University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Theses, Dissertations, and Student Research in Agronomy and Horticulture

Agronomy and Horticulture Department

9-2012

STUDIES OF DROUGHT TOLERANCE OF HARD RED WINTER WHEAT (*TRITICUM AESTIVUM* L.) CULTIVARS IN NEBRASKA

Sumardi Bin Abdul Hamid
University of Nebraska-Lincoln

STUDIES OF DROUGHT TOLERANCE OF HARD RED WINTER WHEAT (*TRITICUM AESTIVUM* L.) CULTIVARS IN NEBRASKA

by

Sumardi Bin Haji Abdul Hamid

A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Agronomy

Under the Supervision of Professors P. Stephen Baenziger and Harkamal Walia

Lincoln, Nebraska

September, 2012

STUDIES OF DROUGHT TOLERANCE OF HARD RED WINTER WHEAT (*TRITICUM AESTIVUM* L.) CULTIVARS IN NEBRASKA

Sumardi Bin Haji Abdul Hamid

University of Nebraska, 2012

Advisors: P. Stephen Baenziger and Harkamal Walia

In Nebraska, about 75% of the wheat production is in the western half of the state, and about 92% of the winter wheat acreage is in dryland production, where productivity is limited by low and/or uncertain rainfall. We have investigated the effects of water stress on few established winter wheat (*Triticum aestivum* L.) cultivars, which are known for their superior adaptation to either rainfed or irrigated wheat production systems in western Nebraska. We also began a study to investigate the variation in the root system architecture to confer drought tolerance in winter wheat. The objectives of this study were to investigate the effects of water stress on root and shoot growth of winter wheat cultivars, and also to characterize the root system architecture (RSA) traits of winter wheat cultivars in order to evaluate their drought tolerance under limiting water conditions. The root length, root dry matter, root-to-shoot length ratio and root-to-shoot mass ratio of the three cultivars were significantly greater in the water stress than well-watered conditions. Results from the water stress experiment showed that Goodstreak is a drought tolerant cultivar due to its longest root length and high root dry matter. Based on the RSA phenotyping of the 3-week old water-stressed plants, Goodstreak had the highest

total root length, total root length density, projected area of roots and network root length distribution. Under water stress conditions, Harry demonstrated a shallow root system with low root and shoot dry matters but displayed the highest root-to-shoot length ratio. It appeared that Harry utilized less water and invested less energy into dry matter under water stress. Our findings support the fact that Wesley performed well in irrigated wheat production systems in Nebraska because of its high shoot and root biomasses. This study leads us to suggest that Wesley is a drought sensitive cultivar because it uses the available soil moisture at ‘uneconomical’ and ‘unsustainable’ rate compared to Harry and Goodstreak.

ACKNOWLEDGEMENTS

This thesis would not have been possible without the assistance of many people whose contributions I gratefully acknowledge.

First and foremost I wish to thank my advisors, Dr. P. Stephen Baenziger and Dr.Harkamal Walia for their guidance, encouragement and friendship. They helped me come up with the thesis topic and guided me. They have supported me not only academically but also emotionally through the rough road to finish this thesis. I owe my deepest gratitude to my other committee member, Dr. Gautam Sarath for serving on my M.S. advisory committee. I also extend my sincere gratitude to lab members in Walia's Lab for helping and sharing their experiences and opinions with me. I am also grateful to Daniel W. McDonald, Co-founder and President of Phenotype Screening Corporation, Knoxville, TN for his generous support and technical advice on the two-dimensional root phenotyping at his facilities. Thank you to Steve's wheat group for their assistance in the greenhouse and seed laboratory, and also to small grains breeding colleagues who have taught me valuable field experience. I would like to thank all the friends who have been of invaluable assistance during this period of study. I am heartily thankful to many friends who have offered me great help, both materially and psychologically.

I would like to thank Fulbright for supporting my scholarship and also the Government of Brunei Darussalam for their continual support in my career and academic study. My deepest gratitude is sent to my lovely parents and family for their warm support and wonderful love which bring belief and hope into my life. Their continuous love and support meant so much to me.

TABLE OF CONTENTS

Acknowledgement……………………………………………………………..……….i

Table of contents……………………………………………………………………..ii

List of tables…………………………………………………………………………....iv

List of figures…………………………………………………………….…………..…vi

List of appendices…………………………………………………………..……..…..viii

Foreword……………………………………………………………………………….ix

Chapter 1: The Influence of Seed Vigor, Fall Stand Establishment and Water Stress on Winter Wheat Cultivars in Nebraska

Abstract………………………………………………………………………..……..….1

Introduction……………………………………………………………………………..2

Materials and methods………………………………………………………….......5

Results and discussion…………………………………………………….…..…10

References……………………………………………………………………….......18

Chapter 2: Phenotyping of Root System Architecture (RSA) As a Tool to Evaluate Drought Tolerance in Winter Wheat Cultivars in Nebraska

Abstract………………………………………………………………………….…….30

Introduction…………………………………………………………………………....31

TABLE OF CONTENTS (Continue)

Materials and methods……………………………………………………………...…34

Results and discussion……………………………………………………………......40

References…………………………………………………………….…………...…..46

LIST OF TABLES

Chapter 1

Table 1 Mean squares from analysis of variance of three winter wheat cultivars for seed vigor and coleoptile length experiments……………………..…………………..21

Table 2 Mean comparisons of seedling vigor and coleoptile length of three winter wheat cultivars……………………………………………………………………..…21

Table 3 Mean squares and interactions between three cultivar and two water regime for shoot length, shoot dry matter, root length, root dry matter, root-to-shoot length ratio and root-to-shoot mass ratio traits from analysis of variance…………………………20

Table 4 Mean comparisons of shoot length, shoot dry matter, root length, root dry matter, root-to-shoot length ratio and root-to-shoot mass ratio traits of three winter wheat cultivars……………………………………………………………………….23

Chapter 2

Table 1 Mean squares from analysis of variance of average root width, maximum number of roots, median number of roots, network bushiness, network length distribution, network length, specific root length, network area, network depth and network volume of three winter wheat cultivars in water-limiting conditions at 10-day old seedling stage, which was analyzed by GiA Roots software……………………….48

Table 2 Mean squares from analysis of variance of maximum number of roots, median number of roots, network bushiness, network width depth and network length distribution of three winter wheat cultivars in water-limiting conditions at 3-week old seedling stage, which was analyzed by GiA Roots software………………...…49

LIST OF TABLES (Continue)

Table 3 Mean squares from analysis of variance of projected area of roots, total root length, total root length density, root count, root diameter, root count density, width at depth and approximate mass density of three winter wheat cultivars in water-limiting conditions at 3-week old seedling stage, which was analyzed by RhizoTraits software……………………………………………………………………………...50

Table 4 Comparisons of average root width, maximum number of roots, median number of roots, network bushiness, network length distribution, network length, specific root length, network area, network depth and network volume for 10-day old seedlings of three winter wheat cultivars analyzed by GiA Roots……………………………….51

Table 5 Comparisons of total root length, total root length density, root count, root diameter, width at depth, root count density and approximate mass density for 3-week old seedlings of three winter wheat cultivars analyzed by RhizoTraits……………...…52

Table 6 Comparisons of maximum number of roots, median number of roots, network bushiness, network width depth ratio and network length distribution for 3-week old plants of three winter wheat cultivars analyzed by GiA Roots………………...……53

LIST OF FIGURES

Chapter 1

Figure 1 The mean seed vigor index for the first-week seedlings of three winter wheat cultivars. Error bars are the standard error of the mean…………………………….....24

Figure 2 The mean seed vigor index for the second-week seedlings of three winter wheat cultivars. Error bars are the standard error of the mean…………………………….....24

Figure 3 The mean coleoptile lengths of three winter wheat cultivars. Error bars are the standard error of the mean……………………………………………………..……25

Figure 4 Means of three winter wheat cultivars for root length, root dry matter, shoot length and shoot dry matter from the water stress experiment. Error bars are the standard error of the mean…………………………………………………………………....26

Figure 5 Interaction plots for shoot length, shoot dry matter, root length, root dry matter, root-to-shoot length ratio and root-to-shoot dry mass ratio traits for three cultivars (G is Goodstreak, H is Harry and W is Wesley) from the water stress experiment, where well-watered condition is WW and limited water condition is LW……………...…27

Chapter 2

Figure 1 Graphs for each RSA parameters (average root width, max. no. of roots, median no. of roots, network bushiness, network length distribution, network length, specific root length, network area, network depth and network volume) of 10-day old plants of three winter wheat cultivars analyzed by GiA Roots. Error bars are the standard error of the mean. Means with the same letter are not significantly different……...54

LIST OF FIGURES (Continue)

Figure 2 Graphs for each RSA parameters (total root length, total root length density, projected area of roots, no. of root counts, root diameter, total root depth, width at depth, root count density and approximate mass density) of 3-week old plants of three winter wheat cultivars analyzed by RhizoTraits. Error bars are the standard error of the mean. Means with the same letter are not significantly different……………………………………………………………………………….56

Figure 3 Graphs for each RSA parameters (max. no. of roots, median no. of roots, network bushiness, network width depth ratio and network length distribution) of 3-week old plants of three winter wheat cultivars analyzed by GiA Roots. Error bars are the standard error of the mean. Means with the same letter are not significantly different……………………………………………………………………………….58

LIST OF APPENDICES

Chapter 1

Appendix 1 Freshly harvested plants of three winter wheat cultivars grown under different water regimes (well-watered and limited water condition) from the water stress experiment………………………………………………………………….....29

Appendix 2 Mean, minimum and maximum values for seed vigor index and coleoptile length of three winter wheat cultivars in Nebraska…………………………...……..29

Chapter 2

Appendix 1 Root images of 10-day old plants of the three winter wheat cultivars (Goodstreak, Harry and Wesley) scanned and analyzed using GiA Roots. (Note: Only 1 root sample for each cultivar was shown for illustration purpose)……………..……59

Appendix 2 Root images of 3-week old plants of the three winter wheat cultivars (Goodstreak, Harry and Wesley) scanned and analyzed using GiA Roots. (Note: Only 1 root sample for each cultivar was shown for illustration purpose)…....…60

Appendix 3 The set-up of the 2-D root phenotyping for the three winter wheat cultivars (Goodstreak, Harry and Wesley) using expanded polystyrene (EPS) beads as a growth substrate under controlled water regime. (With permission from the Phenotyping Screening Corporation)…………………………………..……61

Appendix 4 Root images of 3-week old plants of the three winter wheat cultivars (Goodstreak, Harry and Wesley) scanned and analyzed using RhizoTraits. (Note: Only 1 root sample for each cultivar was shown for illustration purpose)……...62

FOREWORD

This thesis is written for publication in the format required by the Journal of Agronomy and Crop Science.

Chapter 1

The influence of seed vigor, fall stand establishment and water stress on winter wheat cultivars in Nebraska

ABSTRACT

In Nebraska, about 75% of the wheat production is in the western half of the state with approximately 92% of the winter wheat acreage is in dryland production. The seed vigor, fall stand establishment, and also the effect of water deficit on three winter wheat cultivars (‘Goodstreak’, ‘Harry’ and ‘Wesley’) specifically selected due to their superior adaptation to rainfed or irrigated wheat production systems in Nebraska were compared. The results showed that semi-dwarfing allele had an influence on both seed vigor and coleoptile length but did not account for the overall drought tolerance in winter wheat cultivars. The root dry matter, root-to-shoot length ratio and root-to-shoot mass ratio of winter wheat were significantly greater in the water stress than in the well-watered conditions, indicating that root growth had increased under water stress.Under drought stress, the root length of Goodstreak was significantly greater than Harry and Wesley, which could possibly contribute to its drought tolerance during the early growth stage. Harry did not have significantly greater root length, root dry matter, shoot length and shoot dry matter when compared to Goodstreak and Wesley. Our results suggested that Harry may be capable of utilizing limited water resource during the seedling growth because of its low shoot dry matter, shallow and intensive root system, and also its ability to conserve stored soil moisture for use at a later stage of wheat development.

INTRODUCTION

Wheat (*Triticum aestivum* L.), the world's most important and widely adapted crop in terms of area and production and contributes more calories and protein to the world's diet than any other food crop (Hanson *et. al.*, 1982). Drought stress, which is the most serious environmental problem limiting crop production in rainfed agriculture (Bahieldin *et al.*, 2005), can severely impact plant growth and development, limit plant production and the crop performance (Shao *et. al.*, 2009). Although wheat is a relatively drought tolerant species, it produces a small fraction of its yield potential ranging (0.8 to 1.5 ton ha^{-1}) under moisture stress on approximately 60 million hectares in developing countries (Morris *et. al.*, 1991). At the same time, soil water deficit is also a limitation to the wheat productivity in the developed countries, for example in the Midwestern areas of North America. In Nebraska, about 75% of the wheat production is in the western half of the state with approximately 92% of the winter wheat acreage is in dryland production. Some of the challenges faced by the winter wheat producers in Nebraska are to identify cultivars and planting methods that result in successful stand establishment, winter survival and resistance to moisture stress. Theoretically, for winter wheat to grow successfully in the field during the early phase of growth, wheat cultivars should possess excellent germination, vigorous seedling growth, fall establishment, and be able to withstand drought in the field.

Wheat seedlings must be able to emerge from being planted in the soil to the surface for further growth and development. A significant positive correlation was found between the rate of emergence and seed vigor (Kittock and Law, 1967). Therefore, vigorous wheat seedlings will have a higher opportunity for emergence in the field. Under most environmental conditions, early seed vigor is considered an essential component of crop plant development (Ludlow and Muchow, 1990). However, drought stress can decrease the wheat seedling growth during and after germination. Therefore, it is important for wheat grower to select wheat cultivar which has an excellent seedling vigor index during the drought stress. Seeds with an excellent seedling vigor index may be planted at the earliest possible planting date when less than optimum conditions are likely to be encountered. The evaluation of early growth vigor at about the four-leaf stage is a promising technique in screening for early drought tolerance of crops. This approach can be used for a large number of samples with a small labor input (Dhanda *et. al.*, 2004; Turner, 1986; Hafid *et. al.*, 1998). In this study, the early seedling vigor index was determined during the first and second week after germination. Coleoptile length is also a focus of research in wheat improvement because it is often associated with fall stand establishment in winter wheat (Hakizimana *et. al.*, 2000). The degree of seedling establishment is an important factor in determining yield and time of maturity of plants. In Nebraska, wheat seedlings require good stand establishment in the fall so that they have sufficient time to attain the growth necessary for winter survival.

The effects of water stress on the root and shoot growth and development also merit study as water stress is the most important abiotic constraint to increasing grain yield in rainfed wheat growing areas. Wheat plants experience drought stress either when the water supply to roots becomes too little to support growth or when the transpiration rate becomes very high due to wind and temperature. The challenge for wheat breeders where rainfall is either insufficient or unpredictable in its timing and quantity is to produce cultivars that are capable of utilizing available water resources for successful crop production. However, such breeding effort is difficult for a plant breeder because yield and drought tolerance indicators usually have relatively low heritabilities even under ideal conditions and an unpredictable variable water supply reduces heritabilities even further (Quarrie *et. al.*, 1999). Furthermore, the wheat improvement and breeding programs have typically focused on improving above-ground traits with an obvious emphasis on yield. Few studies have investigated the positive relationship between the root and shoot growth of winter wheat, the importance of this relationship has been alluded to in the literature. A positive correlation between shoot and root dry weight in winter wheat was reported by Hoffman and Kolb (1997) and Mian *et. al.* (1993). Therefore, crop yields should be improved by studying root system particularly during the early stage of wheat growth and development. The specific objectives of the present study were: (1), to evaluate the seed vigor and coleoptile length of the drought tolerant and susceptible winter wheat cultivars in Nebraska, and (2), to investigate the effects of water stress on the root as well as shoot systems of winter wheat cultivars during the early phase of growth under controlled conditions in the greenhouse.

MATERIALS AND METHODS

Plant materials

Three hard red winter wheat cultivars, ‘Goodstreak’, ‘Harry’ and ‘Wesley’ grown across Nebraska agroecoregions, were chosen for evaluation of various seedling traits under greenhouse and laboratory conditions. The experiment was conducted at the University of Nebraska-Lincoln, during 2011–2012. The drought tolerant cultivars in this study were Harry and Goodstreak. Harry was selected as the drought tolerant cultivar because it was released primarily for its superior adaptation to rainfed wheat production systems in western Nebraska. In its primary area of adaptation (western Nebraska), Harry (28 environments from 2000 to 2002) has yielded 3310 kg ha^{-1}, which was greater than Wesley (2650 kg ha^{-1}) (Baenziger *et al.*, 2004a). Goodstreak was also chosen in this study because it was released primarily for its superior adaptation to rainfed wheat production systems in western Nebraska with low moisture conditions. The average Nebraska rainfed yield of Goodstreak of 3280 kg ha^{-1} (28 environments) was greater than Wesley (2650 kg ha^{-1}) (Baenziger *et al.*, 2004b). Goodstreak is a conventional-height wheat cultivar and among the most widely grown cultivars in low moisture, rainfed wheat production. Goodstreak had performed well throughout most of Nebraska but was best adapted to low rainfed wheat production systems where conventional height wheat cultivars were grown. The drought sensitive cultivar in this study was Wesley which was lower yielding than Harry and Goodstreak. Wesley appeared to be better suited for irrigated production systems statewide and similar production areas in adjacent states (Peterson *et al.*, 2001).

Measurement of seedling vigor index of winter wheat cultivars

A set of fifty randomly selected seeds of each cultivar were placed in petri dishes on two layers of Whatman no. 2 filter paper of 25 mm diameter and kept in a dark incubator at room temperature for 2 days. Ten seeds of uniform size of each cultivar were randomly placed equidistantly on the brown germination paper that was pre-soaked in distilled water. Another presoaked paper towel was placed on the first one so that the seeds were held in position. The towels were then rolled and wrapped with polythene to prevent drying. The towels were placed in a beaker filled with 250 ml distilled water and kept in a growth chamber for 2 weeks, at average day and night temperature of 22 ± 2 °C. Every week, the towels were unrolled and the numbers of seeds germinated were counted. Germination was recorded when the radicle reached at least 2 mm in length. The length of the root and shoot of individual seedlings was measured to determine the seedling vigor index. Seedling vigor index was calculated by multiplying the sum of the root and shoot lengths by the germination percentage (Anderson and Abdul-Baki, 1973; Dhanda *et. al.*, 2004). The experiment was done once and conducted in a completely randomized design with three replications.

Measurement of coleoptile length of winter wheat cultivars

The coleoptile length was measured following the blotter-paper germination protocol of Hakizimana *et. al.* (2000), with some modifications. A germination towel (no. 76 germination paper, cut to dimensions of 15.2 cm and 20.3 cm; Anchor Paper Co., St. Paul, MN) was moistened with tap water, and placed flat on a table. A guide with marks 1 cm apart was placed 5 cm from the bottom of the moistened paper. Thirty-five seeds were placed on the line with the germ end down. A second wet germination towel was placed on top of the seeds. The towels were rolled loosely from left to right without disturbing the seed position in the paper, and secured with a rubber band in the middle of the towel roll. The binder sheet protectors with dimensions of 20.3 × 27.9 cm were cut to the same height as the plastic tub. Excess water was removed from the rolled towels. The rolled towels were placed into cut sheet protectors and vertically arranged in a covered plastic tub. The tub was then placed in a dark incubator at 4º C for 3 days to reduce dormancy. At the end of this period, samples were placed in a second dark incubator at 15ºC for 16 days. The experiment was performed once and arranged in a randomized complete block design, with three replicates in each experiment. If unable to record the coleoptile lengths on time, at the end of the sixteen-day period, the rolled towels should be stored in a refrigeration unit at 4ºC for an additional two days. Coleoptiles were gently straightened and measured. The average coleoptile length for each cultivar was obtained from the coleoptile lengths of germinated, vigorous seedlings. Non-germinated, vigorous seedlings that were affected by microbial activity were not measured.

Water stress experiment of winter wheat cultivars

The water stress experiment was conducted in pots with perforated plastic bags. Plants were grown in the pot-culture conditions in the greenhouse for 21 days. The factorial arrangement for the treatment design consisted of two qualitative factors, ‘cultivar’ with three levels (Goodstreak, Harry and Wesley) and ‘water regime’ with two levels (well-watered and water stress conditions). Each of the six treatment combinations was assigned to 15 replicate pots in a completely randomized design with four plants per pot. A uniform amount of sand (6.9 kg) was used for each pot. Seeds were sieved in order to get a uniform seed size before they were allowed to germinate. The seeds were germinated in the dark growth incubator for two days at a temperature of 22 ± 2 °C. The 2-day old seedlings of a uniform growth stage were planted in the pots. The plants were watered daily until 14 days after seeding (DAS) which involved alternate watering with either 250 ml of water or half-strength Hoagland solution (Hoagland & Arnon, 1950). Drought stress was imposed to half of the pots for a period of 7 days. After the 21 days (DAS), the plants were harvested by removing the sand by gentle washing from the roots of the plants. This process of removing the sand from the roots was performed for each plant. The root and shoot lengths were measured for each plant immediately after they had been washed thoroughly. Both the root and shoot dry matters were allowed to dry in an oven at a temperature of 160°F for 3 days and then weighed.

Statistical analysis

Data from seed vigor, coleoptile length and water stress experiments were analyzed separately. All statistical computations were made using the PROC GLM procedure in SAS computer packages version 9.2 for Windows (SAS Institute Inc., Cary, NC, USA). The seed vigor and coleoptile length measurements were estimated using one-way analysis of variance procedure. Two-way analysis of variance (ANOVA) was performed to analyze the data from the water stress experiment. Interaction between cultivar and trait (shoot length, shoot dry matter, root length, root dry matter, root-to-shoot length ratio and root-to-shoot mass ratio) in the water stress experiment were analyzed. Tukey multiple comparison test was performed to identify differences among the least-squares means.

RESULTS AND DISCUSSION

We assessed the seed vigor index of three winter wheat cultivars: Goodstreak, Harry and Wesley to evaluate their early growth vigor during the first and second week after germination. Analysis of variance showed that there was a significant difference ($p < 0.0001$) in the 1st week of seed vigor index among the cultivars (Table 1). The 1st week of seed vigor index of Goodstreak (2842) was significantly higher than Wesley (2600), which was significantly higher than Harry (2181). For the 2nd week of seedling stage, there was also a significant difference ($p < 0.0001$) in seed vigor index among cultivars. As in the first week experiment, Goodstreak (3478) had larger value of the 2nd week seed vigor index than Harry (2821) but was not different from Wesley (3313) (Table 2). Based on the seed vigor index experiment, the drought tolerant cultivar, Goodstreak had the highest seed vigor index in both the first and second week after germination (Fig. 1). However, the other drought tolerant cultivar, Harry had the lowest seed vigor index (Fig. 2). The range of values of the 1st week seed vigor was 2480 and 3260 for Goodstreak, and 1620 and 2655 for Harry (Appendix 2). The range of the 2nd week seed vigor was 2980 and 4070 for Goodstreak, and 2450 and 3030 for Harry. Hence, seed vigor index did not account for the overall drought tolerance in winter wheat cultivars because of the low seed vigor index of the drought tolerant cultivar, Harry.

In terms of coleoptile length, there was a significant difference ($p < 0.0001$) in the coleoptile length among cultivars (Table 1). Goodstreak produced the longest coleoptile length (8.4 cm) which was significantly longer than both Harry (5.4 cm) and Wesley (5.6 cm). The coleoptile lengths of Harry and Wesley were not statistically different (Table 2; Fig. 3).

Seed vigor index and coleoptile length are two important traits in the emergence of winter wheat plants. In normal conditions, wheat genotypes with high seed vigor index tend to have better germination percentage, root and shoot lengths, however under water stress condition, seed vigor index was only associated to germination percentage and coleoptile length (Dhanda *et. al.*, 2004). Our study demonstrated that semi-dwarfing genes present in Harry and Wesley had an influence on their seedling vigor and coleoptile length. *Rht1* and *Rht2* were associated with reduced coleoptile length while *Rht8* had no effect on coleoptile length during early growth (Ellis *et. al.*, 2004). Based on analyses of microsatellite markers, both Wesley and Harry possessed the semi-dwarfing alleles of *Rht1* but absence of *Rht2* and *Rht8* while Goodstreak did not possess *Rht1*, *Rht2* and *Rht8* (Guedira *et. al.*, 2009). As expected, Goodstreak had a longer coleoptile length than the semi-dwarfing cultivars, Harry and Wesley. From an agronomic standpoint, depth control is important for planting the semi-dwarf cultivars, Harry and Wesley, due to their shorter coleoptiles. If the coleoptile was shorter than the planting depth, there will be poor emergence, and ultimately, the young seedlings could die before reaching the surface, hence causing stand loss during the early phase of growth. There was a positive correlation between the grain yield and yield components with coleoptile length with marked decline in grain yield with shorter coleoptiles in the deepest sowing (Yagmur and Kaydan, 2009).

This study indicated that tall conventional high cultivar, Goodstreak had better fall stand establishment compared to semi-dwarf cultivars, Harry and Wesley due to its comparatively longer coleoptile length. Goodstreak also had the highest seed vigor index, but was not significantly greater than Wesley. The fact that Harry performed better in the dryland production areas in Nebraska demonstrated that seed vigor index and coleoptile length might be ameliorated by other factors than those measured here in the early stage of growth.

The effect of water regime, cultivar and the interactions between cultivar and water regime for the different agronomic traits were investigated (i.e. shoot length, shoot dry matter, root length, root dry matter, root-to-shoot length ratio and root-to-shoot dry mass ratio) (Table 3). Analysis of variance for the root dry matter showed that water regime (p = 0.0364) and cultivar (p < 0.0001) were significant. The root dry matter of winter wheat was significantly (p = 0.0364) greater in the water stress condition (0.054 g) than in well-watered (0.049 g) condition, indicating that an increase in root growth of winter wheat was observed under water stress (Table 4). When cultivars were compared in terms of root dry matter, we observed that the root dry matter of Wesley (0.059 g) was slightly larger than Goodstreak (0.052 g) but not statistically different (p = 0.0746), but was significantly (p < 0.0001) greater than Harry (0.044 g) (Table 5). There was no significant interaction (p = 0.1326) for the root dry matter between cultivar and water regime (Table 3; Fig. 5). The drought sensitive cultivar, Wesley might be caused by its utilizing more water for dry root matter during the first two week of seedling stage when water was not a limiting factor. This water use could have contributed to Wesley being a well-adapted winter wheat cultivar in irrigated production systems in Nebraska.

For shoot dry matter, no significant difference (p = 0.0679) was observed between well-watered condition (0.098 g) and water stress condition (0.090 g) during the early growth stage of winter wheat (Table 4). The availability of less water did not greatly affect shoot dry matter production during seedling stage. However, a significant effect (p

= 0.0001) for shoot dry matter was observed among cultivars. Goodstreak (0.098 g) and Wesley (0.103 g) had significantly more shoot dry matter than Harry (0.081 g). However, since Harry performed better than Wesley in the rainfed wheat production system in western Nebraska, its low shoot dry matter might be beneficial in dry environment because low shoot dry matter could potentially reduce the total surface area of leaves and transpiration rate, and this would help conserve additional water for use in the later stages of development such as tillering, stem extension, heading and ripening. Analysis of variance showed that there was no significant interaction (p = 0.8075) for the shoot dry matter between cultivar and water regime (Table 3; Fig. 5).

For the root length, the effects of cultivar (p = 0.0333) and water regime (p < 0.0001) were significant in this study (Table 3). Interestingly, there was a significant interaction (p < 0.0001) for the root length between cultivar and water regime (Table 3; Fig. 5). This was the only significant interaction between cultivar and trait observed in this study, indicating that the different water regime could significantly influence the root length trait of a cultivar more effectively than the other traits investigated. For Harry, the root length was not significantly different (p = 0.5973) between the well-watered condition (28.4 cm) and water stress condition (27.7 cm) (Table 4; Fig. 4). Goodstreak produced significantly longer root length in water stress condition (34.3 cm) than in well-watered condition (26.5 cm). Similarly, Wesley also produced significantly (p = 0.0018) longer root length in water stress condition (31.3 cm) than in well-watered condition (27.3 cm). With water stress conditions, the root length was significantly different (p < 0.0001) among cultivars. In water stress condition, Goodstreak (34.3 cm) had significantly longer root length than Wesley (31.3 cm), which was significantly longer than Harry (27.7 cm). Goodstreak produced on average 7.8 cm longer root length in the water stress than in the well-watered conditions. This result confirmed the previous report by Mac Key (1973) which concluded that a tall wheat plant tends to have a deep root system. However, Harry did not have significant difference in root length between the well-watered and water stress conditions. The drought tolerant cultivar, Harry performed well in the rain-fed wheat system based on the West Dryland Wheat Variety tests (2004-2010) in Nebraska (data not shown). Hence, the drought tolerance of Harry in the field could be contributed by the stability of its shallow roots regardless of the different water regimes i.e. well-watered and water stress conditions in the early stage of growth, although Harry might grow deep roots later. Its shallow roots during seedling would provide a better water absorption for water that was available in the subsoil rather than in the deep soil. The benefit of possessing shallow roots initially could be evident from improved performance of bean (*Phaseolus vulgaris* L.), soybean (*Glycine max* L.) and maize (*Zea mays* L.) grown under low P condition due to the increased topsoil foraging by dense shallow roots (Lynch and Brown, 2001). This study suggests that shallow and intensive root system could provide an advantage for wheat cultivars which were grown in the environment with moisture occurring in the surface and subsoil rather than in the deep soil, provided that their root system were responsive to water availability during early crop growth, and effectively extracted water from the shallow soil layers that would be otherwise easily lost by evaporation.

There was also no significant difference ($p = 0.4360$) among water regimes for the shoot length trait. A significant effect was observed among cultivars ($p < 0.0001$). There was no significant difference ($p = 0.0872$) for the shoot length between the drought tolerant cultivars, Goodstreak (24.4 cm) and Harry (23.5 cm) although the shoot dry matter of Goodstreak (0.098 g) was statistically greater than Harry (0.081 g) (Table 4). The shoot length of Wesley (26.7 cm) was significantly greater than Goodstreak (24.4 cm) and Harry (23.5 cm). There was no significant interaction (p = 0.1322) for the shoot length between cultivar and water regime (Table 3; Fig. 5). The final shoot length performance of Harry in the rain-fed production system was greater than Wesley such that the mature plant of Harry was 6 cm taller than Wesley (Baenziger *et al.*, 2004a).

In this study, any changes in the root-to-shoot length ratio and root-to-shoot mass ratio were also analyzed when the three cultivars were subjected to water stress condition. Significant differences were observed for effects of root-to-shoot length ratio among cultivars (p = 0.0356) and also among water regimes (p < 0.0001) (Table 3). The root-to-shoot length ratio of Harry (1.27) was significantly greater than Wesley (1.12) but not significantly different from Goodstreak (1.16) (Table 4). Interestingly, the root-to-shoot length ratio was significantly (p < 0.0001) higher in water stress condition (1.29) than in well-watered condition (1.07). There was no significant interaction (p < 0.1239) for the root-to-shoot length ratio between cultivar and water regime (Table 3; Fig. 5). The increase of the root-to-shoot length ratio from the well-watered to water stress conditions may be attributable to greater root length under stress, probably due to the induction of root to shoot hormonal signaling when the root system was subjected to drought stress.

For the root-to-shoot mass ratio, there was no significant difference (p = 0.6157) among cultivars. On the other hand, a significant difference (p < 0.0001) for root-to-shoot mass ratio was observed among water regimes. The winter wheat cultivars had significantly (p < 0.0001) greater root-to-shoot mass ratio in water stress condition (0.62) than in well-watered (0.42) condition. No significant interaction (p = 0.7403) was observed between cultivar and water regime (Table 3; Fig. 5). Our experiment proved that the root dry matter of winter wheat increased in drying soil in response to drought stress, indicating greater partitioning and preferential accumulation of starch and dry matter from shoot to root as an adaptation to drought. The allocation of dry matter to the roots due to drought stress could enhance water uptake. The winter wheat invested more energy into root dry matter under water stress condition, which did not support the finding from Siddique *et. al.* (1990) who indicated that wheat cultivars invested less root dry matter into root system during early growing season, resulting in a lower root-to-shoot mass ratio.

Traits of winter wheat such as shoot length and shoot dry matter were not significantly different between the well-watered and water stress conditions. Nevertheless, significant differences for root length, root dry matter, root-to-shoot length ratio and root-to-shoot mass ratio were observed among the cultivars between the well-watered and water stress conditions. The results of the current study contrast those who found vigorous wheat genotypes have larger shoot and root dry matters. Based on our study, there was little evidence to support that the drought tolerant cultivars uniformly displayed advantageous traits such denser shoot and root dry matter, and longer shoot and root lengths under water stress. In this case, the drought tolerant cultivar, Harry did not have significantly greater root length, root dry matter, shoot length and shoot dry matter in the limited water conditions at least during the early growth stage. However, Harry does perform well in the water stress condition in the later stage of wheat development. It appeared that Harry has the ability to conserve additional soil moisture during drought stress condition and utilize it more efficiently for water-demanding processes during the later stage of wheat development. Lupton *et. al.* (1974) argued that semi-dwarf root systems did not necessarily cause adverse effect on the amount of water and nutrient absorbed by the plant, and hence grain yield. During early growth, the drought sensitive cultivar, Wesley had higher root dry matter and longer shoot length in the well-watered condition compared to the drought tolerant cultivars, Goodstreak and Harry. Our study suggested that Wesley might have performed better in the well-water condition due to their denser root system during their juvenile phase of growth, making it suitable for the irrigated wheat production system in Nebraska and similar production areas in adjacent states. The drought tolerance of Goodstreak in the field may be attributed to their longer root length under water stress during early stage of growth but probably not their seedling vigor and coleoptile length. We assumed that greater seedling vigor and coleoptile length might help to increase the emergence rate and fall stand establishment of Goodstreak but have less influence on the drought tolerance at later stage of development. It is clear that the drought tolerant cultivars, Goodstreak and Harry displayed different responses in terms of shoot length, shoot dry matter, root length and root dry matter towards drought stress, hence there may be multiple ways to achieve drought tolerance. It appeared that the quality of root system of winter wheat was more critical in determining their efficiency of extracting water from the ground when assessing the drought tolerance of winter wheat during the early growth stage. Furthermore, our results indicated that the effects of semi-dwarfing alleles influenced the fall stand establishment of the winter wheat but not the ability of winter wheat to confer resistance to drought stress once established.

REFERENCES

Anderson, J., & Abdul-Baki, A. (1973). Vigor determination in soyabean seeds by multiple criteria. *Crop Science, 3*, 630-633.

Baenziger, P., Beecher, B., Graybosch, R., Baltensperger, D., Nelson, L., Mcvey, D., Watkins, J., Hatchett, J. & Chen, M. (2004a). Registration of 'Harry' wheat . *Crop Science, 44*, 1474-1475.

Baenziger, P., Beecher, B., Graybosch, R., Baltensperger, D., Nelson, L., Krall, J., McVey, D., Watkins, J., Hatchett, J. & Chen, M. (2004b). Registration of 'Goodstreak' wheat. *Crop Science, 44*, 1473-1474.

Bahieldin, A., Mahfouz, H. T., Eissa, H. F., Saleh, O. M., Ramadan, A. M., Ahmed, I. A., Dyer, W. E., El-Itriby, H. A., & Madkour, M. A. (2005). Field evaluation of transgenic wheat plants stably expressing the HVA1 gene for drought tolerance. *Physiologia Plantarum, 123*, 421-427.

Dhanda, S., Sethi, G., & Behl, R. (2004). Indices of drought tolerance in wheat genotypes at early stages of plant growth. *J. Agronomy & Crop Science, 190*, 6-12.

Ellis, M. H., Rebetzke, G. J., Chandler, P., Bonnett, D., Spielmeyer, W., & Richards, R. A. (2004). The effect of different height reducing genes on the early growth of wheat. *Functional Plant Biology, 31*, 583-589.

Guedira, M., Brown-Guedira, G., Van Sanford, D. S., Souza, E., & Marshall, D. (2009). Distribution of Rht genes in modern and historic winter wheat germplasm from the eastern and central United States. *Crop Science, 50*, 1811-1822.

Hafid, El, R., Smith, D., Karron, M., & Samir, K. (1998). Physiological responses of spring durum wheat cultivars to early-season drought in a Mediterranean environment. *Ann. Bot., 81*, 363-370.

Hakizimana, F., Haley, S. D., & Turnipseed, E. B. (2000). Repeatability and genotype X environment interaction of coleoptile length measurements in winter wheat. *Crop Sci., 40*, 1233-1237.

Hanson, H., Borlaug, N. E., & Anderson, R. (1982). *Wheat in the third world.* Boulder, Colorado: West View Press.

Hoagland, D. R., & Arnon, D. I. (1950). The water-culture method for growing plants without soil. *California Agricultural Experiment Station Circular, 371*, pp. 1-32.

Hoffman, T., & Kolb, F. (1997). Effects of barley yellow dwarf virus on root and shoot growth. *Plant Disease, 81*, 497-500.

Kittock, D., & Law, A. (1967). Relationship of seedling vigor to respiration and tetrazolium chloride reduction by germinating wheat seeds. *Agron. J., 60*(3), 286-288.

Ludlow, M., & Muchow, R. (1990). A critical evaluation of traits for improving crop yields in water-limited environments. *Adv. Agron., 43*, 107–153.

Lupton, F., Oliver, R., & Ellis, F. (1974). Root and shoot growth in semi-dwarf and taller winter wheats. *Annals of Applied Biology, 77*, 129-144.

Lynch, J., & Brown, K. (2001). Topsoil foraging - an architectural adaptation of plants to low phosphorus availability. *Plant Soil, 237*, 225-237.

Mac Key, J. (1973). The wheat root. In S. ER, & S. LMS (Ed.), *Proceedings 4th International Wheat Genetics Symposium*, (pp. 827-842). Columbia, Missouri.

Mian, M., Nafiger, E. D., Frederic, L. K., & Teyker, R. H. (1993). Root growth of wheat genotypes in hydroponic culture and in the greenhouse under different soil moisture regimes. *Crop Science, 33*(2), 283-286.

Morris, M., Belaid, A., & Byerlee, D. (1991). Wheat and barley production in rainfed marginal environments of the developing world. Part 1 of 1990-91. In *CIMMYT world wheat factors and trends: Wheat and barley production in rainfed marginal environments of the developing world.* Mexico: DF: CIMMYT.

Peterson, C., Shelton, D., Baenziger, P., Baltensperger, D., Graybosch, R., Worrall, W., Nelson, L., McVey, D., Watkins, J. and Krall, J. (2001). Registration of 'Wesley' wheat. *Crop Science, 41*(1), 260-261.

Quarrie, S. A., Lazic-Jancic, V., Kovacevic, D., Steed, A., & Pekic, S. (1999). Bulk segregant analysis with molecular markers and its use for improving drought resistance in maize. *Journal of Experimental Botany, 50*(337), 1299-1306.

Shao, H., Chu, L. J., Manivannan, P., Panneerselvam, R., & Shao, M. (2009). Understanding water deficit stress-induced changes in the basic metabolism of higher plants- biotechnologically and sustainably improving agriculture and the ecoenvironment in arid regions of the globe. *Crit. Rev. Biotechnol., 29*, 131-151.

Siddique, H., Belford, R., & Tennant, D. (1990). Root:Shoot ratios of old and modern, tall and semi-dwarf wheats in a mediterranean environment. *Plant and Soil, 121*, 89-98.

Turner, N. (1986). Crop water deficits: a decade of progress. *Adv. Agron., 39*, 1-51.

Yagmur, M., & Kaydan, D. (2009). The effects of different sowing depth on grain yield and some grain yield components in wheat (Triticum aestivum L.) cultivars under dryland conditions. *African Journal of Biotechnology, 8*(2), 196-201.

Table 1. Mean squares from analysis of variance of three winter wheat cultivars for seed vigor and coleoptile length experiments.

Source of Variation	*df*	Mean Squares		*df*	Mean Squares
		Seed Vigor – 1st Week	Seed Vigor – 2nd Week		Coleoptile Length
Cultivar	2	3215473.8*	3396352.1*	2	284.5*
Error	84	55969.2	144301.2	312	0.8

NS: Not significant at $P < 0.05$
* : $p \leq 0.05$

Table 2. Mean comparisons of seedling vigor and coleoptile length of three winter wheat cultivars.

Trait	Cultivar	Least Square Mean	Mean Comparison	Mean Difference	Fr > P
Seedling Vigor – 1st Week	Goodstreak	2842	Goodstreak-Harry	661	< 0.0001*
	Harry	2181	Goodstreak-Wesley	242	0.0006*
	Wesley	2600	Harry-Wesley	-419	< 0.0001*
Seedling Vigor – 2nd Week	Goodstreak	3478	Goodstreak-Harry	657	< 0.0001*
	Harry	2821	Goodstreak-Wesley	165	0.2344^{NS}
	Wesley	3313	Harry-Wesley	-492	< 0.0001*
Coleoptile length	Goodstreak	8.4	Goodstreak-Harry	3.0	< 0.0001*
	Harry	5.4	Goodstreak-Wesley	2.8	< 0.0001*
	Wesley	5.6	Harry-Wesley	-0.2	0.1873^{NS}

NS: Not significant at $P < 0.05$
* : $p \leq 0.05$

Table 3. Mean squares and interactions between three cultivar and two water regime for shoot length, shoot dry matter, root length, root dry matter, root-to-shoot length ratio and root-to-shoot mass ratio traits from analysis of variance.

Source of Variation	**df**	**Mean Squares**					
		Shoot Length	**Root Length**	**Shoot Dry Matter**	**Root Dry Matter**	**Root-to-Shoot Length Ratio**	**Root-to-Shoot Mass Ratio**
Cultivar	2	343.6*	165.5*	0.0163*	0.0073*	0.24*	0.01 ^{NS}
Water Regime	1	6.7^{NS}	1226.9*	0.0058 ^{NS}	0.0032*	1.47*	1.22*
Cultivar × Water Regime	2	22.4 ^{NS}	534.0*	0.0004 ^{NS}	0.0015 ^{NS}	0.15 ^{NS}	0.01 ^{NS}

NS: Not significant at $P < 0.05$
* : $p \le 0.05$

Table 4. Mean comparisons of shoot length, shoot dry matter, root length, root dry matter, root-to-shoot length ratio and root-to-shoot mass ratio traits of three winter wheat cultivars.

Trait: Shoot Length (cm)				
	Cultivar			Water Regime Means
Water Regime	Goodstreak	Harry	Wesley	
Well-Watered	24.05	24.00	26.93	24.99 **(A)**
Limited Water	24.69	22.93	26.55	24.72 **(A)**
Cultivar Means	24.37 **(B)**	23.46 **(B)**	26.74 **(A)**	
Trait: Root Length (cm)				
	Cultivar			Water Regime Means
Water Regime	Goodstreak	Harry	Wesley	
Well-Watered	26.52	28.38	27.32	27.40
Limited Water	34.27	27.71	31.31	31.10
Cultivar Means	30.39	28.05	29.31	
Trait: Shoot Dry Matter (g)				
	Cultivar			Water Regime Means
Water Regime	Goodstreak	Harry	Wesley	
Well-Watered	0.101	0.086	0.108	0.098 **(A)**
Limited Water	0.096	0.076	0.098	0.090 **(A)**
Cultivar Means	0.098 **(A)**	0.081 **(B)**	0.103 **(A)**	
Trait: Root Dry Matter (g)				
	Cultivar			Water Regime Means
Water Regime	Goodstreak	Harry	Wesley	
Well-Watered	0.046	0.040	0.060	0.049 **(B)**
Limited Water	0.057	0.048	0.058	0.054 **(A)**
Cultivar Means	0.052 **(A)**	0.044 **(B)**	0.059 **(A)**	
Trait: Root-to-Shoot Dry Mass Ratio				
	Cultivar			Water Regime Means
Water Regime	Goodstreak	Harry	Wesley	
Well-Watered	0.38	0.42	0.45	0.42 **(B)**
Limited Water	0.61	0.62	0.62	0.62 **(A)**
Cultivar Means	0.50 **(A)**	0.52 **(A)**	0.53 **(A)**	
Trait: Root-to-Shoot Dry Length Ratio				
	Cultivar			Water Regime Means
Water Regime	Goodstreak	Harry	Wesley	
Well-Watered	0.99	1.22	1.00	1.07 **(B)**
Limited Water	1.33	1.32	1.23	1.29 **(A)**
Cultivar Means	1.16 **(A)**	1.27 **(A)**	1.12 **(B)**	

Means with the same letter are non-significantly different at p = 0.05 level as determined by Tukey

Fig. 1. The mean seed vigor index for the first-week seedlings of three winter wheat cultivars. Error bars are the standard error of the mean.

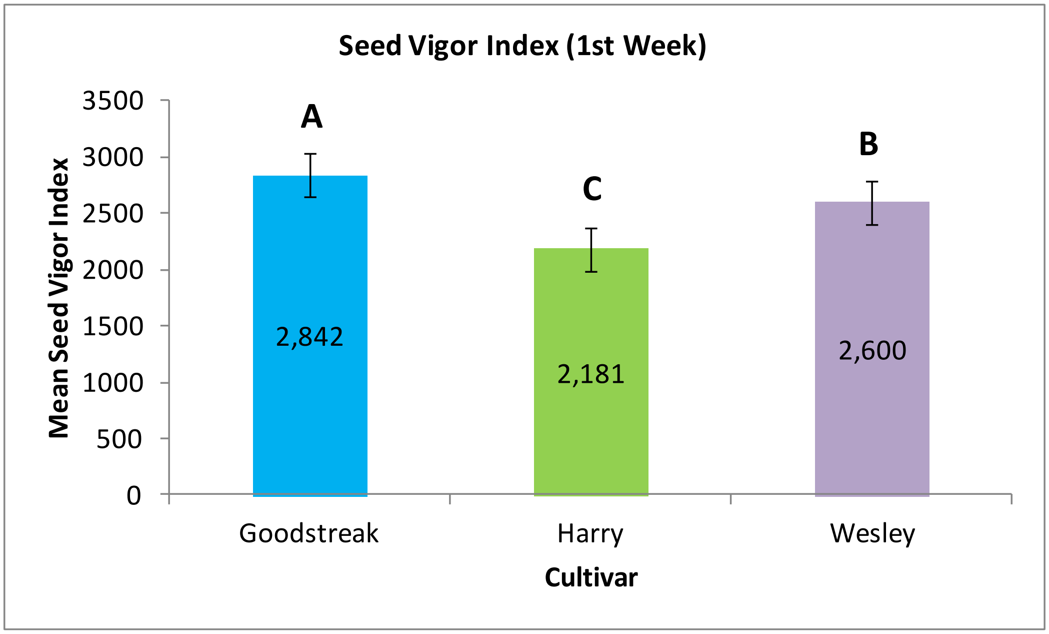


Fig. 2. The mean seed vigor index for the second-week seedlings of three winter wheat cultivars. Error bars are the standard error of the mean.

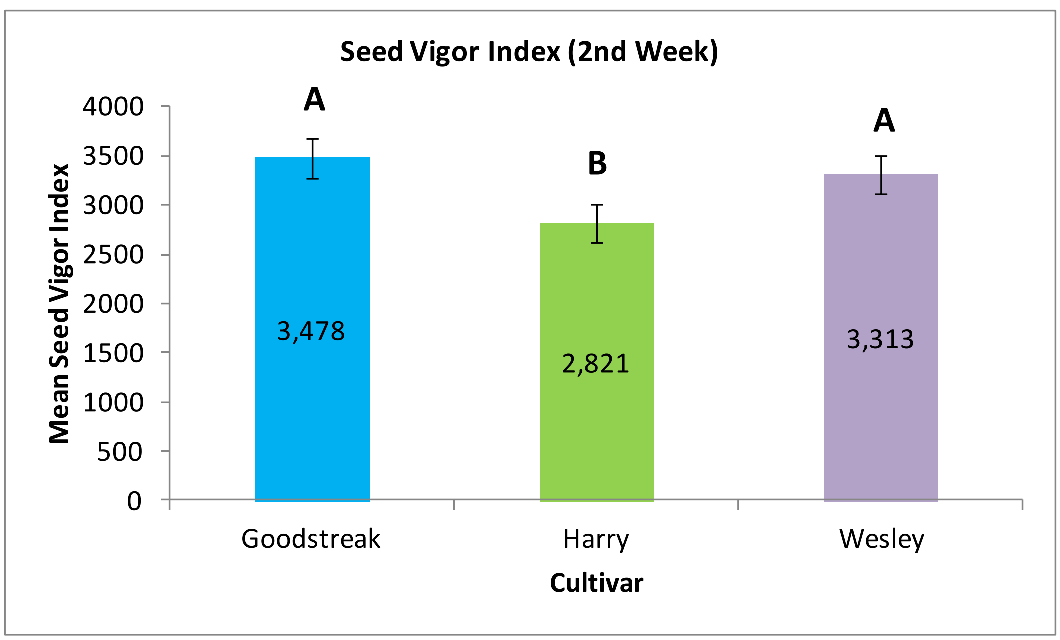


Fig. 3. The mean coleoptile lengths of three winter wheat cultivars. Error bars are the standard error of the mean.

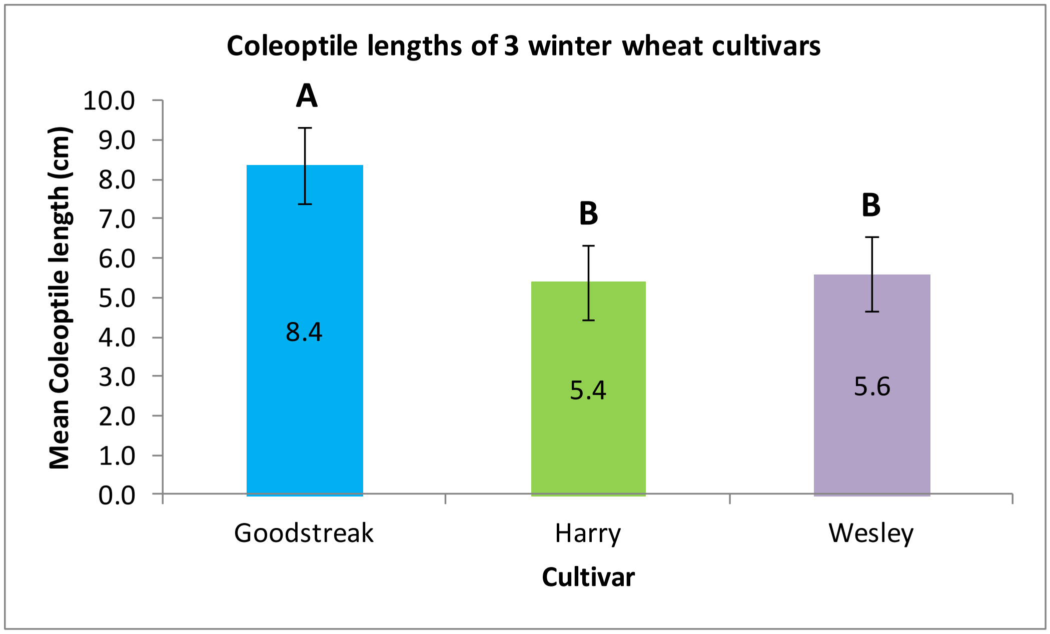
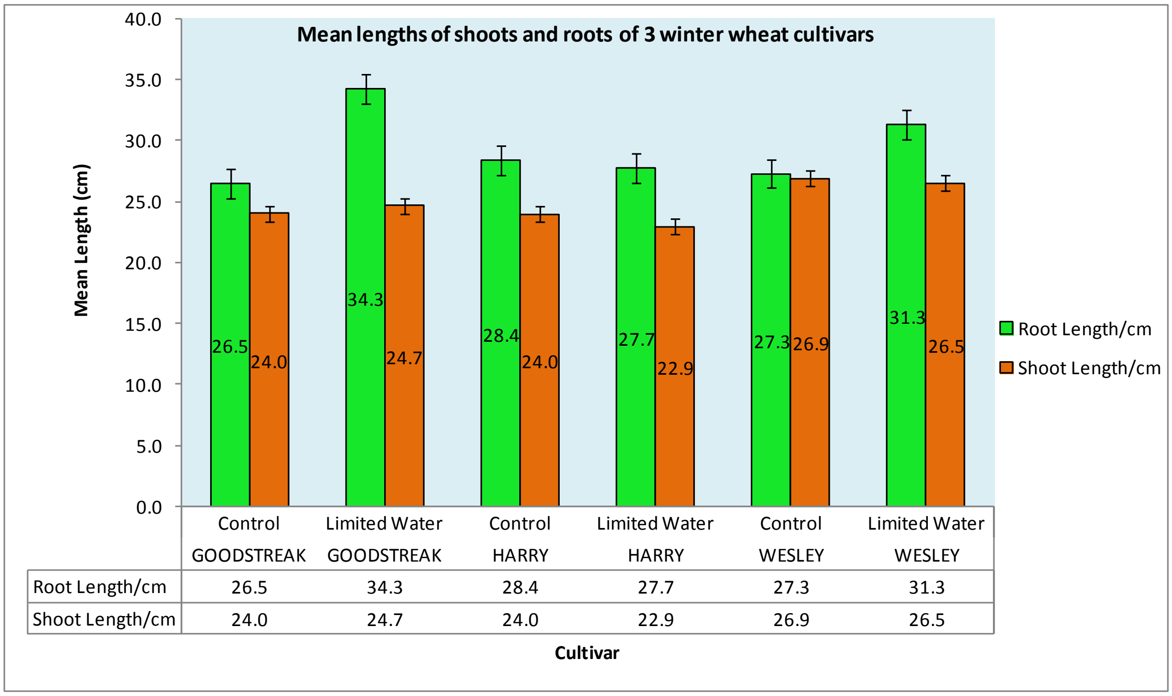


Fig. 4. Means of three winter wheat cultivars for root length, root dry matter, shoot length and shoot dry matter from the water stress experiment. Error bars are the standard error of the mean.



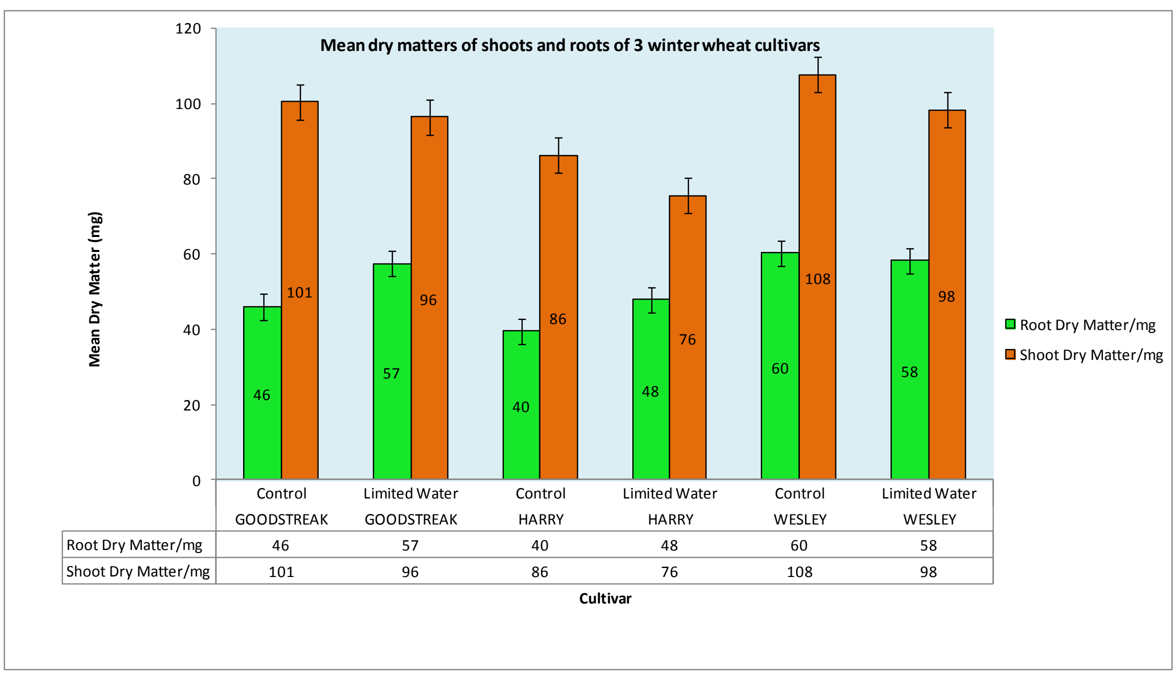
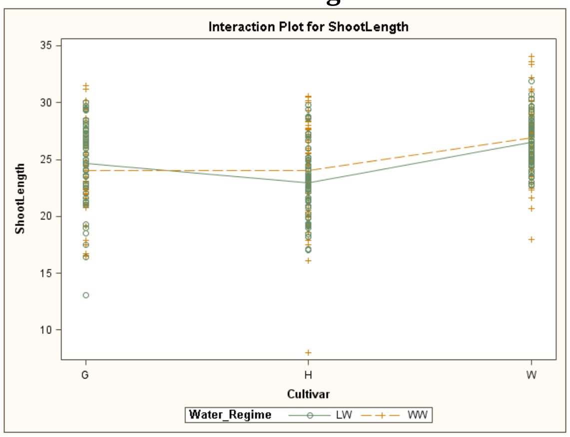
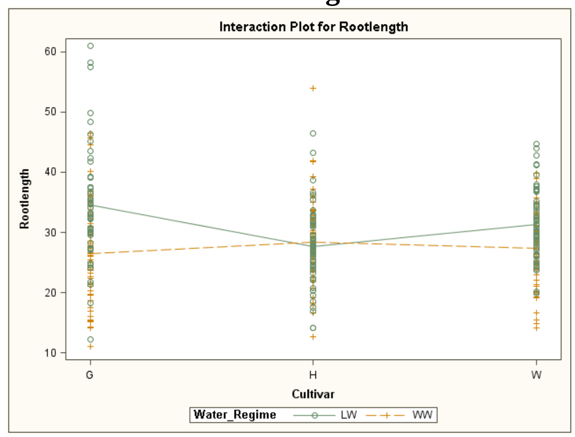


Fig. 5. Interaction plots for shoot length, shoot dry matter, root length, root dry matter, root-to-shoot length ratio and root-to-shoot dry mass ratio traits for three cultivars (G is Goodstreak, H is Harry and W is Wesley) from the water stress experiment, where well-watered condition is WW and limited water condition is LW.

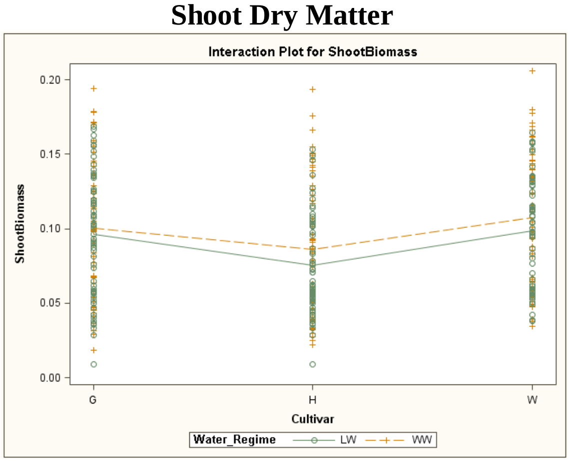
Shoot Length



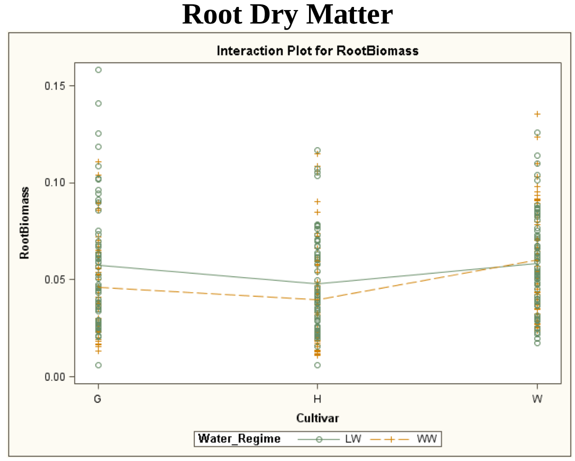
Root Length



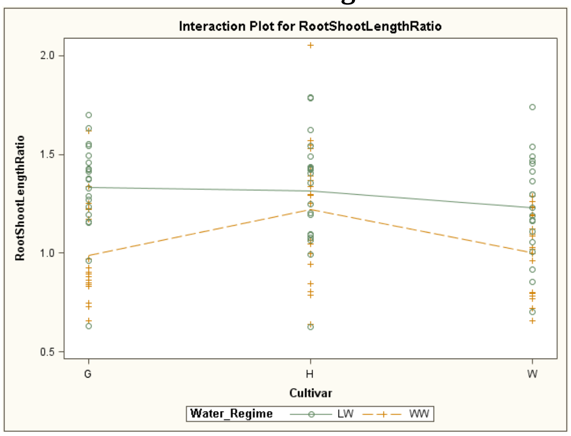
Shoot Dry Matter



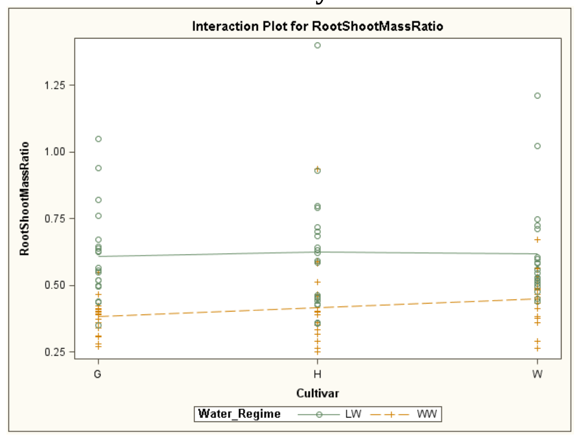
Root Dry Matter



Root-to-Shoot Length Ratio



Root-to-Shoot Dry Mass Ratio



Appendix 1. Freshly harvested plants of three winter wheat cultivars grown under different water regimes (well-watered and limited water condition) from the water stress experiment.

Appendix 2. Mean, minimum and maximum values for seed vigor index and coleoptile length of three winter wheat (*Triticum aestivum* L.) cultivars in Nebraska.

Trait (unit)	Cultivar	Mean ± S.E.	Minimum	Maximum
Seed Vigor Index - 1st week		2842 ± 42	2480	3260
Seed Vigor Index - 2nd week	Goodstreak	3478 ± 65	2980	4070
Coleoptile length (cm)		8.4 ± 1.0	6.2	11.0
Seed Vigor Index - 1st week		2181 ± 49	1620	2655
Seed Vigor Index - 2nd week	Harry	2821 ± 45	2450	3030
Coleoptile length (cm)		5.4 ± 0.7	3.9	7.3
Seed Vigor Index - 1st week		2600 ± 39	2350	3090
Seed Vigor Index - 2nd week	Wesley	3313 ± 92	2580	4120
Coleoptile length (cm)		5.6 ± 1.0	1.9	8.5

Chapter 2

Phenotyping of root system architecture (RSA) as a tool to evaluate drought tolerance in winter wheat cultivars in Nebraska

ABSTRACT

Wheat production is often limited primarily by the availability of water to the root system. To enhance the capability of the winter wheat to survive and proliferate under drought stress during the seedling stage, we performed a high-throughput analysis of the root system architecture of the winter wheat grown under water stress during the early growth stage. Three winter wheat cultivars (‘Goodstreak’, ‘Harry’ and ‘Wesley’) which were known for their superior adaptation to either rain-fed or irrigated wheat production systems in Nebraska were compared in terms of their root system architecture (RSA) traits in response to the drought stress. Two-dimensional root phenotyping methods were employed in the study of RSA of the drought-tolerant and drought-susceptible cultivars grown under water-stress conditions. It appeared that Goodstreak had denser root architecture while Harry had narrow, compact and dense type of root architecture. We speculated that during drought stress, Harry could have invested less energy and utilized less water for accumulating root and shoot biomasses. This study suggests that under water stress, Wesley could have utilized more water and invested more energy for root biomass and root branching. Using a combination of root phenotyping approaches, it was possible to characterize and differentiate the RSA of the drought tolerant and drought sensitive winter wheat cultivars as early as the third week of seedling stage.

INTRODUCTION

Drought stress is one of the major limitations to crop productivity in arid and semi-arid regions worldwide. The growth and function of roots are essential for crop productivity under water-limiting conditions because roots play a vital role in plant growth, development, and fitness such as anchoraging and support for shoots, uptake of water and nutrients, storage organs for carbohydrate and other reserves, and a site for biosynthesis of important hormones necessary for development. Wheat (*Triticum aestivum* L.) genotypes differ in types of root systems and in rooting depths. Some of the most studied root traits are maximum root depth, root diameter, and root to shoot ratio. It was generally accepted that wheat genotypes with extensive root systems were beneficial for environments where moisture is available deep in the soil profile (Hurd, 1974). The ability to grow deep roots is currently the most accepted target trait for improving drought resistance, but genetic variation has been reported for a number of other traits that may affect drought response (Veeresh *et. al.*, 2011). Several studies also suggested that genotypes with deep, coarse roots, high levels of root branching and penetration, leaf rolling, early stomatal closure, and a high cuticle resistance are drought tolerant (Blum *et. al.*, 1989; Samson *et. al.*, 2002; Wang and Yamauchi, 2006).

Roots have been intensively studied for over one hundred years but little is known about root dynamics despite their importance, as root systems are difficult to access and observe under field conditions. As such, there is a growing interest in the study of the spatial distribution, age, and identity of all roots from a single plant, collectively termed as the plant root system architecture (RSA) (Lynch J., 1995; Osmont *et. al.*, 2007; Ingram and Malamy, 2010). It is recognized that the root system architecture (RSA) is a complex trait controlled by many genes through quantitative trait loci (QTL). The genetic basis of RSA in crops is poorly understood, and it is difficult to study RSA because different types of roots have distinct genetic, development, and physiological characteristics (Zobel, 1992).

There are many comprehensive reviews of methods used for root study in crops such as soil cores, monolith, minirhizotrons, pots, solution culture and shovelomics (Bohm, 1979; Smit *et al*., 2000; Gregory, 2006; Samuel *et. al.*, 2010). High throughput root phenotyping has been a bottleneck for genetic analysis of RSA for a number of decades. However recently, there have been advances in root research with the use of computer digital image analysis. The importance of root phenotyping should not be underestimated because it can help to understand the RSA regulation, and potentially also can be used to develop crops with improved agronomic performance. Better root phenotyping methods will ultimately lead to identification of the genetic loci underlying useful agronomic traits in the future. Root traits are critical in increasing yield under soil-related stresses (Serraj *et al.*, 2004; Lynch, 2007). Traditionally, measuring root systems was a labor-intensive and tedious task (Bohm, 1979; Dowdy *et. al.*, 1995; Box J.E., 1996) but now with the availability of digital imaging systems and software, it is possible to study various RSA parameters such as maximum number of roots, root diameter, median number of roots, root volume, bushiness, total root length, and root count density. Due to this advanced technology, researchers now have numerous choices for conducting their root studies in the field, greenhouse and laboratory either using two-dimensional RSA imaging or three-dimensional RSA imaging. The advantage of using root imaging system and software is that it allows the observation of root traits at different soil depths throughout growing season or even to monitor the root development of different cultivars.

In this study, root phenotyping experiments were conducted using three specific hard red winter wheat cultivars based on their adaptation to either rainfed or irrigation wheat production systems. In Nebraska, drought has been a major limiting factor in the wheat production in which about 92% of the winter wheat acreage is in dryland production. Drought can occur throughout the life of the plant. In this study, the effect of drought on the root system during the early growth stage of the winter wheat was studied. Immediately after germination, root growth and activity are of relatively greater importance for plant establishment than is shoot formation. Winter wheat forms a fibrous root system, which contributes to water and nutrient uptake throughout the life cycle of wheat. Basically for the root dry matter trait, our previous study showed that there were significant cultivar and water effects, and the root dry matter of winter wheat was significantly greater in the water stress than in well-watered conditions. We were more interested in the drought response, so that is why we looked at RSA under drought. Hence the objective of this study was to determine the differences in the RSA parameters of drought-tolerant and drought-susceptible winter wheat cultivars at seedling stage under water-limiting condition. In a breeding program, rooting patterns could be established for a series of genotypes for use as parents to improve the RSA. The root system of crops should merit additional attention due to the fact that directed modification of RSA holds particular promise for improving agricultural productivity under low input conditions (Lynch, 2007). In order to improve crop productivity, wheat breeders need to select cultivars with a RSA adapted to the conditions of the target environment.

MATERIALS AND METHODS

Plant materials

Three hard red winter wheat cultivars, ‘Goodstreak’, ‘Harry’ and ‘Wesley’ grown across Nebraska agroecoregions, were selected for evaluation of various RSA parameters at the early stage of growth under greenhouse and laboratory conditions. The experiment was conducted at the University of Nebraska-Lincoln, during 2011–2012. The drought tolerant cultivars in this study were Harry and Goodstreak. Harry, as semi-dwarf cultivar, was selected as the drought tolerant cultivar because it was released primarily for its superior adaptation to rainfed wheat production systems in western Nebraska. Goodstreak, a conventional height cultivar, was chosen in this study because it was released primarily for its superior adaptation to rainfed wheat production systems in western Nebraska with low moisture conditions. Goodstreak was one of the most widely grown cultivars in low moisture, rainfed wheat production, where conventional height wheat cultivars were grown. The drought susceptible cultivar in this study was Wesley which was lower yielding than Harry and Goodstreak. Wesley is best suited for irrigated production systems statewide and similar production areas in adjacent states.

Measurement of RSA parameters of 10-day old water-stressed plants using GiA Roots software

Seeds of Harry, Wesley and Goodstreak were sieved to obtain a uniform seed size before they were germinated in petri dishes in the dark at a temperature of 22 ± 2 °C and placed in the dark growth chamber. Two-day old seedlings of a uniform growth stage were transferred and placed randomly on germination papers and grown using the 'Paper Roll Method' in the growth chamber at average day and night temperature of 22 ± 2 °C for a period of ten days. The germination papers were placed in the one-liter beaker filled with 250 ml distilled water. For the first seven days, the water in the beaker was maintained to avoid water stress due to evaporation. After seven days, three-day water stress treatment was imposed by removing water from the beaker. So, the seedlings were allowed to grow with drought stress in the growth chamber for another three days. The germination papers were randomized every other day within the beaker. After ten days, the plants were removed from germination papers. The roots of each plant were scanned using a flatbed scanner (Epson Expression 836XL; Epson America, Torrance, Calif.) with a resolution of 400 dpi and analyzed using the GiA Root software (Georgia Tech Research Corporation and Duke University). A completely randomized design was used with two seeds per cultivar on each germination paper and replicated three times.

Measurement of RSA parameters of 3-week old water-stressed plants using GiA Roots software

Seeds of Goodstreak, Harry and Wesley were used in this experiment. The experiment was conducted in pots with perforated plastic bags and plants were grown in the greenhouse. Seeds were sieved in order to get a uniform seed size before they were germinated. Two-day old seedlings of a uniform growth stage were planted in the pots. A uniform amount of sand (6.9 kg) was used for each pot. The plants were watered daily until fourteen days after seeding (DAS) which involved alternate watering with either 250 ml of water or half-strength Hoagland solution. The pots were randomized every other day. Drought stress treatment was imposed by withholding water to all the pots for a period of one week. After the twenty one days (DAS), the plants were harvested by removing the sand by gentle washing from the roots of the plants. The cleaned roots of each cultivar were brought to the laboratory immediately and then scanned using a flatbed scanner (Epson Expression 836XL; Epson America, Torrance, Calif.) with a resolution of 400 dpi. The root system architecture (RSA) parameters were analyzed using the GiA Root software (Georgia Tech Research Corporation and Duke University). The experiment was performed once in which two plants per pot and five replicate pots per treatment were employed and arranged in a completely randomized design in the greenhouse.

Measurement of RSA parameters of 3-week old water-stressed plants using X-ray based, RootViz FS imaging system and RhizoTraits software

This experiment was conducted at the Phenotyping Screening Corporation (Knoxville, TN). Seeds of Goodstreak, Harry and Wesley were germinated in germination paper using the "Rag Roll" method. Each germination paper was wet with sterile water and scattered with seeds on half the paper. The other half of the paper was folded over and loosely rolled. The seed roll was then placed in a plastic freezer bag and allowed to germinate. Twenty four seedlings were planted in "Q-30" size containers filled with dry "T" size EPS beads. The seedlings were planted approximately ¼ inch below the top of the surface line. After planting, each plant was watered with 30 ml tap water and placed in a turbogarden that was filled with 50 liters tap water. Note: Each turbogarden housed eight plants (Appendix 3). In this experiment, 250 ml PSC Sterile Stock Nutrient Solution (modified Hoagland's solution prepared using various water-soluble fertilizer ingredients) was added to the water in each turbogarden. The pumps were set for ten seconds ON and four minutes fifty seconds OFF. The grow lights were timed to be on for fourteen hours a day using the bulb intensity of 533 micromoles/m^2/second at the soil line. The pH was adjusted to 6.0 in each turbogarden. Each plant was imaged on this first day of planting. The standard daily growing protocol included: (1) each dripper head was checked to insure that the water was flowing freely and there was no clogs (drippers were checked twice daily, in the morning and afternoon), (2) each plant was rotated within its turbogarden to insure that each plant got a random dosage of light and (3) each plant was examined to make sure there is no disease or insects. On a twice weekly basis, (1) the water was changed in each turbogarden. (2) 250 ml PSC Sterile Stock Nutrient Solution was added to each turbogarden after it was drained and refilled with clean tap water, and then (3) the pH was adjusted in each turbogarden to pH 6.0. Water stress was imposed by withholding water supply to the plants after the 2nd week of normal water regime. The plants were allowed to grow for another 1 week under water stress conditions. For root imaging, each plant was imaged in-situ eight times throughout the experiment beginning from the first day the plants were planted and every three days thereafter. However, on the final day of the experiment, each plant was removed from its container and the bare, shaken roots were imaged (Note: roots were NOT washed).

The RSA parameters were investigated using the combination of X-ray based, RootViz FS imaging system and RhizoTraits© Version 1.0 software (Phenotype Screening Corporation, Knoxville, TN), which include the total root length, projected area of roots, root count, root diameter, width-at-depth, root count density, approximate mass density and total root length density. The software used to analyze the root images required roots to be about 300 microns or larger in diameter before it would conclude that a structure in the image was actually part of the root system The RSA parameters were based upon transect analysis. Transects were imaginary horizontal lines drawn at pre-determined depths that were automatically generated on each image of the root system. For this analysis, transects were drawn at depths with an interval of 25 mm apart. The numbers of root crossings at each transect were counted and the root diameters of crossing root were determined.

The RSA traits that were measured include the total root length (TRL), projected area of roots, total root length density (TRL_d), root count, root diameter, width-at-depth, root count density and approximate mass density of roots. The TRL was the total root length in meters of all root segments. The projected area of the root system was estimated in square pixels. The total root length density was measured by TRL divided by the volume of the plant's container (m/m^3). The root count was based on the count number of each root measured at a specific root depth of the root system determined by RhizoTraits. The root diameter was based on the diameter of each root measured in millimeters at a specific root depth of the root system determined by RhizoTraits. The width-at-depth was based on the width of the root system at a specific root depth of the root system determined by RhizoTraits. The root count density was based on the number of roots per unit width (which was measured as the number of root counts per mm^2) at a specific root depth of the root system determined by RhizoTraits. The approximate mass density of roots measured in mm^2/mm was a measure proportional to the approximate mass density of roots at a specific root depth of the root system determined by RhizoTraits.

Statistical analysis

All data analyses were performed using the SAS computer packages version 9.2 for Windows (SAS Institute Inc., Cary, NC, USA). The data collected from the 10-day old, 2-week old and 3-week old seedling experiments were analyzed separately and statistically using one-way analysis of variance (ANOVA) to test for significant differences for the RSA parameters among cultivars using PROC GLM. Tukey's multiple comparison test was applied at 5 percent level of probability to compare the mean differences among cultivars.

RESULTS AND DISCUSSION

Water stress was imposed by withholding water at different seedling stages. We conducted separate experiments to investigate the effects of water stress to the 10-day old and 3-week old seedlings of three winter wheat cultivars of Nebraska. For the 10-day old wheat plants, we compared 10 RSA parameters using the GiA Roots software, which include average root width, maximum number of roots, median number of roots, network bushiness, network length distribution, network length, specific root length, network area, network depth and network volume. There were 2 RSA traits that showed significant differences among 10-day old cultivars in water-limiting conditions, namely the maximum number of roots (p = 0.0411) and network volume (p = 0.0302). No significance differences were observed for 8 RSA traits, namely the average root width (0.03 pixels), median number of roots (2.3), network bushiness (1.59), network length distribution (0.71 pixels), network length (83.5 pixels), specific root length (521.3 pixels/pixels^2), network area (2.24 pixels) and network depth (29.54 pixels) among the 10-day old cultivars. For the maximum number of roots, Harry (3.7) had a larger maximum number of roots than Goodstreak (2.5) (Fig. 1), indicating that the root system of Harry had greater physical strength and potential for RSA compared to Goodstreak during this early growth stage. No significant difference (p = 0.7191) for the maximum number of roots trait was observed between Harry and Wesley. Since Harry performed better than Wesley in the rainfed wheat production system in western Nebraska, other root-associated avoidance mechanisms besides maximum number of roots (or root counts) may be important to confer drought tolerance in Harry during this seedling stage. A significant difference (p = 0.0302) in the root volume trait was observed between the drought tolerant cultivar, Goodstreak and drought susceptible cultivar, Wesley, whereby Wesley (0.1954 pixels^{-2}) produced larger network volume of roots than Goodstreak (0.1506 pixels^{-2}). However, the root volume of Harry (0.1515 pixels^{-2}) was not significantly different (*p* = 0.0536) from Wesley (0.1954 pixels^{-2}). These data demonstrated that under water stress, the drought sensitive cultivar, Wesley may have consumed more water to produce higher volume of roots in order to permeate a large volume of soil in its early growth stage than the drought tolerant cultivar, Goodstreak.

The results of the 2-D root phenotyping analyses GiA Roots software on the 3-week old plants showed that significant differences was observed among the cultivars for three root traits i.e. the maximum number of roots, median number of roots and network length distribution. For the maximum number of roots of the 3-week old water-stressed plants, interestingly, Goodstreak (18.4) produced two-fold more root counts than Harry (9.4), which was not statistically different from Wesley (13.8). For the median number of roots, Goodstreak (10.9) had significantly larger median number of roots than Harry (5.8), which was not statistically different from Wesley (8.5). In terms of the network length distribution parameter, the drought tolerant cultivar, Goodstreak (0.64 pixels) produced greater network length distribution than Harry (0.56 pixels). Wesley (0.60 pixels) was not significantly different from either Goodstreak (0.64 pixels) or Harry (0.56 pixels). The network length distribution is the fraction of network pixels found in the lower two-third of the network, and the lower two-third of the network is defined based on the network depth. Therefore, this study indicated that the larger root system of Goodstreak tended to be distributed in the lower two-third of its entire root system than Harry did. This result suggests that Goodstreak might have RSA that was capable of growing preferentially in deep soil before severe stress occurred in order for the plant to tolerate water deficit stress. There were no significant differences observed among cultivars for two RSA traits, namely the network bushiness (p = 0.7661) and network width depth ratio (p = 0.2197) (Table 2). The average network bushiness and network width depth ratio for all three cultivars were 1.69 and 1.10, respectively. Bushiness is considered as a measure of global branching complexity of root system. However, in this study, the network bushiness of Harry was not affected by the number of roots it produced. Based on these results and our previous study of the water stress experiment (Chapter 1), we speculated that Harry could have developed a drought avoidance mechanism during water stress by producing less root and shoot biomasses and consuming less water and energy while maintaining its bushiness capability, at least at the early stage of growth.

We used another method of analyzing the RSA by utilizing the RootViz imaging system (a non-destructive, high resolution and high-throughput plant root imaging system). Eight root characteristics traits of the 3-week old seedlings were investigated in this study, namely the total root length (TRL), total root length density (TRL_d), projected area of roots, root count, root diameter, width-at-depth, root count density and approximate mass density. Analysis of variance showed that there were 3 RSA traits that were significantly different among the cultivars, which include TRL, TRL_d and projected area of roots. The drought tolerant cultivar, Goodstreak had the longest total root length (1.29 meters), followed by Wesley (1.11 meters) and Harry (0.91 meters) (Table 5; Fig. 2). It was reported that TRL trait was one of the most important factors regarding turgor maintenance and plant growth under drought condition during the seedling stage of pearl millet (*Pennisetum glaucum* [L.] Leeke) (Kusaka *et. al.*, 2004). It was possible that Goodstreak could also use the same mechanism of TRL as the pearl millet in order to avoid drought stress during the seedling stage. In contrast, the TRL trait did not seem to confer drought tolerance to Harry because it had the lowest TRL compared to Goodstreak and Wesley. The TRL_d of Goodstreak (3.63×10^{-18} m/m^3) was significantly greater than Wesley (3.13×10^{-18} m/m^3), which was significantly greater than Harry (2.52×10^{-18} m/m^3). Both the semi-dwarf cultivars, Harry and Wesley had lower TRL and TRL_d compared to the conventional tall wheat cultivar, Goodstreak. Drought tolerance of chickpea (*Cicer arietinum* L.) indicated that higher root length density was an important trait for coping with terminal drought in chickpea, particularly in deeper soil layer, 30–60 cm depth, and also root length density has been associated with deep root system (Kashiwagi *et. al.*, 2005). Since Goodstreak is a drought tolerant cultivar, it might have performed better by producing higher root length density when subject to water stress condition. Our findings also support Wojciechowski *et. al.* (2009) who postulated that the total root length was altered by reduced height alleles and that the root architecture such as average root diameter was not affected by reduced height alleles. Goodstreak had the highest projected area of roots (471,453 $pixels^2$), which was greater than Wesley (425,605 $pixels^2$) and Harry (332,483 $pixels^2$) (Fig. 2). Wheat genotypes with larger projected area of roots are generally considered as having one of the desirable root traits under water stress. The greater projected area of roots could imply that the number of lateral roots, and therefore the number of root tips could have increased. The greater projected area of roots could potentially increase the size of contact with soil, which can increase its foraging capability for the available water and nutrients in the rhizosphere. Five traits did not show significant difference among the cultivars, namely root count (3.7), root diameter (0.3 mm), width at depth (23.3 mm), root count density (0.18 m per m^3) and approximate mass density of roots (0.34 mm^2 per mm). Interestingly, there was no statistically difference for the root count trait among the cultivars when the roots were scanned using the X-ray imaging system and analyzed using the RhizoTraits software. Based on our previous results that Harry produced less root and shoot dry masses but highest root-to-shoot length ratio (Chapter 1), it appeared that during the seedling stage, Harry invested less dry root matter without compromising the RSA traits it produced under water stress such as root count, root diameter, width at depth, root count density and approximate mass density of roots. This result may be important for row spacing and fertilizer application. Harry might have performed better in drought stress due to its sustainable way of utilizing water and energy but would have produced more roots when water becomes available.

In conclusion, our results indicated that the drought tolerant cultivar, Goodstreak ranked first in the TRL, TRL_d and projected area of roots during its early stage of growth and development. Goodstreak was able to generate deep root architecture with greater number of roots, median number of roots, TRL, TRL_d and projected area of roots, which can grow vertically downwards to absorb water and nutrients available in the deep soil when water was a limitation to growth. We found that although Harry and Wesley were semi-dwarf wheat cultivars, their RSA were different under water stress during the seedling stage. Even though the drought sensitive cultivar, Wesley produced greater TRL, TRL_d and projected area of roots than Harry, we postulated that Harry performed better than Wesley under limited water condition because Harry could have invested less energy into branching of roots, penetration of roots into dry soil and hydraulic conductivity. It is interesting to note that Harry, which performed well in the wheat dryland production areas in Nebraska compared to Wesley, possessed a compact, narrow and intensive type of root system. We speculated that a compact, narrow RSA might be beneficial for dryland wheat production areas where the crops depend on the stored soil moisture that is available in subsoil and also from occasional precipitation. We were able to characterize and differentiate the drought tolerant and drought sensitive winter wheat cultivars using a combination of RSA phenotyping approaches. Using the non-destructive RSA phenotyping via RootViz imaging system, we were able to find additional differences for the RSA traits among cultivars. When the results from the RSA phenotyping were combined with our water stress experiment, we were able to supply not only the basic information on the root characteristics but also speculate on the energy investment and assimilate allocation of each winter wheat cultivar. Evaluation of the RSA of cereal crops will become a valuable tool in tailoring crop root systems to specific environments.

REFERENCES

Blum, A., Mayer, S., & Galon, G. (1989). Agronomic and physiological assessments of genotypic variation for drought tolerance in sorghum. *Aust. J. Agric., 40*, 49-61.

Bohm, W. (1979). *Methods of Studying Root Systems.* Berlin: Springer.

Box J.E., J. (1996). Modern methods of root investigations. In Y. Waisel, A. Eshel, & U. Kafkafi (Eds.), *Plant Roots: The Hidden Half* (2nd ed., pp. 193-237). New York: Marcel Dekker.

Dowdy, R., Nater, E., & Dolan, M. (1995). Quantification of length and diameter of root segments with public domain software. *Commu. Soil Sci. Plant Anal., 26*, 459-468.

Gregory, P. (2006). *Plant Roots: Their Growth, Activity, and Interactions with Soils.* Oxford, UK: Blackwell Publishing.

Hurd, E. (1974). Phenotype and drought tolerance in wheat. *Agric. Meteorol., 14*, 39-55.

Ingram, P., & Malamy, J. (2010). Root system architecture. In J.-C. Kader, & M. Delseny (Eds.), *In Advances in Botanical Research* (Vol. 55). Elsevier Ltd.

Kashiwagi, J., Krishnamurthy, L., Crouch, H., & Serraj, R. (2005). Variability of root length density and its contributions to seed yield in chickpea (*Cicer arietinum* L.) under terminal drought stress. *Field Crops Research, 95*(2-3), 171–181.

Kusaka, M., Lalusin, A. G., & Fujimura, T. (2004). The maintenance of growth and turgor in pearl millet (*Pennisetum glaucum* [L.] Leeke) cultivars with different root structures and osmo-regulation under drought stress. *Plant Science, 168*, 1-14.

Lynch, J. (1995). Root architecture and plant productivity. *Plant Physiol, 109*, 7-13.

Lynch, J. (2007). Roots of the second Green Revolution. *Aust J Bot, 55*, 493-512.

Osmont, K., Sibout, R., & Hardtke, C. (2007). Hidden branches: developments in root system architecture. *Annu Rev Plant Biol, 58*, 93-113.

Samson, B., Hasan, H., & Wade, L. (2002). Penetration of hardpans by rice lines in the rainded lowlands. *Field Crops Res., 76*, 175-188.

Samuel, T., Shawn M., K., Kathleen M., B., & Jonathan P., L. (2010). Shovelomics: high throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. *Plant Soil, 341*, 75-87 .

Serraj, R., Krishnamurthy, L., Kashiwagi, J., Kumar, J., Chandra, S., & Crouch, J. (2004). Variation in root taits of chickpea (*Cicer arietinum* L.) grown under terminal drought. *Field Crops Res., 88*, 115-127.

Smit, A., Bengough, A., Engels, C., Van Noorwijk, M., Pellerin, S., & Van de Geijn, S. (2000). *Root Methods: A Handbook.* Berlin: Springer-Verlag.

Veeresh R.P., G., Amelia, H., Akira, Y., Shashidhar, H., & Rachid, S. (2011). Root biology and genetic improvement for drought avoidance in rice. *Field Crops Research, 122*, 1-13.

Wang, H., & Yamauchi, A. (2006). Growth and function of roots under abiotic stress in soil. In B. Huang, *Plant-Environmental Interactions* (3rd ed.). New York: CRC Press.

Wojciechowski, T., Gooding, M., Ramsay, L., & Gregory, P. (2009). The effects of dwarfing genes on seedling root growth of wheat. *Journal of Experimental Botany, 60*(9), 2565-2573.

Zobel, R. (1992). Root morphology and development. *Journal of Plant Nutrition, 15*(6-7), 677-684.

Table 1. Mean squares from analysis of variance of average root width, maximum number of roots, median number of roots, network bushiness, network length distribution, network length, specific root length, network area, network depth and network volume of three winter wheat cultivars in water-limiting conditions at 10-day old seedling stage, which was analyzed by GiA Roots software.

Source of Variation	*df*	Mean Squares									
		Average Root Width	Average Max. # of Roots	Average Median # of Roots	Average Network Bushiness	Average Network Length Distribution	Average Network Length	Average Specific Root Length	Average Network Area	Average Network Depth	Average Network Volume
Cultivar	2	9.1×10^{6NS}	2.167*	1.722^{NS}	0.400^{NS}	0.075^{NS}	194.968^{NS}	19620.764^{NS}	0.531^{NS}	62.631^{NS}	3.93×10^{3}*
Error	15	1.49×10^{5}	0.544	0.544	0.740	0.037	560.503	32872.955	0.221	35.747	8.81×10^{4}

NS: Not significant at $P < 0.05$

* : $p \leq 0.05$

Table 2. Mean squares from analysis of variance of maximum number of roots, median number of roots, network bushiness, network width depth and network length distribution of three winter wheat cultivars in water-limiting conditions at 3-week old seedling stage, which was analyzed by GiA Roots software.

Source of Variation	*df*	Mean Squares				
		Average Max. # of Roots	Average Median # of Roots	Average Network Bushiness	Average Network Width Depth	Average Network Length Distribution
Cultivar	2	210.70909*	68.3661932*	0.02402898^{NS}	0.05587455^{NS}	0.01885702*
Error	29	32.98558	12.3184953	0.08936175	0.03497242	0.00409029

NS: Not significant at $P < 0.05$
 * : $p \leq 0.05$

Table 3. Mean squares from analysis of variance of projected area of roots, total root length, total root length density, root count, root diameter, root count density, width at depth and approximate mass density of three winter wheat cultivars in water-limiting conditions at 3-week old seedling stage, which was analyzed by RhizoTraits software.

Source of Variation	*df*	Mean Squares							
		Average Projected Area of roots	Average Total Root Length	Average Total Root Length Density	Average Root Counts	Average Root Diameter	Average Root Count Density	Average Width at Depth	Average Approximate Mass Density
Cultivar	2	3.17×10^{11}*	2.435*	2.02×10^{-35}*	6.857^{NS}	0.018^{NS}	0.097^{NS}	1243.312^{NS}	0.165^{NS}
Error	198	1.74×10^{10}	0.127	9.70×10^{-37}	9.495	0.010	0.124	596.880	0.128

NS: Not significant at $P < 0.05$
* : $p \leq 0.05$

Table 4. Comparisons of average root width, maximum number of roots, median number of roots, network bushiness, network length distribution, network length, specific root length, network area, network depth and network volume for 10-day old seedlings of three winter wheat cultivars analyzed by GiA Roots.

RSA Parameter	Cultivar	Growth Stage	Least Square Mean	Mean Comparison	Mean Difference	Fr > P
Average Root Width (pixels)	Goodstreak	10-day old	0.033	Goodstreak-Harry	0.002	0.9567^{NS}
	Harry		0.031	Goodstreak-Wesley	0.002	0.5488^{NS}
	Wesley		0.031	Harry-Wesley	0	0.7193^{NS}
Max. No. of Roots	Goodstreak	10-day old	2.5	Goodstreak-Harry	-1.2	0.0382*
	Harry		3.7	Goodstreak-Wesley	-0.8	0.1576^{NS}
	Wesley		3.3	Harry-Wesley	0.4	0.7191^{NS}
Median No. of Roots	Goodstreak	10-day old	1.7	Goodstreak-Harry	-0.8	0.1576^{NS}
	Harry		2.5	Goodstreak-Wesley	-1.0	0.0795^{NS}
	Wesley		2.7	Harry-Wesley	-0.1	0.9195^{NS}
Network Bushiness	Goodstreak	10-day old	1.67	Goodstreak-Harry	-0.14	0.9579^{NS}
	Harry		1.81	Goodstreak-Wesley	0.36	0.7516^{NS}
	Wesley		1.31	Harry-Wesley	0.50	0.5841^{NS}
Network Length Distribution (pixels)	Goodstreak	10-day old	0.78	Goodstreak-Harry	0.02	0.9716^{NS}
	Harry		0.76	Goodstreak-Wesley	0.20	0.1869^{NS}
	Wesley		0.58	Harry-Wesley	0.18	0.2667^{NS}
Network Length (pixels)	Goodstreak	10-day old	82.1	Goodstreak-Harry	3.4	0.9665^{NS}
	Harry		78.7	Goodstreak-Wesley	-7.7	0.8405^{NS}
	Wesley		89.8	Harry-Wesley	-11.1	0.7004^{NS}
Specific Root Length (pixels/pixels^2)	Goodstreak	10-day old	579.4	Goodstreak-Harry	60.0	0.8365^{NS}
	Harry		519.4	Goodstreak-Wesley	114.4	0.5332^{NS}
	Wesley		465.0	Harry-Wesley	54.4	0.8631^{NS}
Network Area (pixels)	Goodstreak	10-day old	2.01	Goodstreak-Harry	-0.13	0.8821^{NS}
	Harry		2.14	Goodstreak-Wesley	-0.56	0.1255^{NS}
	Wesley		2.57	Harry-Wesley	-0.43	0.2715^{NS}
Network Depth (pixels)	Goodstreak	10-day old	33.16	Goodstreak-Harry	6.21	0.2037^{NS}
	Harry		26.95	Goodstreak-Wesley	4.66	0.3914^{NS}
	Wesley		28.50	Harry-Wesley	-1.55	0.8953^{NS}
Network Volume (pixels^{-2})	Goodstreak	10-day old	0.1506	Goodstreak-Harry	-0.0009	0.9985^{NS}
	Harry		0.1515	Goodstreak-Wesley	-0.0448	0.0486*
	Wesley		0.1954	Harry-Wesley	-0.0439	0.0536^{NS}

NS : not significant at $P < 0.05$
* : significant at $P < 0.05$

Table 5. Comparisons of total root length, total root length density, root count, root diameter, width at depth, root count density and approximate mass density for 3-week old seedlings of three winter wheat cultivars analyzed by RhizoTraits.

RSA Parameter	Cultivar	Growth Stage	Least Square Mean	Mean Comparison	Mean Difference	Fr > P
Total Root Length (m)	Goodstreak	3-week old	1.29	Goodstreak-Harry	0.38	<.0001*
	Harry		0.91	Goodstreak-Wesley	0.18	0.0074*
	Wesley		1.11	Harry-Wesley	-0.2	0.0040*
Total Root Length Density (m/m^3)	Goodstreak	3-week old	3.63×10^{-18}	Goodstreak-Harry	1.11×10^{-18}	<.0001*
	Harry		2.52×10^{-18}	Goodstreak-Wesley	0.5×10^{-18}	0.0072*
	Wesley		3.13×10^{-18}	Harry-Wesley	-0.61×10^{-18}	0.0017*
Projected Area of Roots ($pixels^2$)	Goodstreak	3-week old	471453	Goodstreak-Harry	138970	<.0001*
	Harry		332483	Goodstreak-Wesley	45848	0.0957^{NS}
	Wesley		425605	Harry-Wesley	-93122	0.0003*
Root Counts	Goodstreak	3-week old	4.0	Goodstreak-Harry	0.6	0.4553^{NS}
	Harry		3.4	Goodstreak-Wesley	0.3	0.8092^{NS}
	Wesley		3.7	Harry-Wesley	-0.3	0.8209^{NS}
Root Diameter (mm)	Goodstreak	3-week old	0.3	Goodstreak-Harry	0	0.9291^{NS}
	Harry		0.3	Goodstreak-Wesley	0	0.3095^{NS}
	Wesley		0.3	Harry-Wesley	0	0.1984^{NS}
Width at Depth (mm)	Goodstreak	3-week old	21.6	Goodstreak-Harry	1.9	0.8978^{NS}
	Harry		19.7	Goodstreak-Wesley	-6.4	0.2596^{NS}
	Wesley		28.0	Harry-Wesley	-8.3	0.1412^{NS}
Root Count Density (per mm^2)	Goodstreak	3-week old	0.18	Goodstreak-Harry	-0.04	0.8194^{NS}
	Harry		0.22	Goodstreak-Wesley	0.04	0.7617^{NS}
	Wesley		0.14	Harry-Wesley	0.08	0.4274^{NS}
Approximate Mass Density (mm^2 per mm)	Goodstreak	3-week old	0.35	Goodstreak-Harry	0.06	0.6132^{NS}
	Harry		0.29	Goodstreak-Wesley	-0.04	0.7465^{NS}
	Wesley		0.39	Harry-Wesley	-0.1	0.2454^{NS}

NS : not significant at $P < 0.05$

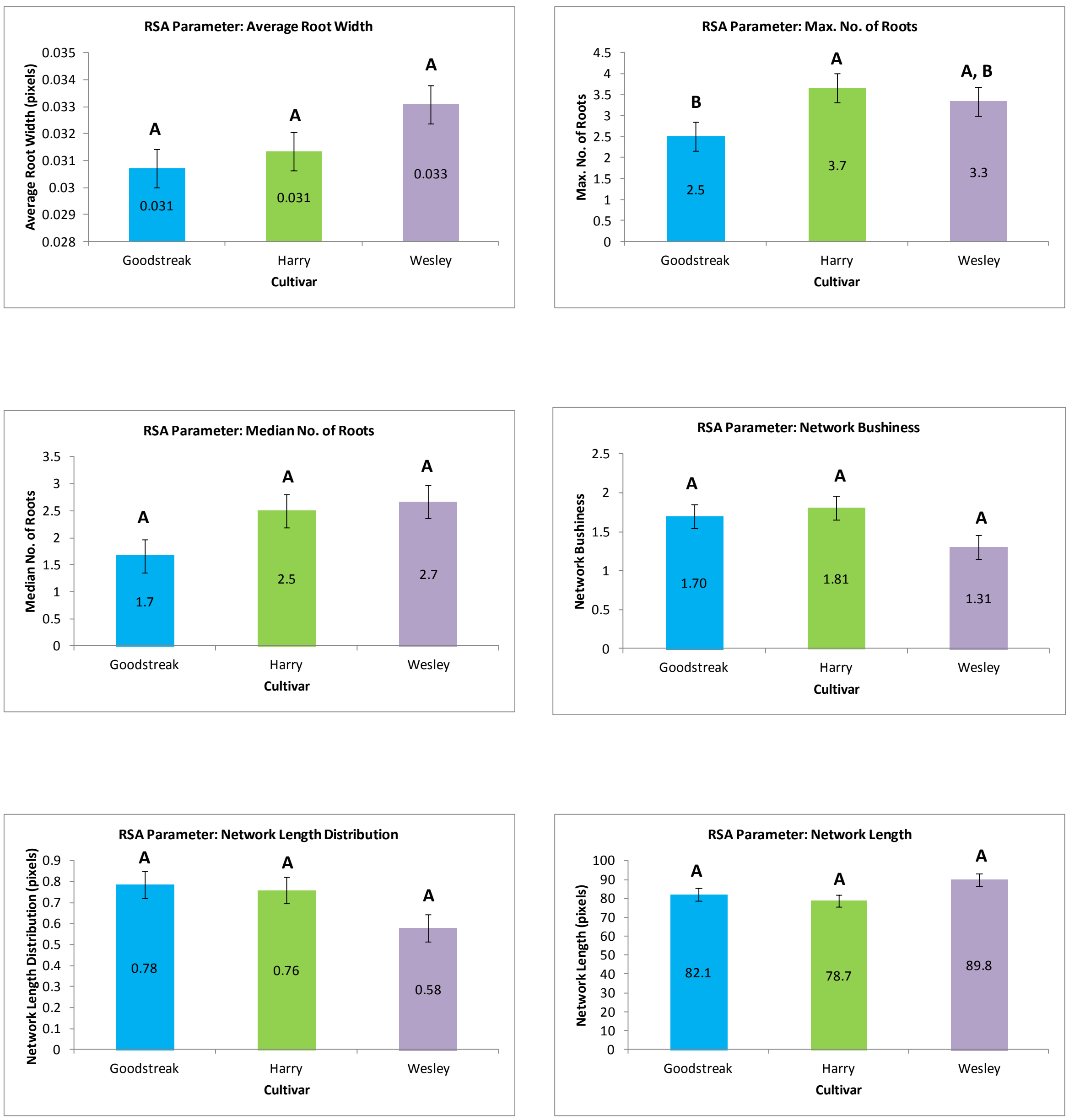
* : significant at $P < 0.05$

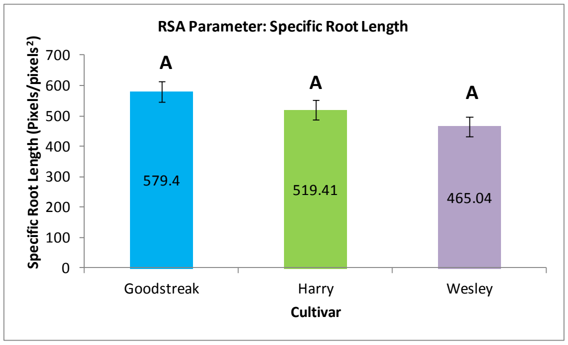
Table 6. Comparisons of maximum number of roots, median number of roots, network bushiness, network width depth ratio and network length distribution for 3-week old plants of three winter wheat cultivars analyzed by GiA Roots.

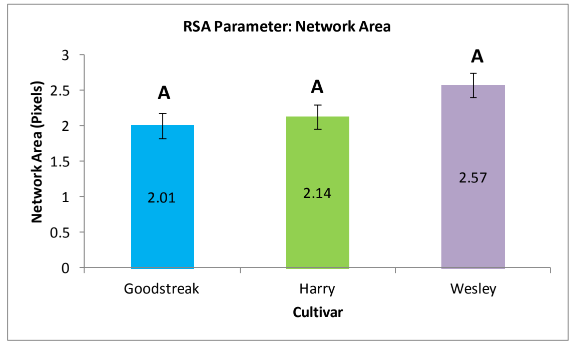
RSA Parameter	Cultivar	Growth Stage	Least Square Mean	Mean Comparison	Mean Difference	Fr > P
Max. No. of Roots	Goodstreak	3-week old	18.4	Goodstreak-Harry	9.0	0.0035*
	Harry		9.4	Goodstreak-Wesley	4.6	0.1697^{NS}
	Wesley		13.8	Harry-Wesley	-4.4	0.2006^{NS}
Median No. of Roots	Goodstreak	3-week old	10.9	Goodstreak-Harry	5.1	0.0065*
	Harry		5.8	Goodstreak-Wesley	2.4	0.2454^{NS}
	Wesley		8.5	Harry-Wesley	-2.7	0.211^{NS}
Network Bushiness	Goodstreak	3-week old	1.74	Goodstreak-Harry	0.07	0.8369^{NS}
	Harry		1.67	Goodstreak-Wesley	0.09	0.7765^{NS}
	Wesley		1.65	Harry-Wesley	0.02	0.9951^{NS}
Network Width Depth Ratio	Goodstreak	3-week old	1.02	Goodstreak-Harry	-0.14	0.2^{NS}
	Harry		1.16	Goodstreak-Wesley	-0.09	0.509^{NS}
	Wesley		1.11	Harry-Wesley	0.05	0.7837^{NS}
Network Length Distribution (pixels)	Goodstreak	3-week old	0.64	Goodstreak-Harry	0.08	0.0137*
	Harry		0.56	Goodstreak-Wesley	0.04	0.2513^{NS}
	Wesley		0.60	Harry-Wesley	-0.04	0.3315^{NS}

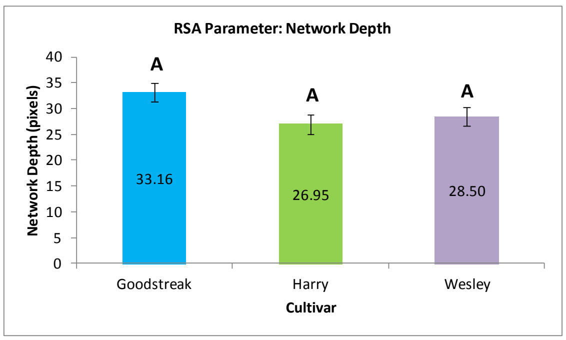
NS : not significant at $P < 0.05$
* : significant at $P < 0.05$

Fig. 1. Graphs for each RSA parameters (average root width, max. no. of roots, median no. of roots, network bushiness, network length distribution, network length, specific root length, network area, network depth and network volume) of 10-day old plants of three winter wheat cultivars analyzed by GiA Roots. Error bars are the standard error of the mean. Means with the same letter are not significantly different.




RSA Parameter: Specific Root Length
Specific Root Length (Pixels/pixels^{2})
A
A
A
579.4
519.41
465.04
Goodstreak
Harry
Wesley
Cultivar


RSA Parameter: Network Area
Network Area (Pixels)
A
A
A
2.01
2.14
2.57
Goodstreak
Harry
Wesley
Cultivar


RSA Parameter: Network Depth
Network Depth (pixels)
A
A
A
33.16
26.95
28.50
Goodstreak
Harry
Wesley
Cultivar

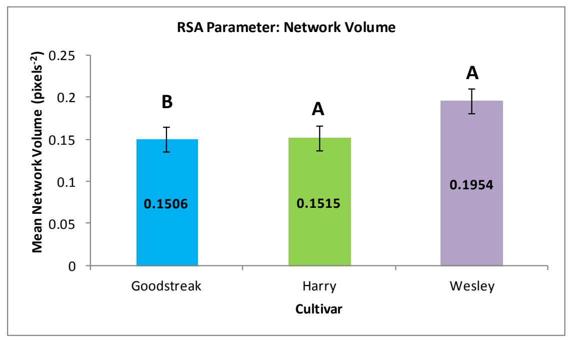
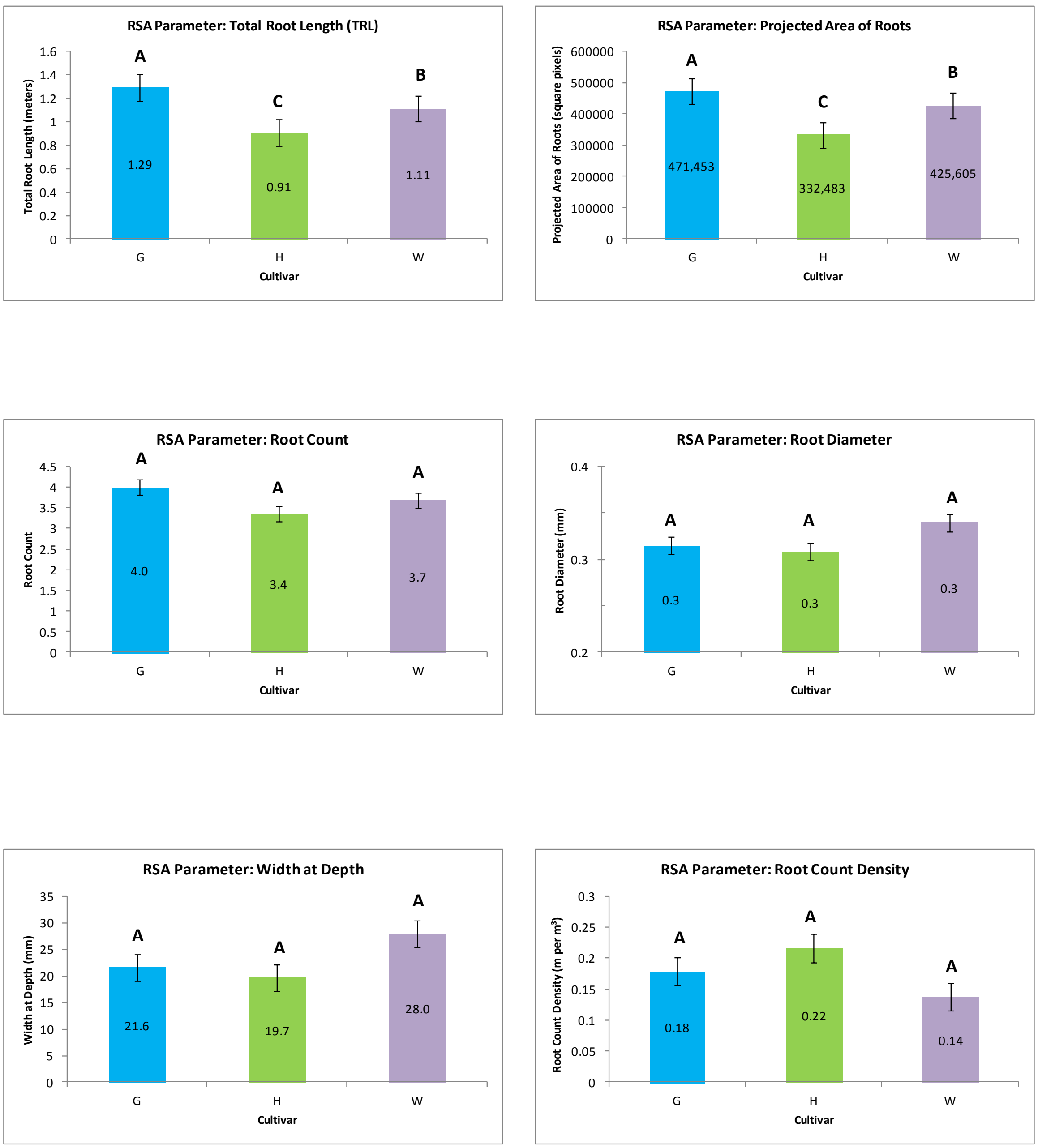
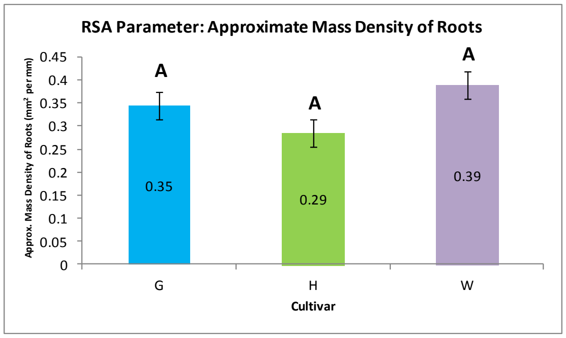

RSA Parameter: Network Volume
Mean Network Volume (pixels^{-2})
B
A
A
0.1506
0.1515
0.1954
Goodstreak
Harry
Wesley
Cultivar

Fig. 2. Graphs for each RSA parameters (total root length, total root length density, projected area of roots, no. of root counts, root diameter, total root depth, width at depth, root count density and approximate mass density) of 3-week old plants of three winter wheat cultivars analyzed by RhizoTraits. Error bars are the standard error of the mean. Means with the same letter are not significantly different.




RSA Parameter: Approximate Mass Density of Roots
Approx. Mass Density of Roots (mm² per mm)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
A
A
A
0.35
0.29
0.39
G
H
W
Cultivar

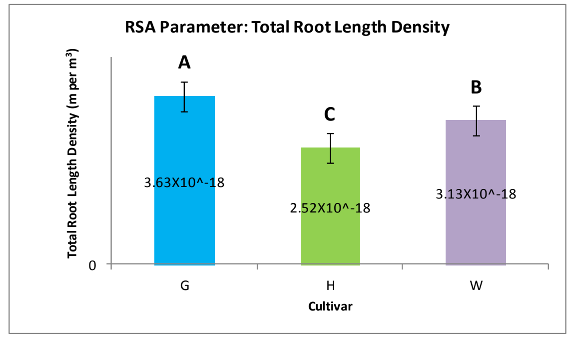
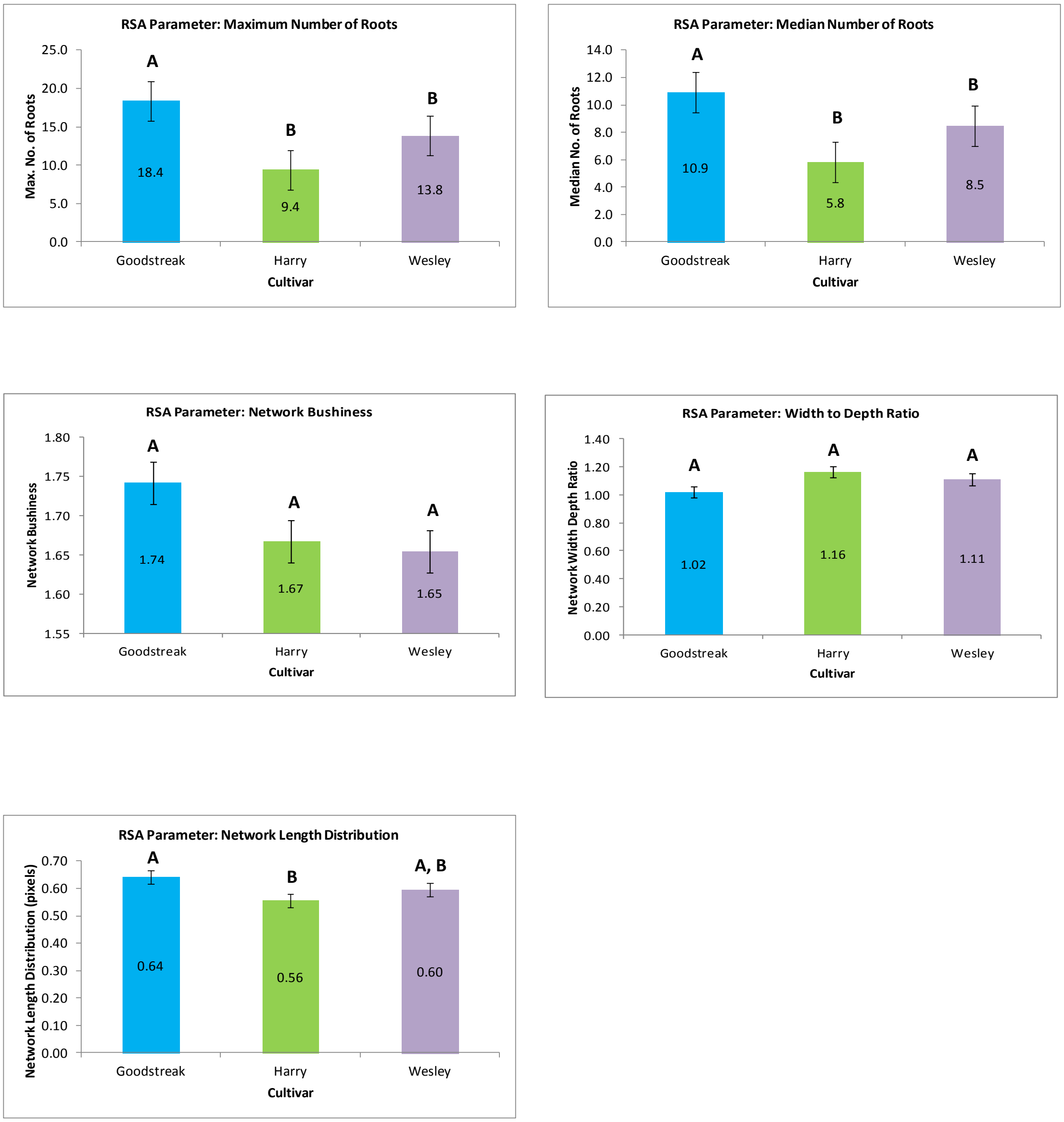
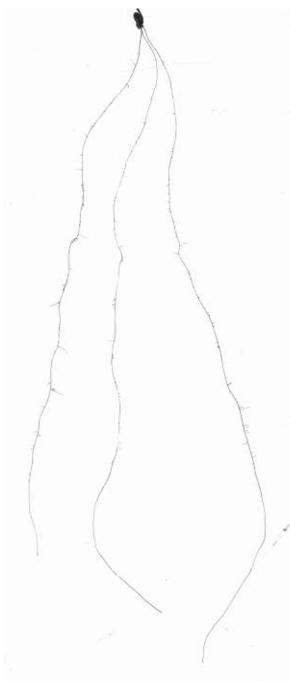
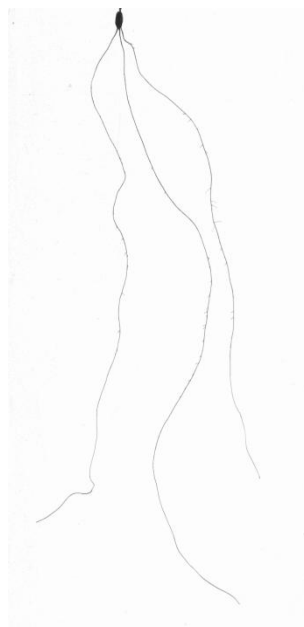
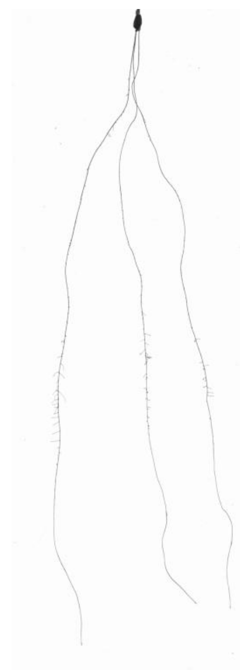

RSA Parameter: Total Root Length Density
Total Root Length Density (m per m³)
0
A
C
B
3.63X10^-18
2.52X10^-18
3.13X10^-18
G
H
W
Cultivar

Fig. 3. Graphs for each RSA parameters (max. no. of roots, median no. of roots, network bushiness, network width depth ratio and network length distribution) of 3-week old plants of three winter wheat cultivars analyzed by GiA Roots. Error bars are the standard error of the mean. Means with the same letter are not significantly different.

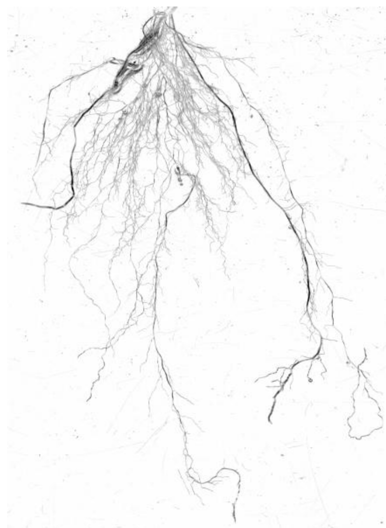


Appendix 1 Root images of 10-day old plants of the three winter wheat cultivars (Goodstreak, Harry and Wesley) scanned and analyzed using GiA Roots. (Note: Only 1 root sample for each cultivar was shown for illustration purpose)

Goodstreak	Harry	Wesley
		

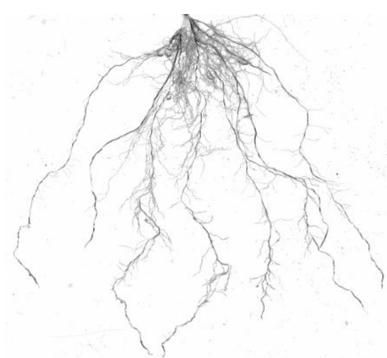
Appendix 2 Root images of 3-week old plants of the three winter wheat cultivars (Goodstreak, Harry and Wesley) scanned and analyzed using GiA Roots. (Note: Only 1 root sample for each cultivar was shown for illustration purpose)

Goodstreak



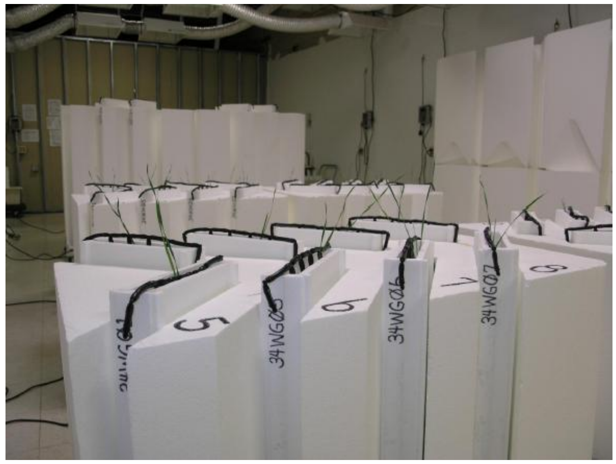
Harry

Wesley



Appendix 3 The set-up of the 2-D root phenotyping for the three winter wheat cultivars (Goodstreak, Harry and Wesley) using expanded polystyrene (EPS) beads as a growth substrate under controlled water regime. (With permission from the Phenotyping Screening Corporation)

Cultivar: Goodstreak



Cultivar: Harry

Cultivar: Wesley

Appendix 4 Root images of 3-week old plants of the three winter wheat cultivars (Goodstreak, Harry and Wesley) scanned and analyzed using RhizoTraits. (Note: Only 1 root sample for each cultivar was shown for illustration purpose)

Goodstreak **Harry** **Wesley**