plot of marker data along three principal components; (c), plot of pair-wise LD ($r^2$) values of markers as a function of their probability ($P$-value) of LD; (d), genome-wide average linkage disequilibrium (LD) measured by $r^2$ over genetic distances (cM).
7.5.3 Genome wide association scan

7.5.3.1 Under stress condition

GWAS based on CMLM model identified two DArT markers significantly associated with root length under stress growing conditions (Table 7.3, Fig. 7.2a). The DArT markers \textit{wPt6278} (2B) and \textit{wPt1159} (3B) were significantly associated with root length. Quantile-quantile (QQ) plot representing expected and observed probability showed fit of the model which enabled reducing any spurious association of markers with root length (Fig. 7.3b). Genomic regions on chromosomes 2B and 3B showed the highest peak with \(P\)-values of 3E-04 and 1.1E-3, explaining 17% and 14 % of phenotypic variation, respectively (Table 7.3).

Figure 7.3 Manhattan plot (a) and quantile-quantile (QQ) plot of \(P\)-values (b) of 91 wheat genotypes tested under stress condition. The shaded area along the dotted points (b) showed the 95% confidence interval. RL stands for root length.
7.5.3.2 Under non-stress condition

Three significant marker-trait associations were detected under non-stress condition (Fig 7.4a). QQ plot of observed and expected log10 probability of P-values showed the appropriateness of CMLM in the analysis (Fig. 7.4b). Markers wPt0021 (3B), wPt4487 (4A) and wPt8890 (5B) showed significant association with root length under the non-stress condition, explaining 22%, 22% and 19% of phenotypic variation, respectively. The minor allele frequency (MAF) of DArT markers ranged from 0.09 to 0.43 while the highest P-value was 3E-4.

Figure 7.4 Manhattan plot (a) and quantile-quantile (QQ) plot of P-values (b) of 91 wheat genotypes tested under stress condition. The shaded area along the dotted points (b) showed the 95% confidence interval. RL stands for root length.

It was interesting to note that chromosome 3B harboured two loci significantly associated with root length each explaining 14% and 22% of phenotypic variation, under stress and non-stress conditions, respectively (Table 7.3).
Table 7.3. Significantly associated DArT markers, their chromosomal positions and level of phenotypic variation ($R^2$) of root length under stress and non-stress growing conditions.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Marker name</th>
<th>Chromosome</th>
<th>Position</th>
<th>MAF</th>
<th>$R^2$ (%)</th>
<th>$p$</th>
<th>$q$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress</td>
<td>wPt6278</td>
<td>2B</td>
<td>83.9</td>
<td>0.08</td>
<td>17</td>
<td>3E-04</td>
<td>1E-03</td>
</tr>
<tr>
<td></td>
<td>wPt1159</td>
<td>3B</td>
<td>44.4</td>
<td>0.06</td>
<td>14</td>
<td>1.1E-03</td>
<td>2E-03</td>
</tr>
<tr>
<td>Non-stress</td>
<td>wPt0021</td>
<td>3B</td>
<td>96.64</td>
<td>0.11</td>
<td>22</td>
<td>5E-04</td>
<td>1E-03</td>
</tr>
<tr>
<td></td>
<td>wPt4487</td>
<td>4A</td>
<td>174.62</td>
<td>0.43</td>
<td>22</td>
<td>5E-04</td>
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<tr>
<td></td>
<td>wPt8890</td>
<td>5B</td>
<td>77.95</td>
<td>0.09</td>
<td>19</td>
<td>1.6E-03</td>
<td>3E-03</td>
</tr>
</tbody>
</table>

MAF stands for minor allele frequency; $p$ = level of significance without adjustment; $q$ = adjusted level of significance based Benjamini-Hochberg false discovery rate controlling procedure; $R^2$ = percentage phenotypic expression.

7.6 Discussion

7.6.1 Two significant QTLs were located on the B genome of wheat

Selection for improved crop traits can be accelerated when high genetic variability is coupled with molecular markers tightly linked to trait of interest. Detecting significant association between molecular markers and various traits has been the focus of many crop breeders (Abdelraheem et al. 2015, Bac-Molenaar et al. 2015, Qin et al. 2015). The present study was one of similar efforts in the search for QTLs that control root length, which is helpful for better adaptation in a drying world. A total of five DArT markers were found significantly associated with root length under the two water regimes (Table 7.2). The B genome of wheat harboured two of the significant QTLs identified under water stress condition (Table 7.3). The pattern of marker trait association in the two growing conditions were different that none of the significant associations were common between the two water regimes despite the fact that chromosome 3B had two significant QTLs with one for each growing conditions located about 52 cM apart. This might be due to the differential gene expression pattern.
triggered by the induced stress which is in agreement with Bac-Molenaar et al. (Bac-Molenaar et al. 2015). Genomic regions of chromosomes 2B and 3B have previously been reported to harbour QTLs related to high grain yield (Waktola 1999, Lopes et al. 2013, Bellucci et al. 2015, Lopes et al. 2015, Tadesse et al. 2015).

One of the major problems hindering root research is the uneasy access to roots due to their natural underground exposure. Marker assisted selection (MAS) is one of the promising approaches which will minimize the labour and time required for physical examination of roots once a reliable marker trait association is established. DArT markers \textit{wPt0021} (3B), \textit{wPt4487} (4A) and \textit{wPt8890} (5B) were significantly associated with root length under non-stress growing condition which can be incorporated into breeding programs. Similar previous research (Qin et al. 2015, Ryan et al. 2015) reported significant early vigour QTLs located on chromosomes 3B and 4A which was in agreement with the present study. The marker trait associations in this study tended to be on the B genome and none were on D genome could be partly due to the reason that D genome was poorly populated by markers. The B genome of wheat showed the highest frequency of significant associations in both water regimes indicating its role in controlling early root vigour and water stress resistance.

7.6.2 Linkage disequilibrium and genetic structure

Unweighted pair group method with arithmetic mean (UPGMA) clustering resulted in three major clusters (Fig. 7.2d). Principal component analysis (PCA) also showed large genetic diversity in genotypes calling for the use of mixed linear model for GWAS analysis in order to account for population structure and relatedness (Zhang et al. 2010). Thorough understanding on the genetic structure and linkage disequilibrium is vital for
successful genome-wide marker trait association analysis (GWAS). The present study found significant LD ($P < 0.05$) in 3,877 of 25,125 (15%) intra chromosomal marker pairs with an average $r^2$ value of 0.25. Comparable LD values of 19% were reported in cotton association studies (Qin et al. 2015), while in wheat it was 15% (Neumann et al. 2011, Dreisigacker et al. 2012, Edae et al. 2014). In this study, LD decreased rapidly at genetic distances of less than 15 cM (Fig. 7.2c & d). The average genetic distance between markers with $r^2 > 0.1$ was 15.3 cM. Average LD decay for all chromosomes was estimated at approximately 35 cM, when the value of the cut-off value for $r^2$ was set to 0.25 (Fig. 7.2d). This relatively long LD might be caused by inbreeding which limits the number of heterozygotes and the number of effective recombination rates leading to correlated genetic polymorphisms, hence long physical LD (Gaut and Long 2003). Findings in this study were in agreement with previous studies which reported LD decay extending up to 35 cM (Abdurakhmonov et al. 2008, Tadesse et al. 2015). Nearly 16% of the significant LDs were found among inter chromosomal marker pairs which were caused by factors other than linkage. Breeding and selection, population stratification and relatedness, genetic drift and bottlenecks were reported to be among the factors that could cause LD among non-collinear markers (Abdurakhmonov et al. 2008, Chao et al. 2010).

7.6.3 Phenotypic variation and potential for genetic improvement

Early crop growth stage is one of the most succulent, and delicate stages which are highly vulnerable to environmental hazards like drought. As evidenced in this study early stage water stress can inflict up to 50% reduction in crop performance. Comparable reductions (40 - 54%) in root length were reported on segregating populations and diverse wheat collections in previous research (Ud-Din et al. 1992,
Ayalew et al. 2015, Ayalew et al. 2016b). Root traits hold the promise for successful crop adaptation in arid areas through their obvious role in water acquisition (Burdon et al. 2012, Tanaka et al. 2013). Water stress can induce signals in different genotypes differently. Some genotypes respond by halting growth while others keep on non-stress physiology by defying the stress. This growth differential was observed by the change in the ranking of genotypes across the two water regimes in this study. This differential response/ranking which was also observed in our previous experiments (Ayalew et al. 2015) indicated the appropriateness of selecting stress resistant genotypes under the target environment (Blum 2011a). Previous findings (Blum 2011a, Bac-Molenaar et al. 2015) reported the complex and dynamic nature of plant growth high lighting the different roles played by specific genes at a specific growth stage. Broad sense heritability was moderate to high (59%-73%) in this study which indicated the possibility for successful genetic improvement of root length using diverse germplasm.

7.7 Conclusion

The B genome of hexaploid harboured two significant QTLs for root length under stress condition which calls for further genetic characterization of this genome. The importance of the B genome for early root vigour is also indicated by the presence of two out of three significantly associated loci with root length under non-stress condition in chromosomes 3B and 5B. The present study indicated the possibility of improving root length under water stress and non-stress growing conditions. Genetic improvement programs for the two water conditions need to be done separately as the marker trait associations are quite different under the two growing conditions. The identified markers can be incorporated into a MAS program for root length improvement.
Chapter 8  General discussion

8.1  Introduction

Drought is one of the major abiotic stresses limiting wheat production in many parts of the world. Genetic variation and understanding on the mechanism of inheritance of drought resistance are the fundamental requirements for efficient stress resistance breeding.

This research has made the following contributions:

a) A reliable and quick root phenotyping system that enables evaluation of large numbers of genotypes was developed.

b) A total of 832 wheat genotypes collected all over the world including some wheat wild relatives were evaluated. Genotypes, Colotana 296-52, Santa Elena, Tamarin rock and AUS103 showed the longest roots under stress, while two hexaploid genotypes Camm and Tincurrin were found to have the shortest roots.

c) Root length under water stress is controlled by dominant gene actions which are additive in effect.

d) Bi-parental QTL mapping identified eight QTLs and phenotypic effects of two major QTLs were validated under other genetic backgrounds.

e) Genome wide association analysis identified five QTLs significantly associated with DArT markers and most of them are in the B genome of hexaploid wheat.

8.2  New root phenotyping system was developed and divergent genotypes were identified.

Developing a reliable phenotyping technique for root traits is one of the active areas of research. Measuring root traits is not as direct and simple as measuring above-ground
plant parts. In this research, a hydroponic system was developed enabling induction of water stress while the plants were grown undisturbed. The use of hydroponic systems was given sound practical backup by various researchers (Szira et al. 2008, Hoffmann et al. 2012). Standard commercial wheat varieties for low rainfall areas were used as benchmarks/controls both for genotype comparison and system performance control. The consistency in the performance of the control varieties under stress indicated that the hydroponic system was successful in simulating the stress and in discriminating genotypes for early stage water stress. It also implies the importance of early stage resistance as an indicator of later stage performance, as the control varieties were found good at early stage resistance in addition to their claimed resistance at later stages (Wheeler 2013).

Wheat genotypes with diverse genetic backgrounds were evaluated to identify extreme genotypes for further evaluation and genetic studies. Root length was chosen as a measure of water stress resistance in this study for environments that experience dry spells at the start of a cropping season after successful germination. In such a situation it is not the amount of rainfall that matters, it is its distribution in the season. Precipitation from the early showers can be effectively used if plants have the means to reach to the water that has gone into deeper soil profile. In this study, the wheat collections tested showed very diverse response to water stress in terms of their root elongation. Deep rooting is also helpful for early vigour which is believed to counter-balance water loss through early ground cover and transpiring the available water through the leaves rather than wasting it in the form of soil transpiration (Burdon et al. 2012, Gupta et al. 2012). The present study is in accordance with the idea that genotypes with deep rooting ability have the potential to maintain their cellular hydration through the extra water from

Genotypic differences as a function of level of domestication showed that highly selected and domesticated plants are vigorous and grow quicker as compared to wild relatives (del Blanco et al. 2000). Hexaploid wheat and other high ploidy artificial wheat hybrids showed longer roots under both stress and non-stress condition which indicates that higher ploidy level is favourable for better growth. This corroborates with the idea that hybridization (alloploidy) of two or more genomes increases allelic diversity which provides genetic buffer and also expression of novel phenotypes which is helpful for environmental stress resistance (Udall and Wendel 2006).

In conclusion, data from this study showed the presence of ample genetic variability which can be used as a source of resistance for early stage water stress resistance breeding, and also to study the genetics of root traits. The hydroponic system was found to be a handy tool for root phenotyping for large number of genotypes.

8.3 Natures of gene actions controlling root length under water stress were identified.

Following the identification of promising genotypes, the next logical question will be how heritable this variation is and how it is genetically controlled. Gene actions controlling root growth under stress were quantitatively analysed in a diallel cross. Analysis of variance indicated the diversity of both parental and progeny lines in their reaction to water stress. The performance of parental lines was in agreement with the first experiment showing repeatability of the screening technique developed. Both additive and dominant gene effects showed significant role in the control of root length
under water stress. The general combining ability was not purely due to additive gene effects which showed the role of other forms of gene actions that can be harnessed by develop hybrid line. In addition to the prominently dominant gene action, over dominance was also observed which may be due to complementarity of additive genes. Additive complementarity of genes can inflate complete dominance into over dominance which increases variance (Vr) in relation to parent-offspring co-variance (Wr), particularly for more recessive parents (Allard 1956, Blum 1996, Lippman and Zamir 2007). Most of the hybrids in this study showed heterosis greater than the better parent, best explained by the additive complementarity of dominant genes. Over dominance can also be caused by tightly linked genes in repulsion phase which gives non-inheritable genetic component (Fasoula and Fasoula 2002, Lippman and Zamir 2007). Over dominance was reported to be important in the inheritance of yield and other yield related traits in rice and other crops (Sio-Se Mardeh et al. 2006, Li et al. 2008).

From the present study it can be conclude that seedling root length is under dominant genetic control. Root length under water stress can be improved through hybridization and recurrent selection of genotypes concentrating desirable dominant genes (Sio-Se Mardeh et al. 2006, Blum 2011a). The most promising hybrids Santa Elena × Pato, Santa Elena × Tincurri and Colotana 296-52 × Pato can be advanced into pure lines with high chance of getting an inbred line that have fixed the hybrid vigour from the hybridization. Genotypes with high proportion of recessive genes may be suitable for environments having shallow soil profile. Developing structured mapping population from these contrasting genotypes facilitates to effectively map QTLs contributing to root length and other traits.
8.4 QTLs explaining large proportion of phenotypic variation were identified and transferred into other cultivars thereby improving water stress resistance.

Selection for improved crop traits can be accelerated when high genetic variability is coupled with molecular markers tightly linked to trait of interest. Detecting significant association between molecular markers and various traits has been the focus of many crop breeders (Abdelraheem et al. 2015, Bac-Molenaar et al. 2015, Qin et al. 2015). This research was one of similar efforts in the search for QTLs that control root length, which is helpful for better adaptation in a drying world. QTLs were identified and mapped using both structured (RILs) and unstructured (collections) populations. Mapping using RIL populations enabled the identification of eight QTLs in total while GWAS revealed a total of five DArT significant marker-root length associations under the two water regimes. The marker-trait association pattern under stressed and non-stressed growing conditions were different for both GWAS and RIL mapping experiments except the fact that GWAS detected two significant associations on chromosome 3B one significant association under stress and non-stress conditions each.

Water stress resistance QTLs on chromosomes 3A (Chapter 6) and 3B (Chapter 7) collocated with the DREB1A and DREB1B genes, respectively pointing out the need to study these QTL regions further and develop functional markers for reliable MAS. This difference in the association patterns between the two water conditions highlights the differential gene expressions triggered by the induced stress which is in agreement with Bac-Molenaar et al. (2015). Genomic regions of chromosomes 2B and 3B have previously been reported to harbour QTLs related to high grain yield (Waktola 1999, Lopes et al. 2013, Bellucci et al. 2015, Lopes et al. 2015, Tadesse et al. 2015).
One of the major problems hindering root research is the uneasy access to roots due to their natural underground exposure. Marker assisted selection (MAS) is one of the promising approaches which will minimize the labour and time required for physical examination of roots once a reliable marker trait association is established. Confirming the phenotypic effect of identified QTLs on genetic backgrounds other than the mapping population is a major step toward for the utilization molecular genetics in plant breeding (Zhou et al. 2003, Collard et al. 2006, Palomeque et al. 2010). The major water stress resistance QTLs identified by RIL mapping were transferred into three other cultivars and the phenotypic effect of these QTLs were examined. SSR markers Xbarc148 and Xgwm391, closely linked to the QTLs Qdrl.uwa.1AS and Qdrl.uwa.3AL, respectively, were used to track down the acquired QTLs in F_{2.3} (Synthetic x Yitpi) and F_{3.4} (Synthetic x Chara, and Synthetic x Lang) progenies. Validation progenies having the positive alleles of Qdrl.uwa.1AS and Qdrl.uwa.3AL showed 11% and 13% increases in root length, respectively. Heterozygous (Aa) genotypes were generally higher in average root length than their homozygous recessive (aa) parents suggesting that long root is dominant over short root (Fig 7.4). However, the average phenotypic performance of the homozygous dominant types (AA) was not significantly different from the heterozygous (Aa) types. This result was in agreement with our previous study (chapter 5) on the genetics of gene actions controlling root length under water stress (Ayalew et al. 2016b). The highest increase in root length was found among Synthetic x Yitpi progenies as a result of Qdrl.uwa.3AL validation indicating the presence of high proportion of dominant genes in these parental lines. The validation lines produced in this study can be further selected to maintain desirable traits of the recipient parental genotypes through background selection and fixing the transferred genes. The SSR markers validated in this study can be used to identify genotypes with the desired root
phenotype. As Xgwm391 was mapped in the same linkage group as DREB1A gene which plays a key role in drought tolerance at reproductive stage, further study of this QTL region might provide possibility of elucidating the correlation of early vigour and late stage stress tolerance.

The present study indicated the possibility of improving root length under water stress and non-stress growing conditions. Genetic improvement programs for the two water conditions need to be done separately as the marker trait associations are quite different under the two growing conditions. The identified markers can be incorporated into a MAS program for root length improvement. Seedling root length can also be used as an indicator for final crop yield performance as the QTLs identified in this study collocated with various grain yield QTLs reported in the literature. However, a comprehensive study using the same set of germplasm needs to be done to confirm the importance of seedling resistance as an indicator for later stage crop performance.

8.5 Future prospects

Breeding for drought resistance will remain to be the major challenge in crop production and agriculture in general. Improving the adaptability of current cultivars through gene pyramiding and diversification of the wheat gene pool for drought resistance need extra attention in the face of changing/warming climate.

For more effective breeding of wheat cultivars resistant to drought stress:
a) Exploration for more sources of variation for drought resistance needs to be done and results from the newly optimized hydroponic culture need to be extended to field condition, both for genetic studies and for incorporation into breeding programs.

b) Hybrid wheat development needs to be emphasised as one of the potentials to improve deep rooting as there is large dominant gene effect.

c) The identified and validated QTLs are proven to be significant enough for fine mapping and positional cloning. MAS using the identified markers facilitate selection for deep rooting.

d) The gene expression of the contrasting genotypes and the mapped genomic regions will give more fine-tuned information on the gene networks controlling the development of long roots and water stress.

e) QTLs found in the present study conferring drought resistance at the early growth stage can be further tested under field and terminal drought conditions to draw conclusions about their importance for later stage drought resistance.
References


Appendices

Appendix 1. Published articles resulting from research for this thesis
DROUGHT STRESS

Screening Wheat (Triticum spp.) Genotypes for Root Length under Contrasting Water Regimes: Potential Sources of Variability for Drought Resistance Breeding

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Keywords
hydroponic; osmotic stress; seedling resistance; wheat collections

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Abstract
Screening for root traits has been one of the most difficult areas to practise over large number of genotypes. Hydroponic systems enable easy access to roots while high-molecular weight polyethylene glycol (PEG) is used to induce water stress. A total of 839 genotypes were evaluated for root length in a hydroponic trial under PEG-induced stress and non-stress growing conditions. Augmented complete block design with seven blocks and six standard control varieties was used. Root length differences were highly significant (P < 0.01) under both stress and non-stress growing conditions among genotypes. Osmotic stress has caused an average reduction of 54% in root length. Among the genotypes, root length ranged from 1.4 to 13.3 cm under stress, and 4.4 to 23.3 cm under non-stress conditions, respectively. The best control variety for drought resistance was significantly (P < 0.05) outperformed by four new entries namely Goztana 296-52, Compare, Santa Elena and Tammarin Rock, while the shortest roots were measured on genotypes Aus 16356, Elia, Camm, Portugal 3, and Sentinel. Differences among ploidy levels, domesticated and wild forms were also significant (P < 0.05). Hexaploid wheat showed significantly longer roots in both growing conditions while wild tetraploids showed the shortest roots under stress. There was a change in the ranking of genotypes under the two water regimes, which indicates the difficulty of selecting drought resistant varieties under optimum environments.

Introduction
Drought is one of the most serious environmental constraints limiting crop growth and productivity in many parts of the world (Balonechi 2010, Comas et al. 2013). Breeding for drought resistant genotypes through identification of new sources of resistance and pyramiding resistance related genes into a cultivar is an efficient and cost-effective way of adaptation (Fleury et al. 2010). Plants use different mechanisms to avoid damage from a dehydrating environment. Based on mechanisms of survival, plants can be classified into dehydration-avoidant, dehydration-tolerant and drought-escaping types (Blum 2011b). Among them, dehydration avoidance is the most desirable mechanism of drought resistance for crop productivity improvement.

Constitutive traits such as deep root system (Marschall et al. 2006, Lilley and Kirkegaard 2011), fine roots with small diameters, root length density (Blum 2011b, Comas et al. 2013), leaf rolling, leaf waxy layer and osmotic adjustment (Blum 2011b) are among the frequently studied traits that confer dehydration avoidance mechanism to plants. Root traits can be improved by screening genotypes at early growing stage, which can be used as an indicator for later stage performance (Hofmann et al. 2012, Comas et al. 2013). Improving the resistance of seedlings to water-deficit stress has a twofold benefit. The first and direct benefit is that it enables crop establishment through withstanding early season drought (Blum 1996, Albrecht et al. 2012, Passiouha 2012) that happens shortly after successful germination. Water from precipitation or irrigation can be lost in the form of crop respiration, soil evaporation and
percolation into deeper soil layers (Shaxson and Barber 2003). Plants can re-access the water that has gone into deep percolation only if they have long and vigorous root growth at early stage. The second advantage is that water stress resistance at early stage can also be indicative of resistance at later growth stages (Comas et al. 2013), which makes root evaluation easier. However, many researchers warned the need to be cautious in extrapolating early-stage results for later stage resistance unless it is tested and proved in the field (Passiourea et al. 2012, Wasson et al. 2012, Comas et al. 2013).

Germplasm collections from water-deficient environments are highly likely to harbour genes that help their adaptation and survival in such environments. Evaluating diverse germplasm is at the very start of any breeding programme. However, evaluation of root traits is a challenging task especially when targeting large number of genotypes. As a result, there is no best way for root phenotyping to date. Therefore, this research was undertaken (i) to develop an efficient root phenotyping technique, (ii) to find genotypes which can grow deep root system under early-stage water stress and (iii) to identify contrasting (resistant and susceptible) genotypes for genetic studies and molecular breeding.

Materials and Methods

Screening was carried out in a constant temperature room in the school of plant biology, The University of Western Australia. A hydroponic system was developed from plastic boxes (3000 ml) with holes of about 8 mm diameter drilled on lids that supported plant growth on the surface of a solution (Fig. 1). The experiment was set up in a way that the boxes were filled with water/solutions, and the lids were perforated and lined with filter paper to keep plants in place and the surface moist. A total of 838 wheat lines, including control varieties, were evaluated. Genotypes consisted of bread, and durum wheats, and some wild relatives from six different continents (Africa, Asia, Australia, Europe, N/America and S/America), accessed through the Australian winter cereals collection. Augmented complete block design with seven blocks (planting time) and six released control varieties was used. The control varieties – Drysdale (CSIRO 2008), Gladiator, Young, Wyalkatchem, Guardian and Mace (Wheeler 2013) – are among the most popular wheat varieties for their adaptation to low rainfall areas in Australia.

Seeds were first germinated in Petri dishes lined with filter paper soaked with distilled water for 48 h, and then seedlings were transferred to the hydroponic system. Osmotic stress of −0.82 MPa was induced using PEG 6000 (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China). Plants were grown in water for the first seven days followed by either in half strength Hoagland solution alone (control) or half strength Hoagland’s solution and PEG6000 (treatment). The pH of the solution was adjusted to 5.5-5.7 and the relative humidity was 65–70 % while the temperature was 25/22 °C day/night. Light intensity of 300 μmol m⁻² s⁻¹ was supplied using cool fluorescent lamps in 10/14 dark and light timing using an automatic timer. The solution was being constantly aerated using an electric air bubbler. Data were recorded on root length 14 days after planting (seven days after stress treatment) using a graduated ruler.

Data analysis

Analysis of variance was carried out based on augmented complete block design using CRISPSTAT 6.1 software (International Rice Research Institute 2007) accounting for both inter- and intrablock differences. The plot numbers were considered in the residual to account for any measurement errors. The following mixed model was used: \( y_{ij} = \mu + G_j + B_i + e_{ij} \), where \( y_{ij} \) is the observed mean, \( \mu \) is the general mean, \( G_j \) is the genotype, \( B_i \) is the effect of the jth control variety, \( e_{ij} \) is the error effects. The test genotypes and the random error were considered random while the block and control genotypes were considered fixed. Means were adjusted for inter- and intrablock variations and were compared based on the standard errors of the differences between two means with controls and with entries. Due to the imbalance created owing to the occurrence of new entries in a block, four different standard errors (Federer and Raghavarao 1975) were computed as follows:

- Between two controls = \( \sqrt{2MSe/b} \)
- Between two adjusted means in the same block = \( \sqrt{2MSe} \)
- Between two adjusted means in different blocks = \( \sqrt{2MSe(1 + \frac{1}{b})} \)
- Between adjusted means and control mean = \( \sqrt{MSe(b + 1/c + 1)/bc} \), where MSe is mean square of
error, \( b \) is the number of blocks, and \( r \) is number of control varieties.

Adjusted mean values across the six continents and the different ploidy levels were compared using one-tailed t-test.

**Results**

**Seedling performance under stress condition**

Root length reduction of up to 54% was observed as a result of the induced water stress compared to the non-stressed growing condition (Fig. 2c). Analysis of variance depicted a highly significant difference (\( P < 0.01 \)) among both control varieties and new entries (lines other than control varieties). The adjusted mean values ranged from 1.4 cm to 13.3 cm (Fig. 2a) while the overall mean was 6.6 cm.

The six control varieties performed generally better than most of the genotypes tested. Differences among five of six control varieties (Gladius, Drysdale, Wyalkatchem, Gaurdian, and Mace) were not significant except Young, which showed a significantly (\( P < 0.05 \)) shorter root. Therefore, the numerically longest rooted variety (Mace) was used to compare with the new entries. Four genotypes namely Conotana 296-52, Compare, Santa Elena and Tammarin Rock significantly (\( P < 0.05 \)) outperformed Mace, the longest rooted control variety. Genotypes Aus 16596, Elia, Cann, Portugal 3 and Sentell were among the shortest rooted genotypes.

**Seedling performance under non-stress condition**

Analysis of variance did not show any significant difference among the control varieties under non-stress conditions. Adjusted mean values of new entries were compared with check varieties and with other new entries based on calculated LSD values (Table 1). There was a very high spread of mean values ranging from 4.4 cm to 23.3 cm (Fig. 2b) with an overall mean value of 14.5 cm. Genotypes Persia 28, W20A, Iraq 33, SST 3 and Triticum spelta vulpinum Alef showed the longest roots ranging from 23.3–22.5 cm in that order. In the lowest end of the spread (Fig. 2b), genotypes Persia 6, Tucorin, LA Florida, Datatine and Secalis fricans showed the shortest root lengths ranging from 4.4 to 6.4 cm.

The mean difference between any of those genotypes from the two extremes was in the range of 16.4 to 18.9 cm, which was far larger than 10.8, the LSD value at 5% level of significance (Table 1).

**Genotypic differences based on continents of origin**

The combined means of genotypes from the respective continents of origin showed comparable root lengths under the

| Table 1 Critical values for root length mean comparisons under stress (left) and non-stress (right) conditions |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Comparison                      | Stressed (SE)   | LSD (5 %)       | Non-stressed (SE) | LSD (5 %)     |
| Between control means           | 0.9             | 1.8             | 1.8             | 3.8            |
| Between two adjusted means      | 2.3             | 4.8             | 4.8             | 9.9            |
| Between two adjusted means in  | 2.5             | 5.2             | 5.2             | 10.8           |
| different blocks                |                 |                 |                 |                |
| Between adjusted mean           | 1.9             | 3.9             | 3.9             | 8.2            |
| control mean                    |                 |                 |                 |                |

SED, standard error of the difference between two means; LSD, least significant difference at 5 % probability.
non-stress condition. However, the difference was much pronounced under the stress condition. The overall mean root lengths of Australian and Asian genotypes were significantly (P < 0.05) shorter under stress as compared to the rest of the continents. Genotypes from South America, Africa and Europe showed relatively smaller reduction in root length as a result of the stress. The highest reduction in root length was observed among Australian germplasm.

Genotypic differences based on ploidy levels and level of domestication

Mean differences among the different ploidy levels and domesticated and wild forms were significant (P < 0.05) both under stress and non-stress conditions. Generally, the domesticated forms were the ones with longer roots under stress (Fig. 4). Common wheat was the most prevalent in the tested germplasm and had significantly (P < 0.01) longer roots in both growing conditions. The wild tetraploid forms (T. turgidum dicoccoides and T. timopheovii) showed the shortest roots under stress. The distant hybrids of wheat (wheat by rye and other synthetic species) showed relatively longer roots than other wild forms of wheat. T. monococcum showed a comparable root length with common, and durum wheat under the stress condition.

Discussion

In this particular study, wheat genotypes with diverse genetic backgrounds were evaluated for root length both under stress and non-stress conditions to identify genotypes for further evaluation and genetic studies. It is the target environment that dictates “what and how” to select for water stress resistance (Blum 1996, 2005; 2011b; Passiora 2012; Tardieu 2012). As a result, root length was the main focus of this study keeping in mind environments having initial rainfall enough for germination but followed by a dry spell before the actual rainy season resumes. Results from this study showed that the wheat collections tested do vary significantly (P < 0.01) for root length under −0.82 MPa osmotic stress, which is halfway to permanent wilting. There was a significant variation under the non-stress condition as well. Soils at field capacity (−0.01 to −0.03 MPa) are generally considered to be optimum for plant growth and soil microbial respiration. If water is continuously taken up by plants or lost in the form of soil evaporation, then the water potential of the soil will drop to about −1.5 MPa, which eventually causes permanent wilting (Kricz et al. 2004). Deep rooting is also helpful for early vigour, which in turn is believed to counter balance water loss through early ground cover and transpiring the available water through the leaves than gain it in the form of soil evaporation (Burdon et al. 2012, Gupta et al. 2012). The present study is in accordance with the idea that genotypes with deep rooting ability have the potential to maintain their cellular hydration through the extra water from deeper soil profile, which in turn improves productivity (Blum 2005, 2011a, Burdon et al. 2012, Henry 2013).

Root phenotyping has been the most challenging task so far, especially for large number of genotypes. The use of hydroponic systems was given sound practical backup by various researchers (Siwa et al. 2008, Hoffmann et al. 2012). In this study, standard commercial wheat varieties for low rainfall areas were used as benchmarks/controls both for genotype comparison and system performance control. The consistency in the performance of the control varieties under stress indicates that the hydroponic system was successful in simulating the stress and in discriminating genotypes for early-stage water stress. It also indicates the importance of early-stage resistance as an indicator of later stage performance; the control varieties were found good at early-stage resistance in addition to their claimed resistance at later stages (Wheeler 2013). The newly optimized phenotyping hydroponic set-up coupled with augmented complete block design (Federer and Crossa 2013, Mejia and Mejia 2013) enabled us to successfully and objectively compare large numbers of genotypes with relatively low difficulty to access plant roots at low cost.

Comparison based on continents of origin of genotypes did not show any significant variation under non-stress condition (Fig. 3). However, the stress simulated had differentiated genotypes from the six continents into extremely tolerant and susceptible groups. Genotypes from S/ America grew the longest roots both under stress and non-stress conditions followed by African and European genotypes. The longest rooted genotype under stress, Collotana 296-52, was from Brazil while the shortest rooted was from Australia. Collotana 296-52 is a late maturing line with very thick culm and profuse tillers (Unpublished). The sample size across continents, however, was not the same to make valid continent to continent comparisons. Contrary to our expectations, the Australian genotypes showed

![Fig. 3](image-url) The relative reduction in root length among continents of origin under stress and non-stress conditions.
the lowest average root length under stress, which might be
due to the adaptation of these genotypes to a Mediter-
anean ecology where early/dry sowing is practised that might
have conditioned the seedlings to respond slowly to stress
at early-stage.
Differences as a function of level of domestication are in
accordance with the established facts that highly selected
and domesticated plants are vigorous and grow quicker as
compared to wild relatives (del Blanco et al. 2000). Hexa-
ploid wheat and other high polyploid artificial wheat
hybrids showed longer roots under the stress condition,
which indicates that higher ploidy level is favourable for
better growth (Fig. 4). This corroborates with the idea that
hybridization (allopolyploidy) of two or more genomes
increases allelic diversity which provides genetic buffer and
also expression of novel phenotypes, which is helpful for
environmental stress resistance (Udall and Wendel 2006).

All polyploid wheats have inherited their AA genome
from the same progenitor, T. sartu (A*A’) (Goncharov
et al. 2009, Goncharov 2011). This gives the same ground
to compare the effect of the GG, BB, and DD genomes for
rooting capacity. The wild tetraploids, T. turgidum dicocc-
coids (BBA’A’) and T. timopheevii araraticum (GGG’A’)
showed significantly shorter roots than that of durum
wheat (T. turgidum durum) and other wheat classes, which
indicates that the GG and/or BB genomes, both from Ag.
speltoides, were not contributing much for early root vigour.
This difference may also indicate that the A’A’ genome in
the wild forms is different from the one in cultivated wheat
(tetraploid and hexaploid). The A’A’ and BB genomes in T.
durum and T. aestivum might have undergone other forms
of evolution/changes before they attain their present forms.
Hexaploid wheat showed the longest root in overall
performance under stress, which indicates that the DD


genome was positively contributing to long rooting in
addition to the A’A’ genome, which was instrumental in
T. durum. Diploid wheats with DD (Ae. squarrosus) and
A’A’ (T. monococcum) genomes showed comparable root
length to that of common and durum wheats, which indi-
cates the significant contribution of the respective genomes
for early root vigour than that of the BB genome. Unlike all
other tetraploids, durum wheat showed relatively longer
roots which might be due to changes in either or both the
A’A’ and BB genomes in the process of natural evolution
or artificial selection.

The differential root lengths among the different ploidy
levels might also be due to competition for water and
nutrients as all the lines were grown together (Fig. 1) in
the hydroponic system, which might have exposed them
for fierce competition among and within themselves
(Song et al. 2010, Narazyan et al. 2014). Competition
might have also triggered a naturally short rooting geno-
type to grow long roots and vice versa as access to water
and nutrients is a function of root systems (Eldiae et al.
2010).

In conclusion, data from this study showed the presence
of ample genetic variability, which can be used as a source
of resistance for early-stage water stress resistance breeding
and also to study the genetics of root traits. The hydro-
ponic system was found to be a handy tool for root phenoty-
ing for large number of genotypes. The most
contrasting genotypes are being hybridized to obtain segre-
gating populations for further genetic studies and also for
drought resistance breeding.

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References
Albrecht, E., M. Robertson, and M. O’Connor, 2012: Managing
the break of the season: Risks and opportunities for innovative
sowing strategies. 2012 WA Agribusiness Crop Updates. West-
tern Australian Agriculture Authority, Perth.
Balouchi, H., 2010: Screening wheat parents of mapping popula-
tion for heat and drought tolerance, detection of wheat
2000: Physiological performance of synthetic hexaploid
wheat-derived populations technical paper no. 11546 of the
Oregon state Univ. Agric. Exp. Sta. This paper is part of a dis-
sertation submitted by L.A. Del blanco in partial fulfilment of

![Fig. 4 The relative reduction in root length among different ploidy lev-
els and different levels of domestication under stress and non stress
conditions.](image)
the requirements for the Ph.D. Degree at Oregon state Univ.,
Blum, A., 1996: Crop responses to drought and the interpre-
tation of adaptation. Plant Growth Regul. 20, 135–148.
Blum, A., 2011a: Drought resistance—is it really a complex traits?
Blum, A., 2011b: Plant Breeding for Water-Limited Environ-
ments. Springer, New York, USA.
Burdon, J.L., G. Robetke, and M. Morell, 2012: Targets and traits in the pre-breeding development pipeline at cairi 2012.
WA Agribusiness Crop Updates. Western Australian Agriculture Authority, Perth.
Connie, J.H., S.R. Becker, V.M.V. Cruz, P.F. Byrne, and D.A.
Ebdie, B., D.J. Merhart, S. Ahmedian, A.C. Hoops, T. Khunong,
Federer, W.T., and J. Gross, 2012: Screening experimental designs for quantitative trait loci, association mapping, geno-
type-by-environment interaction, and other investigations.
Front. Physiol. 3, 4.
Flurkey, D., S. Jeffries, H. Kuchel, and P. Langridge, 2010:
Goschavar, N., 2011: Genus tritici L. taxonomy: the present
Goschavar, N.P, K.A. Golovina, and E.Y. Kondratenko, 2009:
Gupta, P.K., H.S. Balyah, V. Gahtani, and P.L. Kubel, 2012:
Phenotyping, genetic dissection, and breeding for drought and heat tolerance in common wheat: status and prospects.
International Rice Research Institute, 2007: Croppstat for win-
Krizek, M.K., L.D. Wiseman, and D. Gaumont-Guay, 2004: Soil-
web200: an on-line teaching tool for APH 200 course.
Narayanan, S., A. Mohan, K.S. Gill, and P.V.V. Prasad, 2014:
Song, L., D.W. Zhang, F.M. Li, X.W. Fan, Q. Ma, and N.C.
Tuma, 2010: Drought stress: soil water availability alters the inter- and intra-cultivar competition of three spring wheat cul-
improvement abbreviations: Ks, synonymous sites; Kc, flower-
Wasson, A.P., R.A. Richards, R. Chatrath, S.C. Morra, S.V.S.
Prasad, G.J. Rebetzke, J.A. Kirkpatrick, J. Christopher, and M.
Watt, 2012: Traits and selection strategies to improve root sys-
tems and water uptake in water-limited wheat crops. J. Exp.
Bot. 63, 1385–1398.
Whedon, R., 2013: Wheat variety sowing guide, South Australian Research and Development Institute (SADRI), New Variety
Agronomy Group.
Performance of Ethiopian bread wheat (*Triticum aestivum* L.) genotypes under contrasting water regimes: potential sources of variability for drought resistance breeding

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Abstract

Drought is a common abiotic stress in Ethiopian agriculture. Crop yield is at risk due to drought that happens at various developmental stages of the crop. This experiment evaluated 248 Ethiopian bread wheat genotypes under water stress and non-stress growing conditions. Augmented complete block design with three blocks and eight replicated entries was used. Analysis of variance showed significant diversity among the genotypes in reaction to water stress. The average root and shoot lengths were reduced by 33.4% and 28.8%, respectively, due to water stress. The average fresh biomass per plant was 192 mg for non-stressed and 116 mg for stressed treatments, suffering a 40.5% reduction due to stress. Accessions 8314, 204463, 204454 and 204521 showed the longest roots while accessions 222381, 222405, 222439 and 204586 showed the shortest roots under stress conditions. Drought tolerance indices were calculated based on root length. Geometric mean performance (GMP) index was found helpful in identifying the relatively stable genotypes across the two water regimes. High GMP indices were observed for genotypes 8314, 204521, 231614, and KSN81 which were long rooting genotypes under both stress and non-stress conditions. ANOVA based on region of collection showed that genotypes from Southern Nations Nationalities and Peoples Region had the longest roots. Elevation of origin did not show any significant difference for any of the traits measured. This study demonstrated the presence of large variations for water stress response in the Ethiopian bread wheat germplasm. The identified stress resistant genotypes can be used as potential breeding stocks to develop drought resistant cultivars.

Keywords: hydroponics; osmotic stress; seedling resistance; wheat collections.

Abbreviations: DRI, Relative drought resistance index; GMP, Geometric mean performance; KSN, Kulumsa screening nursery; PEG, Polyethylene glycol; SSL, Stress susceptibility index; SFI, Stress tolerance index.

Introduction

Agriculture is the largest sector of employment and main source of livelihood in Ethiopia. Nearly 85% of the population depends directly on farming. Grain production constitutes the major share of the domestic agricultural production. Nearly 98% of cereals are produced by small holder farmers (USDA, 2014). Ethiopia is the largest wheat producing country in Sub-Saharan Africa, with annual production of more than 4 million tons of grain on 1.6 million hectares of land which accounted for 13% of total land allotted to cereals (CSA, 2014; USAID, 2014). Wheat is mainly grown in the central and south eastern highlands during the main rainy season (June to September) (Hailu et al., 1991). The Ethiopian agriculture is mainly rain-fed in that its performance is highly dependent on the timing, amount and distribution of rainfall (Cheung et al., 2008). This makes the sector vulnerable to drought and other natural calamities. Due to the changing global climate, the rain fall trend is also changing (Funk et al., 2012; Hellin et al., 2012; Schlenker and Lobell, 2010; Stroosnijder et al., 2012). The rains are becoming more erratic with a trend of starting late and ceasing early in the season. This has posed an eminent danger for crop production. The production loss due to both biotic and abiotic factors coupled with the increasing population has made it difficult to attain food security in the country. Improving the adaptability of crop varieties to a changing environment supported by appropriate crop management strategies is the working principle worldwide in ensuring crop productivity (Blum, 2011a; Fanooq et al., 2015; Stroosnijder et al., 2012; Wassen et al., 2012). However, crop improvement for water stress is a much complicated task as drought damage is manifested in various forms at various crop growing stages making breeding for drought resistance uneasy (Blum, 2005; Fischer et al., 2012; Szira et al., 2008; Tubersa, 2012). Therefore, breeding for drought resistance has to integrate all methodologies that help in genotype evaluation and selection at all stages of the crop instead of one final stage (Qu et al., 2008). Seedling or early vigour, and deep root system are believed to contribute for better drought resistance (Al Karaki, 1998; Adkinson et al., 2015; Chloupek et al., 2010; Comas et al., 2013; Lilley and Kirkegaard, 2013). Some genes that contribute to seedling drought resistance may also contribute to later stage resistance (Comas et al., 2013; Hoffmann et al., 2012). Sarkar et al. (2005) have reported in lentil that long root and shoot lengths...
at seedling stage were highly correlated with high grain yield. Initial root parameters and above-ground biomass were also reported to be positively correlated in wheat (Atkinson et al., 2015). Among the seedling traits that enable plants withstand drought are early establishment and ground cover, deep root system and leaf waxiness (Blum, 2005). Genotypes with deep roots are able to extract water from lower soil profiles there by making use of water lost in the form of deep percolation (Cornas, 2013). Selection for root length; however, is dependent on the expectation of soil water availability (Blum, 2011). If soil water is expected to be close to the surface like that of irrigation setups, selection for deep root will not be an objective and vice versa. For environments that are dependent on remnant moisture, short roots are more preferable.

Genetic variability (or any trait of interest is the first and foremost requirement for the success of any breeding program (El-Beltagy and Madkour, 2012; Tadesse et al., 2012). The Ethiopian wheat germplasm was extensively studied for its variability in agro-morphological and molecular traits (Alamereew et al., 2004; Belay et al., 1995; Hailtu et al., 2000; Fecci and Damania, 1996; Tesfaye et al., 1991). However, most of the previous studies were focused on the final crop growth stage such as yield and yield related traits, which had overlooked the importance of seedling evaluation for water stress resistance. It was hypothesised that Ethiopia might harbour valuable genetic resources for water stress resistance as a result of the spade dry spells that have stricken the country for many decades and long history of wheat production in the country (Corway and Schipper, 2011; Hailtu et al., 1991; Kassie et al., 2011). Therefore, the present research was undertaken to evaluate the phenotypic variability among Ethiopian bread wheat genotypes and to identify the most tolerant genotypes for early-stage water stress, and to assess the relationship between underground and aboveground plant biomass in response to water stress.

Results

Analysis of variance for the two growing conditions was done separately after checking the error heterogeneity. ANOVA did not show any significant difference among the replicated entries in both growing conditions. However, the genotypes varied significantly in both stress and non-stress growing conditions based on the calculated LSD values (Fig. 2a, b, and c; Supplementary Table 2).

Phenotypic variation under non-stress condition

Root length variation ranged from 3.6 cm to 21.1 cm while shoot length was from 6.7 cm to 22.7 cm. The longest roots were found in genotypes KSN 85, KSN 6, KSN 15 and KSN 34 (20 - 21 cm) while the shortest roots were found in accessions KSN55, 204585, 222439, and 231553 (3.6 - 7.7 cm) (Supplementary Table 1, Fig. 2a). The average biomass yield was 192 mg per plant with the highest biomass yields measured on genotypes KSN 51 and KSN 78, 226931, and 243714 (353.7 - 392.5 mg per plant) and the lowest biomass yield was measured on genotypes KSN 38, KSN 55, KSN 56, KSN 8 and 226236 (22.5 - 53.8 mg per plant) (Fig. 2c).

Phenotypic variation under stress condition

The induced stress caused reduction in the performance of genotypes for all the three traits. The average root and shoot lengths were reduced by 33.4% and 28.8%, respectively, while fresh biomass yield was reduced by 40.5% (Fig. 3b). Root length ranged from 2.0 cm to 19.6 cm while the range for shoot length was from 2.6 cm to 20.6 cm (Fig. 2b). The longest root length was recorded in accessions 8314, 204463, 204545 and 204521 while the shortest was in 222381, 222405, 222439 and 204586 (Fig. 2a). Biomass yield ranged from 33 mg to 273 mg with an average yield of 119.6 mg per plant. The highest biomass yield was measured in accessions 226941 and 226261 (273 mg per plant) (Fig. 2c).

Genetic variation based on geographic locations

Out of the total 248 tested bread wheat genotypes, only 160 had geographic data of collection. One-way ANOVA based on regions of collection showed a significant (P<0.05) difference for root length among genotypes (Fig. 3a). Shoot length and fresh biomass yield did not show any significant difference among regions of collection. Genotypes from SNNP region showed significantly longer (P<0.05) roots as compared to the root lengths of accessions from other regions (Fig. 3a). The collection sites were arbitrarily grouped into four elevation/altitude categories viz: < 2,000 masl, 2,000 - 2,500 masl, 2,500 - 3,000 masl and > 3,000 masl. One-way ANOVA was conducted based on this grouping, but no significant difference was found for any of the traits (data not shown).

Correlation among traits

All the traits were significantly (P<0.01) and positively correlated in both stress and non-stress conditions. The magnitude of correlation between fresh biomass yield and root length was higher under stress condition while correlation between shoot length and fresh biomass was higher under non-stress condition (Table 1).

Drought resistance indices

The relative importance of the calculated indices was weighed in their ability to differentiate genotypes that perform better in both stress and non-stress growing conditions. SSI index was good in differentiating genotypes that are very sensitive to water stress while GMP was higher in identifying the most stable genotypes across the two water regimes. Higher GMP values were associated with genotypes that were long rooted and at the same time less affected by the stress and a small GMP value indicated genotypes that were short rooted but not much affected due to stress (Supplementary Table 1). Based on the ISIS index genotypes KSN55, 204454, 204463, 221735 and 2243696 showed the lowest susceptibility index while accessions 226235, 16352, 222405, 222439, and 204586 were with the highest susceptibility indices. High GMP index was observed for genotypes 8314, 204521, 231614, and KSN81 while genotypes 204585, 222405, 204586, 231553 and 226939 were with the smallest GMP values. No meaningful association of genotypic performance was possible with the root of the indices.

Discussion

Root phenotyping is among the most marginalised area of crop improvement research mainly because of the difficulty of root traits measurement (Passioura, 2012; Tuberosa, 2012). Hydroponic systems were reported to be handy tools for root phenotyping (Atkinson et al., 2015; Ajayew et al., 2015). The
Table 1. Simple correlation of traits under non-stress (below diagonal) and stress (above diagonal) conditions.

<table>
<thead>
<tr>
<th>Root Length</th>
<th>Shoot length</th>
<th>Fresh biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root Length</td>
<td>0.39**</td>
<td>0.36**</td>
</tr>
<tr>
<td>Shoot length</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fresh biomass</td>
<td>0.37**</td>
<td>0.62**</td>
</tr>
</tbody>
</table>

** indicates significant correlation at P<0.01

Fig 1. Geographical distribution of germplasm collection sites across the four administrative regions in Ethiopia.

Fig 2. Phenotypic distribution of 248 wheat genotypes for root length (a) shoot length (b) and fresh biomass yield (c) under water stress and non-stress conditions. The arrows indicate locations of the genotypes in the spread of root length values across genotypes.
Fig 3. The mean performance of 248 bread wheat genotypes for root and shoot length (a) and fresh biomass weight (b) under stress and non-stress growing conditions.

Fig 4. The relative difference in the performance of wheat genotypes for root length (a), shoot length (b) and fresh biomass yield (c) based on regions of landrace collection.
present study employed hydroponic culture to get easy access to intact roots. Seeding is one of the vital stages in plants which determines the level of crop establishment and crop stand performance in dry seasons. However, this succulent stage of crop plants was less emphasised in research literature partly because phenotyping for seedling resistance is presumed unattainable.

Results from this study indicated that the Ethiopian bread wheat genotypes are highly diverse in terms of root length, shoot length and fresh biomass yield. Previous studies have also found significant variability in the Ethiopian wheat germplasm for several agro-morphological traits such as days to heading and maturity, plant height, grain yield, and harvest index (Relay et al., 1993; Haile et al., 2006; Tesfaye et al., 1993). Depending on a target drought scenario, the identified genotypes can be further evaluated to develop varieties through line selection or hybridization to pyramid different favourable genes into a cultivar. Genotypes with long roots at early stage can be valuable assets for breeding drought tolerant lines in environments with early-growing-season rainfall and with soil types that can retain water at deeper layers. The less vigorous genotypes can be targeted for environments where farming is dependent on rampant moisture that requires water saving for later stage crop growth (Blum, 2005). The regions of collection did not show any significant difference which might be due to the fact that bread wheat is an exotic cereal to Ethiopia and did not differentiate into diverse ecotypes except for root length (Engels et al., 1991). Genotypes from Southern Nations, Nationalities and Peoples Region were significantly long rooted than genotypes from the other regions. This might be due to the many years of exposure of genotypes to low precipitation and the thick soil that can hold water in its deeper layers (Fink et al., 2012; Kasse et al., 2014). All the three traits were highly and positively correlated which enables simultaneous genetic improvement. Biomass yield can be used as a good indicator of long roots under stress condition as the level of magnitude and significance of correlation between these traits were higher as compared to the case with the rest of trains. This finding is in agreement with (Abendrieh et al., 2014). Among the drought resistance indices geometric mean performance index (GMP) was helpful in identifying the most stable genotypes in this study which was in agreement with previous findings (Mohammadi et al., 2011; Sio-Se Mardeh et al., 2006). The use of drought tolerance indices is dependent on the selection strategy one follows to improve drought resistance (Sio-Se Mardeh et al., 2006). Selecting genotypes that yield highest at optimum moisture and again are able to give reasonable yield under stress are favourable for environments which generally have enough precipitation for most part of the years/seasons but are impacted by sporadic drought (Blum, 2011b; Ud-Din et al., 1992). However, if any two environments are characterized by marked differences in terms of moisture availability, selection and breeding needs to be done separately (Coccarelli and Grando, 1991). Stress susceptibility index (SSI) enabled identifying resistant genotypes under stress conditions, however, it was not helpful in the non-stressed situation.

Materials and Methods

**Plant materials**

A total of 248 bread wheat genotypes were evaluated; 160 landrace collections from the Biodiversity Institute of Ethiopia and 88 breeding lines from Kulauma Agricultural research centre. The landraces were collected from four administrative regions in Ethiopia (Amhara, Oromia, Tigray, and Southern Nations Nationalities and Peoples Region (SNNPR) (Supplementary table 1, Fig.1) while the breeding lines were under observation and characterization nursery at Kulauma Agricultural Research Centre (Represented as KSN for Kulauma Screening Nursery).

**Experiment setup and traits measured**

Germplasm evaluation for seedling water stress resistance was conducted at Debre Markos University in a laboratory of the Department of Horticulture. A hydroponic system was developed from plastic boxes (3,000 ml of volume each) with linum diameter holes drilled on lids that supported plant growth on the surface of the solution, following the same methodology as described in Ayalew et al. (2015). The experiment was set up in a way that the boxes were filled with water/solutions and the lids were perforated and lined with filter paper to keep plants in place and the surface moist. An augmented complete block design was set up with three blocks (planting time) and eight randomly selected genotypes as repeated checks/controls. Seeds were first germinated in petri dishes lined with filter paper soaked with tap water for 48 hours and seedlings were transferred to the hydroponic system. Osmotic stress of -0.82 MPa was induced using PEG 6000 (SinoPharm Chemical Reagent Co. Ltd). Plants were grown in water for the first seven days followed by either in half strength Hoagland’s solution alone (control) or half strength Hoagland’s solution with PEG 6000 (treatment). Natural light was used and the solution was being aerated using manual agitation. Data were recorded on root length and shoot length using a scaled ruler and fresh biomass using a sensitive balance 14 days after planting.

**Statistical analysis**

Analysis of variance (ANOVA) was carried out based on augmented complete block design using CropStat version 7.2 statistical software (International Rice Research Institute, 2007) accounting for inter- and intra-block differences. The following linear model was used: \[ y_{ij} = \mu + \alpha_i + b_j + \epsilon_{ij} \]
where \( y_{ij} \) is the observed phenotype, \( \mu \) is the population mean, \( \alpha_i \) is the genotype effect, \( b_j \) is the block effect and \( \epsilon_{ij} \) is the random error. The plot numbers were considered in the residual to account for any measurement errors. Pearson's simple correlation was also calculated among the traits measured based on Dewey and Lu (1959). Means were adjusted for inter- and intra-block variations and were compared based on the standard errors of the differences between two means with controls and with new entries. Due to the imbalance created owing to the occurrence of new entries in a block, four different standard errors (Federer and Raghavaram, 1975) were computed as follows:

- Between two controls: \[ MSe_t \]
- Between two adjusted means in the same block: \[ \sqrt{2MSe} \]
- Between adjusted means in different blocks: \[ \sqrt{2MSe(1 + \frac{1}{b})} \]

Between adjusted means and control mean = \[ MSe(b + 1)(c + 1)/bc \] where MSe is mean square of error, \( b \) is the number of blocks and \( c \) is number of control varieties.

One-way ANOVA was used to compare differences among the four regions of landrace collection and four altitude
groups (< 2,000 mad, 2,000 - 2,500 mad, 2,500 - 3,000 mad and > 3,000 mad).

The following drought indices were calculated based on root length as indicated by the following formulae:

1) Stress susceptibility index (SSI) = \left(1 - \frac{Y}{Y_p}\right) \times \left(1 - \frac{Y}{Y_s}\right) (Fischer and Maurer, 1976)

2) Stress tolerance index (STI) = \frac{Y}{Y_p} (Fernandez, 1992)

3) Relative drought resistance index (DRI) = \frac{Y}{Y_p} (Fischer and Wood, 1979)

4) Geometric mean performance index (GMP) = \left(\frac{Y}{Y_p} \times \frac{Y}{Y_s}\right) (Fernandez, 1992), where in all the above equations Ys is yield of cultivar under stress, Yp is yield of cultivar under non-stress condition, Ys and Yp are the mean yields of all cultivars under stress and non-stress conditions, respectively.

Conclusion

In conclusion, the present study has found the presence of genetic variation among Ethiopian bread wheat genotypes both under severe water stress and non-stress conditions. There was a change in the ranking of genotypes under the two water regimes which calls for a separate breeding strategy for stress and non-stress conditions. The contrasting genotypes can be used as parental lines for further genetic study and as breeding lines based on different drought scenarios.

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The Australian Development Scholarship is highly appreciated for funding this research. The Ethiopian Institute of Biodiversity and Kulauma Agricultural Research Centre, Wheat Centre of Excellence kindly gave us the landrace collections and breeding lines. Mr. Etawgaw Aham helped in making the geographical map of collection sites. Prof. Liyong Hu and Mr. Jing Zhang of Huazhong Agricultural University helped us in accessing PEG.

References


Quantitative analysis of gene actions controlling root length under water stress in spring wheat (Triticum aestivum L.) genotypes

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Abstract. Understanding the genetic control of agronomic traits is important in designing crop improvement programs. Study was conducted to analyse the genetic control of root length under water stress. A full diallel cross of four spring wheat lines, along with their F\textsubscript{1} progenies was evaluated under −0.82 MPa water stress in a hydroponic culture. Analysis of variance showed highly significant (\(P<0.01\)) difference among the parental lines and their F\textsubscript{1} progenies. Genotypes Santa Elena, Colotana 290–52 and Pato showed comparable longer roots whereas Tincurra grew significantly (\(P<0.05\)) shorter roots. Genotypes with long roots were found to have more dominant genes than those with shorter roots. Both general and specific combining abilities were highly significant (\(P<0.01\)) indicating the importance of additive and dominant gene effects in the control of root length under water stress. Genotype Santa Elena was found to be the best general combiner whereas the specific cross Santa Elena × Pato was the best hybrid. Moderate narrow-sense heritability (38\%) was observed indicating the possibility of improving root length under water stress. The highly significant specific combining ability value (dominant genetic control) suggests that genotypes with more dominant genes should be selected as parents for hybridisation and the hybrid wheat approach might be helpful in improving water stress resistance.

Additional keywords: crop improvement, diallel analysis, drought tolerance.

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Introduction

Bread wheat (Triticum aestivum L.) has been one of the most important cereals since the dawn of civilization. It is a staple food for more than 33\% of the global population providing nearly 20\% of daily carbohydrate requirements (Shiferaw et al. 2013). Its production, however, suffers from many adverse environmental conditions, which can cause drastic yield reductions. Water stress is one of the most pressing environmental problems in dry land agriculture. As water stress is manifested in various forms at various plant developmental stages, it is apparently logical to work at various crop growth stages (Qu et al. 2008; Kumar et al. 2012; Passioura 2012; Tuberosa 2012; Ayalew et al. 2015). Several researchers have reported that different crop growth phases have different stress resistance mechanisms, which showed the need to work on different stages of crop development. The drought scenario is the dictating factor in designing any breeding and selection program for drought-prone environments (Sio-Sc Mardeh et al. 2006; Blum 2011a; Pata et al. 2011). In areas where the nature of rainfall is more erratic at the start of the season, drought resistance at seedling stage rather than reproductive stage is what research should target (Blum 1996; Pata et al. 2011; Passioura 2012). Genotypes with good seedling vigour can better resist water stress through more effective use of the available moisture and better vegetative ground cover to avoid soil transpiration losses (Blum 2005; Cattivelli et al. 2008; Chloupek et al. 2010; Elhage et al. 2010; Abdel-Ghani et al. 2015).

Previous studies on seedling water stress resistance showed the presence of huge genetic diversity in wheat genotypes (Balouchi 2010; Ayalew et al. 2015). One of the approaches to make use of this genetic variability is to directly select drought-resistant varieties from the existing genetic resources (Moore and Munnn 2008). The other approach is to make crosses with the superior or contrasting genotypes and select transgressive progenies, which is also an important way to analyze how the traits are genetically controlled in the population (Pridham 2015). Knowledge of the genetic control of a trait is essential for designing a successful breeding strategy. Even in this era of genomics, making crosses among contrasting genotypes followed by quantitative analysis is frequently used to study the different gene actions and mechanisms of their inheritance, especially for wheat (hexaploid) with a large...
genome size (Danenhauer et al. 2007; Lipman and Zamir 2007; Fan et al. 2014; Song et al. 2015).

Researchers have developed different crossing designs and data analysis methods depending on the nature of the data and mating design used (Hayman 1954; Grifﬁng 1956; Mother and Jinks 1982). Among them, diallel mating design is the most explored and frequently used (Hallauer et al. 2010). It works by partitioning the phenotypic variance into different sources of variation.

Having done genotype evaluation for water stress resistance on 838 wheat genotypes (Ayalew et al. 2015), we targeted making crosses among the divergent lines and study the nature of gene action and inheritance of root traits under the same nature of stress that we used for the screening experiment. The present research, therefore, was to investigate the nature of genetic control of root length under early stage water stress in contrasting genotypes.

Materials and methods

Materials

The parents for this study were identiﬁed following the screening of 838 wheat genotypes for water stress resistance (Ayalew et al. 2015). Based on the previous screening experiment, two of the parents, namely Colotanua 296–52 and Santa Elena, were among the top performing genotypes under water stress. Genotype Pato performed moderately whereas Tincurnin was among the shortest rooted genotypes.

A 4 × 4 full diallel cross was conducted following a standard crossing procedure (Simmonds 1986) and seeds from individual crosses were kept separately. All possible cross combinations (P1, and their reciprocals) and their parental lines were used for the diallel analysis.

Methods

Randomized complete block design with three replications was used. Seeds were ﬁrst germinated in Petri dishes lined with ﬁlter paper soaked with tap water for 48 h and seedlings were transferred to the hydroponic system. Osmotic stress of −0.82 MPa was induced using PEG 6000 (Sinopharm Chemical Reagent Co. Ltd, Shanghai, China). Plants were grown in water for the ﬁrst 7 days followed by either in half-strength Hoagland solution alone (Control) or half-strength Hoagland solution with added PEG 6000 to a level of −0.82 MPa water stress (treatment). The pH of the solution was adjusted between 5.5 and 5.7. The plants in the hydroponic system were kept in a constant temperature room at the School of Plant Biology, The University of Western Australia in 2015. The relative humidity of the room was between 65% and 70% and the temperature was 25°C day/22°C night. Light intensity of 300 µmol m−2 s−1 was supplied using cool (constant lamp with a daily photoperiodic cycle of 14 h light/10 h dark. The solution was being constantly aerated using an electric air bubbler. Data were recorded on root length using a graduated ruler on the 17-day-old seedlings. Three individual plants were used to calculate the mean of an entry in each block.

Statistical analyses

ANOVA was conducted on the parental lines and their F1 progenies including reciprocal crosses. ANOVA and estimation of genetic components were conducted based on Hayman and Jinks’ ﬁxed model (Hayman 1954; Jinks 1954). General and speciﬁc combining abilities were calculated based on Grifﬁng’s method I of model I (Grifﬁng 1956). The pooled error variance across blocks was used to test for the signiﬁcance of each source of variation. All statistical analyses were conducted using Genstat statistical software 17th edition (VSN International 2014). The covariance (Vr) variance (Vt) regression graph was used to study the nature of gene actions and their distribution (dominant and recessive) among the parental lines. Analysis of variance over (Wr + Vr) values for each parent across blocks was used to assess the nature of recessive–dominance gene distribution among parents whereas the ANOVA on (Wr − Vr) value for each parent and the three blocks was performed to test adequacy of the additive–dominance model. Further test for validity of diallel assumptions was done using parent–offspring Wr × Vr regression statistics. In the absence of epistasis and with independent distribution of genes among the parents, the linear regression of Wr × Vr has a slope of one and the Wr × Vr array points would remain along the regression line, and within an area delimited by the parallel Wr = Vp × Vr where Vp is the variance of the parental means (Hayman 1954; Povilaitis 1966; Mather and Jinks 1982). Regression line between the parental mean values and the recessive–dominance indicator (Wr − Vr) values was used to differentiate parental lines with high proportions of dominant and recessive genes and to determine the genetic control of long root under early stage water stress.

Results

Analysis of variance

The ANOVA showed highly signiﬁcant (P < 0.01) differences among the parental lines and their ﬁrst ﬁlial generation. Genotype Tincurnin showed signiﬁcantly (P < 0.05) shorter roots as compared with the rest of the parents (Table 1, Fig. 1). All of the progenies from all the crosses showed longer roots as compared with the short-rooted common parent. The speciﬁc cross ‘Pato × Santa Elena’ was the most outstanding cross with signiﬁcantly longer roots.

Combining ability analyses

All of the genetic components, except the average maternal effect, showed highly (P < 0.01) signiﬁcant variation (Tables 2 and 3). The general and speciﬁc combining abilities were highly signiﬁcant indicating the importance of both additive (a) and dominance (b) gene effects in the control of root length under early stage water stress. Genotype Santa Elena was found to be the

| Table 1. Mean performances of parental lines (diagonal) and their F1 hybrids (off diagonal) tested for root length (cm) under water stress in a 4 × 4 diallel cross |
|-----------------|---------------|---------------|-------------|---------|
| Genotype        | Colotanua 296–52 | Tincurnin | Santa Elena | Pato    |
| Colotanua 296–52 | 17.6          | 22.8         | 20.0        | 21.2    |
| Tincurnin       | 24.7          | 5.9          | 20.8        | 18.5    |
| Santa Elena     | 19.3          | 23.5         | 19.5        | 19.8    |
| Pato            | 16.0          | 20.0         | 29.3        | 19.0    |
| s.e.            | 1.86          | 3.56         | 5.37        |         |
best general combiner whereas the cross between genotypes Santa Elena and Pato produced the best hybrids followed by the cross between Santa Elena and Tuncurry (Table 1). There was a significant directional dominance effect (b1) between the parental and hybrid means, which gave significant hybrid vigour in the F1 progeny. The distribution of dominant genes across the four parents was significantly variable. Parents Colotana 296–52, Santa Elena and Pato showed more dominant genes than Tuncurry. Some of the progenies showed a highly significant dominance effect, which has led to a significant discrepancy between the non-maternal components of reciprocal variation.

**Graphical analyses – model adequacy**

ANOVA on the (Wr − Yr) values over arrays and blocks did not show any significant difference, which is consistent with the adequacy of the additive-dominance model (Table 4). Both the slope and intercept of the Wr/Yr regression line were significantly (P<0.05) different from zero and the slope was close to unity. This indicated that epistasis is not a significant player in the control of root length under water stress.

**Graphical analyses – Wr/Yr plot analyses**

The relative order of the parents along the Wr/Yr regression line indicated the distribution of dominant and recessive genes among the parents. Genotypes Colotana 296–52, Pato and Santa Elena were close to the origin showing excess of dominant genes whereas genotype Tuncurry was furthest away from the origin, which showed that it had an excess of recessive genes (Fig. 2).

The intercept of the Wr/Yr regression line passed through the Wr axis below the origin (Fig. 2) indicating over dominance gene action. This was also in conformity with the mean degree of dominance value (Table 5), which was greater than unity. Genotype Tuncurry showed the lowest mean root length and gave the largest (Wr + Yr) value (Fig. 3), which is characteristic of genotypes having excess of recessive genes. There was a negative correlation between (Wr + Yr) values and mid parents. Negative correlation values indicate that the increase in recessiveness is associated with lower performance of the parents.

**Genetic components and heritability**

Additive genetic variance (D) and the two components of dominance variance (H1 and H2) were highly significant, indicating the importance of both additive and non-additive genetic actions in the control of root length under water stress. Variation due to dominance effects (H1) and the dominance variance indicating asymmetry of positive and negative gene effects (H2) were different from each other, showing the difference in the frequencies of the genes of interest among parental lines. The value of relative frequency and magnitude of dominant and recessive alleles (F) was a significantly large positive number (56.2), which showed the frequency of the dominant alleles was higher than that of recessive alleles (Table 5). The average gene frequency value (DF) was different from 0.25 (value expected in equal frequency of the two gene effects) indicating the unequal frequency of dominant and recessive alleles. The gene asymmetry

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**Table 2. The general and specific combining abilities calculated on parental, F1 and reciprocal progenies from a 4 x 4 full diallel cross based on Griffing’s model I, method 1 diallel analysis in spring wheat genotypes grown under water stress**

<table>
<thead>
<tr>
<th>Genetic component</th>
<th>Sum squares</th>
<th>Mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>General combining ability</td>
<td>175.00</td>
<td>58.33***</td>
</tr>
<tr>
<td>Specific combining ability</td>
<td>695.15</td>
<td>115.86**</td>
</tr>
<tr>
<td>Maternal effect</td>
<td>43.08</td>
<td>14.36 ns</td>
</tr>
<tr>
<td>Reciprocal effect</td>
<td>141.28</td>
<td>47.13*</td>
</tr>
<tr>
<td>Residual</td>
<td>310.87</td>
<td>103.64</td>
</tr>
</tbody>
</table>

**Table 3. Mean squares of genetic components calculated on parental, F1 and reciprocal progenies from a 4 x 4 full diallel cross in spring wheat genotypes grown under water stress**

<table>
<thead>
<tr>
<th>Genetic component</th>
<th>Representation</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total additive genetic effect</td>
<td>a</td>
<td>58.54**</td>
</tr>
<tr>
<td>Dominance genetic effect</td>
<td>b</td>
<td>115.86**</td>
</tr>
<tr>
<td>Mean dominance deviation (Difference between parental and progeny mean)</td>
<td>b1</td>
<td>335.84**</td>
</tr>
<tr>
<td>Asymmetry of the gene distribution at the loci exhibiting dominance</td>
<td>b2</td>
<td>71.85**</td>
</tr>
<tr>
<td>Discrepancy in reciprocal due to dominance</td>
<td>b3</td>
<td>71.73**</td>
</tr>
<tr>
<td>Average maternal effects of each parental line</td>
<td>c</td>
<td>14.36 ns</td>
</tr>
<tr>
<td>Reciprocal differences not accountable to maternal effect</td>
<td>d</td>
<td>47.13*</td>
</tr>
<tr>
<td>Total</td>
<td>t</td>
<td>70.35**</td>
</tr>
<tr>
<td>Residual (non-genetic effect)</td>
<td>Block 2</td>
<td>10.36</td>
</tr>
</tbody>
</table>
was also significantly different among parental lines. The value of the average degree of dominance (HI/D)** was greater than unity, which indicated the presence of over dominant gene action. The proportion of additive genes was moderate as it was reflected in the moderate narrow-sense heritability (38%) value whereas broad-sense heritability was 60% (Table 5).

Discussion

ANOVA indicated the diversity of both parental and progeny lines in their reaction to water stress. The performance of parental lines was in agreement with Ayalew et al. (2015) showing repeatability of the screening technique developed. Santa Elena showed the highest general combining ability whereas Santa Elena and Pato were the best specific combiners, which gave rise to the highest hybrid vigour (Table 1).

Both additive and dominant gene effects showed significant role in the control of root length under water stress. Genetic asymmetry (b1) was significant at loci exhibiting dominance, which implied that the general combining ability (a) was not purely additive variation (Povilaitis 1986). This was reflected in the moderate level of narrow-sense heritability (Table 4). Reciprocal differences other than the maternal effect were significant. This difference was purely environmental, which might have been caused by block interactions, or any other systematic error in the run of the experiment (Hayman 1954).

Over dominant gene action was found to be the prominent player in the control of root length with the aggregate effects additively dominant genes. Over dominance may be true or pseudo depending on what has brought about it (Lippman and Zamir 2007). Complementarity of additive genes may inflate complete dominance into over dominance, which increases variance (Wv) in relation to parent-offspring co-variance (Wv), particularly for more recessive parents (Hayman 1954; Allard 1956). Most of the hybrids in this study showed heterosis greater than the better parent, which can be best explained by the additive complementarity of dominant genes from the parental lines. Over dominance can also be caused by tightly linked genes in repulsion phase, which gives uninheritable genetic component (Fasoula and Fasoula 2002; Lippman and Zamir 2007). However, the latter did not seem to be the case in this study as the genetic model assumptions were met during the analysis. Over dominance was reported to be important in the inheritance of yield and other yield-related traits in rice and other crops (Ma et al. 2006; Li et al. 2008; Fridman 2015).

Parental arrays (Vp) and the recessive-dominance indicator (Wv+Vr) showed negative correlation, which indicated the association of short roots with increasing number of recessive genes and vice versa (Povilaitis 1966; Singh and Raje 2011). This showed that long root under water stress was controlled by dominant genes.

The relative position of parental lines along the regression line between parental means and (Wv+Vr) graph shows the proportion of recessive and dominant genes in each parent. Genotypes Tincuirm, Pato and Santa Elena lay above the horizontal (parental performance) line, which indicated the

<p>| Table 4. ANOVA on (Wv − Vr) and (Wv+Vr) values to test model validity and the presence of dominance, respectively, on root length under water stress from a 4 × 4 full diallel cross in spring wheat genotypes. *P&lt;0.05; n.s., not significant; d.f. = degree of freedom and MS is mean of squares. |
|-----------------|---------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>MS (Wv − Vr)</th>
<th>MS (Wv + Vr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>2</td>
<td>232.1</td>
<td>216</td>
</tr>
<tr>
<td>Array</td>
<td>3</td>
<td>216.2a,b</td>
<td>96a</td>
</tr>
<tr>
<td>Residual</td>
<td>6</td>
<td>146.4</td>
<td>136</td>
</tr>
</tbody>
</table>

![Graph](image)

Fig. 2. Parent-offspring covariance (Wv) plotted against the variance of all F1 hybrids in each parental array (Vr) for root length in a 4 × 4 full diallel cross of spring wheat genotypes tested under water stress. The right opening parabola Wv = WvVr limits all the data points.

| Table 8. Estimates of genetic variance components for root length under water stress from a 4 × 4 full diallel cross in spring wheat genotypes. **P<0.01. |
|-----------------|---------|-----------------|-----------------|
| Genetic components of variance | Representation | Value |
|-----------------|---------|-----------------|-----------------|
| Variance due to additive effects | D       | 39.03**          |
| Variation due to dominance effects | HI      | 79.34**          |
| Dominance indicating asymmetry of gene effects | R2      | 60.87**          |
| Relative frequency of recessive and dominance | F       | 56.24**          |
| Mean degree of dominance | √HI/D   | 1.40             |
| Average gene frequency over all loci, max. 0.25 | 0.19   |
| Heritability | Narrow-sense | 38%              |
|                  | Broad-sense | 60%              |
presence of high proportion of recessive genes whereas genotype Pato with high proportion of dominant genes was below this line (Fig. 3). Based on this graphical analysis, recessiveness was more pronounced for Tircunin than for the rest of the genotypes whereas dominant genes were more pronounced for Colotana 296-52. The remaining two parental lines were above the x-axis but still close to the y=0 line, which again showed large proportion of dominant genes (Fig. 3).

Heritability was moderate (38%) showing that selection among pure lines coming out of these hybrids may not be rapid in bringing genetic improvement in the progeny. The broad-sense heritability seemed to be affected by a large proportion of non-genetic component that had rendered it only 60% in value. Focald (1996) has also reported moderate narrow-sense heritability in root length. This is usually the case for quantitatively inherited traits that are influenced by both the frequency of favourable genes and interaction with the environment.

From the present study it can be concluded that seedling root length is under dominant genetic control. Root length under water stress can be improved through hybridisation and recurrent selection of genotypes concentrating desirable dominant genes in a cultivar thereby increasing the chance of extracting water from deeper soil profiles (Sio-Se Marche et al. 2006; Blam 2011f). The most promising hybrids Santa Elena × Pato, Santa Elena × Tircunin and Colotana 296-52 × Pato can be advanced into pure lines with high chance of getting an imbed vector that have fixed the hybrid vigour from the hybridisation.

Genotypes with high proportion of recessive genes (shorter roots) can be evaluated for environments having shallow soil profile and for environments that use residual moisture for crop production. Developing structured mapping population from these contrasting genotypes is underway to effectively map QTL for root length and other traits.

Acknowledgements

Australian development scholarship is deeply acknowledged for sponsoring the study of the first author. We would like to thank Mr. Hameed Ahsanmardy for helping in the diallel data analysis.

References


Simmonds N (1986) 'Principles of crop improvement.' (Longman Science and Technology: New York)
Appendix 2. The chromosomal map and position of markers as was reported in the GrainGenes website, a data base for *Triticaceae* and *Avena* research.
Appendix 3. The chromosomal map and position of DArT markers used for GWAS analysis as was reported by Neumann et al. 2011.