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Study of Root System Architectural Traits of Oat and Response to Endophyte Inoculation and Drought Stress

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STUDY OF ROOT SYSTEM ARCHITECTURAL TRAITS OF OAT AND RESPONSE TO ENDOPHYTE INOCULATION AND DROUGHT STRESS

BY

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This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

STUDY OF ROOT SYSTEM ARCHITECTURAL TRAITS OF OAT AND RESPONSE TO ENDOPHYTE INOCULATION AND DROUGHT STRESS

KRISHNA GHIMIRE

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Oat is an important cereal crop grown worldwide. Oats have the potential to contribute to human health due to their unique nutritional attributes. Developing oat cultivars with efficient root systems able to extract heterogeneously distributed soil resources can help maintain yield under drought conditions and in nutrient poor soil. Various root traits determine the soil volume that is explored by the root system for resource acquisition. Knowledge about the genetic control of oat root traits and response to biotic and abiotic environmental factors is lacking. Identifying quantitative trait loci associated with root traits and understanding the response of roots to abiotic and biotic environmental factors such as drought and endophytic bacteria may enable plant breeders to develop oat cultivars with efficient roots that can maintain yield under unstable climates. To understand the genetic basis of various root traits in oats and how the oat root and shoot development is impacted by drought and by plant growth-promoting endophytic bacteria, we conducted three different experiments. First, we studied the response of oat root and shoot development to endophytic bacterial inoculation by conducting a root vigor assay and a greenhouse experiment. Several endophytic bacteria significantly increased the root length, root area and root volume for one of the two oat cultivars evaluated in the root

vigor assay. The greenhouse study revealed that the response of oat cultivars to endophytic bacterial inoculation varied depending on the growth parameters evaluated, the nitrogen fertilization level, the oat genotype, and their interactions. Thus, identifying a specific strain of bacteria for overall growth promotion in oats might be difficult.

To gain a better understanding of the extent of phenotypic differences in roots among oat genotypes and how those variations are controlled genetically, a genome-wide association study of root system architectural traits was conducted. Root traits were phenotyped at the seedling stage using a germination paper-based growth platform and a high-throughput image analysis system. Significant variability in root traits among the 285 genotypes evaluated was observed and broad-sense heritability ranged from 0.17 to 0.59 depending on the trait. We identified 82 significant marker-trait associations using a mixed linear model approach. Markers significantly associated with root traits explained from 7.6 to 19.9 % of the phenotypic variation. We identified multiple candidate genes located close to the significant markers that are known to have a role in root development.

Finally, we evaluated the morphological and physiological responses of root and shoot development of ten oat genotypes under drought stress. After withholding watering for two weeks on 21 days old seedlings, we measured chlorophyll content, relative water content, stomatal conductance, stomata number, shoot dry weight, root dry weight, root length, root area, and root volume. Seed yield per plant was also collected by continuing the drying and rewatering cycle until physiological maturity. All traits measured were significantly impacted by the water regime. Oat cultivar Hayden showed the smallest reduction in yield in response to drought treatment. Hayden also showed a smaller

reduction in relative water content, chlorophyll content, and a strong reduction in stomata number. Results indicated that the larger root system may not necessarily provide a yield advantage under drought conditions in oats. The importance of root mass distribution into lower and upper soil layers should be investigated to improve our understanding of mechanisms involved in coping with drought.

CHAPTER 1

Literature review

Oat overview

Oats (*Avena sativa* L.) are annual grasses that belong to the tribe Aveneae of the family Gramineae. The genus *Avena* comprises polyploid species of wild weedy and cultivated species distributed across six continents. The area with the most diverse species is situated between 25^{o} and 45^{o} N latitude and 20^{o} W and 90^{o} E longitude that extends from the canary island, the Mediterranean basin, and the middle east to the Himalayan Mass (Murphy & Hoffman, 1992). Oat is the sixth-largest crop globally based on production (Statista, 2019). Oats only account for about 2% of world grain production and the bulk of it is used as feed. Global oat production in 2019/20 was 22.5 million metric tons with a total cultivated area of 9.7 million hectares. Most of the production takes place in Europe, Russia, and Canada (USDA, 2019). Because of the health benefits of oat consumption, interest in oats is increasing globally.

Importance of oats

Oat is a multipurpose crop grown for grain, pasture, forage, and as cover crop. Oat has economic value in human nutrition and health care (Kapoor & Batra, 2016). Oats have been used in the development of many food products such as oat bread, oat yogurt, oat cookies, pasta, flat bread (naan), oat milk, breakfast cereal and instant formula (Adhikari, 2022; Deswal et al., 2014; Duta & Culetu, 2015; Hager et al., 2013; Luana et al., 2014). Oats are a good source of protein, fiber, and minerals. Oat consumption provides beneficial health effects because of its high macronutrients, micronutrients, soluble fiber

(β-glucan), and polyphenolic content (Essa et al., 2012). Oat is useful in controlling diabetes and lipid profile.

In 2019, the USA exported 30 metric tons of oats and imported 1,700 metric tons of oats. Oat use in the USA is much higher than production; in 2020, oat production totaled 771 metric tons and total oat consumption was 2,491 metric tons (USDA, 2020). In 2019, USA produced oats worth $162,711,000 (Statista, 2022). Given the rise in oat consumption, there is a growing market for oats and oat products in the USA.

Root system architecture and crop yield

Roots provide an interface between plant and complex soil environments. The root system provides anchorage and plays an important role in water and nutrient uptake from soil that is required for plant productivity. Root system architecture (RSA) refers to the spatial configuration of the root system in the soil and describes the shape, structure of the root system, and geometric deployment of root axes (Lynch, 1995). The RSA is vital for plant productivity because the soil resources are heterogeneously distributed in the soil and the spatial deployment of the roots will substantially determine the ability of plants to secure edaphic resources. The root traits are influenced by genetics, environmental factors and their interactions. Both monocotyledons and dicotyledons have an abundance of natural variation in root traits attributed to different genotypes. Variations in RSA traits have been reported in lentils, rice, barley, maize, sorghum, and wheat (Gahoonia et al., 2006; Henry et al., 2011; Jia et al., 2019; Li et al., 2015; Mace et al., 2012; Manschadi et al., 2008; Richard et al., 2015). There are several reports of QTLs for root features overlapping with QTLs for productivity (yield, water use or nutrient

use), thus suggesting the possible role of root features in determining the plant productivity (Steele et al., 2007; Tuberosa et al., 2002). In certain environmental settings such as drought, specific RSA can provide growth benefits and impact aerial plant parts that contribute to yield (Rogers & Benfey, 2015). Roots are the first organ to sense drying soil and initiate a signaling cascade that leads to the overall plant's response to drought stress (Schachtman & Goodger, 2008). Root and leaf organize the defense mechanisms both internally and externally in response to abiotic stress (Kim et al., 2020).

Some important root traits that help to maintain yield under drought include small fine root diameter, greater specific root length, and increased root hair density and length (Comas et al., 2013). Similarly, deeper root systems, increased root density at depth, decreased root density at the surface, and increased root hair and xylem diameter can improve productivity (A. P. Wasson et al., 2012). Since increasing water uptake is an urgent need in drought conditions, a reduction in horizontal proliferation of lateral roots in topsoil and allocation of more resources to the growth of primary roots would allow plants to expand their domain of water supply (Xiong et al., 2006).

Root traits and soil resource acquisition

The root serves many functions such as providing support for a plant and absorption of water and nutrient. Different root traits play different roles in improving crop productivity and different types of root system architectural traits are suited for different functions. Thin roots with long specific root lengths (SRL) can extract water and nutrients more efficiently (Comas et al., 2012). In addition to providing support for plants in soil, nodal roots are useful to harvest late-season precipitations (Rostamza et al., 2013).

Large crown roots can help in top-soil foraging in maize. Root hairs assist in root contact with soil particles and the absorption of water and nutrient (A. P. Wasson et al., 2012). Root angle determines the direction of root elongation in soil and affects the area in which roots capture water and nutrients. Deeper roots achieved by root growth angle and root plasticity in response to nitrogen distribution may enhance nitrogen acquisition from deeper soil layers along with water absorption. Crops with a deep root system can also improve soil structure, and its steady-state carbon water and nutrient retention and thus contribute to crop production (Kell, 2011). Nutrient efficient crops are solutions to two major challenges of modern agriculture, improving global food security, and reducing the environmental impacts of chemical fertilizers (Lynch, 2019). The steep, cheap, deep root ideotype for subsoil foraging is useful for N and water capture. Steep, cheap, and deep root ideotype that helps in nitrogen capture in maize consists of root that promotes exploration of deep soil domains to capture nitrate as it leaches through root zones (Lynch, 2013). Architectural traits include steep root growth angles, few nodal roots, sparse lateral branching, and low architectural plasticity in response to environmental cues. Higher yield, plant growth, root depth, and N capture were correlated with steep root growth angle in maize (Trachsel et al., 2013). The breeding targets to increase N efficiency, in crops with substantial natural variation, are steep growth angle, few axial roots, reduced lateral branching, and longer/denser root hairs.

Since the majority of P in soil is highly immobile, to increase P acquisition it is necessary to improve foraging in P rich soil layers and improve the exploitation of those layers through P solubilization. Topsoil foraging would increase P acquisition since P is greatest in topsoil due to P decomposition from plant residues, limited P leaching to deeper soil

layer, and greater biotic activity in topsoil (Lynch & Brown, 2001). Shallower axial root growth angle, greater lateral root density, greater root hair density, and greater root length can increase top-soil forage and P acquisition. Under low P soil conditions, maize genotype with greater production of crown roots showed greater topsoil foraging, P capture, growth, and yield (Sun et al., 2018). Top-soil foraging ideotype is beneficial for P capture along with the capture of K, Ca, and Mg in acid soils. Fe bioavailability is reduced in alkaline soil and is subject to interaction with an array of soil chemical and biological agents (Hansen et al., 2004). Root tissue density controls the length and surface area of the root system for a given root biomass and thus controls the amount of root surface directly interacting with soil and the amount of root surface colonized by mycorrhizal fungi assisting plant nutrient acquisition (Smith & Read, 2010).

Genetics of root system architecture

Genes have been characterized and genetic control of RSA has been reported in many crops such as rice, corn, wheat, and soybean. The expression of a specific gene regulating RSA can confer a growth advantage under specific conditions. In rice, *CRL5* is demonstrated to be essential for crown root initiation (Kitomi et al., 2011). In soybean, *GmEXPB2* is involved in hair root elongation and subsequently affects plant growth and P uptake (Guo et al., 2011). In *Arabidopsis*, *GmEXPB2* is a critical root β-expansin gene involved in root system architecture response to abiotic stress including P, Fe, and water deficiency (Guo et al., 2011). In barley, silencing *HvCKX1* gene leads to increased yield and root weight (Zalewski et al., 2010). In wheat, overexpression of *GmbZIP1* gene leads to an increase in drought tolerance, and increased root and shoot growth (Gao et al., 2011).

In some cases, genes that modify RSA can increase nutrient (N, P) and water use efficiency that leads to higher yield. A rice quantitative trait locus controlling root growth angle, *DEEPER ROOTING 1* (*DRO1*), can alter RSA and improve drought avoidance. Overexpression of *DRO1* increases the root growth angle and results in root growth in more downward direction (Uga et al., 2013). These findings in the literature suggest that genetic manipulation of root system architectural traits is possible, and manipulation of root traits can help maintain yield under drought conditions and nutrient-poor soil.

Measurement of RSA traits

Despite their importance in capturing resources from soil, roots are hidden for phenotyping. Field phenotyping of root traits is very difficult and time-consuming (A. P. Wasson et al., 2012). Some traditional studies have relied on excavation techniques to determine root depth and root length density. There are several non-invasive methods to phenotype roots, some of these include growing plants in gel-based growth platform (Bengough et al., 2004; Iyer-Pascuzzi et al., 2010; Joshi et al., 2017), using seed germination blotting paper or geotextile capillary mat (Atkinson et al., 2015; Hund et al., 2009). In the greenhouse, to better access the root system, plants can be grown in soil or sand-filled pots or PVC tubes (Lafitte et al., 2001). The plants can be grown in liquid cultures as well to visualize the roots (Tuberosa et al., 2002). These non-destructive visualizations of RSA may not recapitulate the three-dimensional nature of RSA in the soil since phenotyping is done in the early stage of growth.

Quantification of the RSA trait is done by image analysis of the root system captured by digital cameras or scanners. For non-soil-grown plants, the image acquisition is easy whereas plants grown in soil or sand must be separated from the soil and imaged. Several

image analysis programs have been developed to increase the number and complexity of RSA traits to be analyzed and to quantify RSA traits across the entire root system. These include WinRhizo (www.regentinstruments.com), Delta-T-Scan (www.delta-t.co.uk), WR-RIPL (http://rootimage.msu.edu), Root Measurement System (Ingram & Leers, 2001), RooTracker (www.biology.duke.edu/rootracker), EZ-Rhizo (Armengaud et al., 2009), DART (Le Bot et al., 2010), ARIA (Pace et al., 2014), DIRT (Das et al., 2015), RootNav (Pound et al., 2013).

Endophytes

Plants provide a spatially and temporally complex habitat to microbes. Endophytes are microorganisms that spend at least parts of their life cycle inside plants. The definition of endophytes has changed in the past and will evolve in the future. The term endophyte means an organism living inside the plant (i.e., "endo" is derived from the Greek word "endon" meaning within, and "phyte" is derived from the Greek word "phyton" meaning plant) (Chanway, 1996). The word has evolved to mean specific microbe plant association, referring to fungi that invade the plants and cause no disease symptoms (Wennström, 1994). The term endophyte has been used for fungi living inside plants and researchers later realized the interior of plants can also be colonized by bacteria. Thus, the new definition of endophytes was proposed to incorporate the bacteria. Wilson (1995b) defined the endophytes as “fungi or bacteria which, for all or part of their life cycle, invade the tissues of living plants and cause unapparent and asymptomatic infections entirely within plant tissues but cause no symptoms of disease”. Endophytes can be classified into three categories based on their plant inhabiting strategies. Obligate

endophytes cannot live outside the host plant and are transmitted through seeds. Facultative endophytes live in soil and can infect the host plants when opportunities arise. Third types of endophytes, the passive endophytes do not actively seek to colonize the plant but can do as a result of stochastic events such as open wounds (Hardoim et al., 2008). The passive endophytes lack the machinery to infect the plant and thus are less appropriate as plant growth promoters (Gaiero et al., 2013).

Bacterial niches inside the host plant

The distribution of endophytes within plants depends upon the ability of endophytes to colonize and the allocation of plant resources. Openings in roots in and around the root hair emergence zone, lateral root emergence zone, lateral root cracks, wounds, stomata, and hydathodes in the shoot are considered as the main entry sites that bacterial endophytes use to enter the host (Hardoim et al., 2015). Some bacterial endophytes can secrete cell wall modifying cellulolytic enzymes like cellulases, xylanases, pectinases, and endoglucanases that helps the bacterial entry and its spread into plant tissue (Compant, Reiter, et al., 2005; Reinhold-Hurek et al., 2006). Bacterial endophytes mostly occupy intercellular spaces in plant due to the abundance of carbohydrates, amino acids and inorganic nutrients (Elbeltagy et al., 2001). They can colonize the intercellular spaces of various plant parts like roots, leaves, stems, flowers, and seeds. This colonization can be localized at tissue level or systemically throughout the plant (Kandel et al., 2017). Endophytes are first observed in root hairs and subsequently in root cortex during early state of colonization (Castanheira et al., 2017; Prieto et al., 2011). Although colonization by endophytes is almost exclusively intercellular, some intracellular colonization has

been reported. Intracellular colonization includes presence of bacteria in root cortical cells of grapes, shoot tips of banana, root of *Arabidopsis,* and seedling roots of switch grass (Compant, Reiter, et al., 2005; Thomas & Reddy, 2013; Van der Meij et al., 2018; White Jr et al., 2014). After initial colonization, some endophytes can move to other plant parts through vascular tissue (Johnston-Monje & Raizada, 2011). The distribution of resources throughout the plant influences the distribution of endophytes. Garbeva et al. (2001) reported diverse bacterial communities in potato, with communities from potato stem differing from communities from stem peel and roots. The bacterial endophytes are more influenced by plant tissue type than fungal endophytes which are more affected by host habitat and biogeography (Coleman-Derr et al., 2016).

Biodiversity of endophytes

Most diversity of life on the planet is accounted for by microbes. Endophytic microbes are ubiquitous and are reported for most crops. Predominant and most studied endophytic bacteria belong to Proteobacteria followed by Firmicutes and then by Actinobacteria (Rana et al., 2020). Some of the most studied genera in leguminous and non-leguminous plants include *Bacillus, Pseudomonas, Fusarium, Burkholderia, Rhizobium,* and *Klebsiella* (Rana et al., 2020).

The microbiome in the root endosphere is considerably less diverse than the microbiome in the rhizosphere and soil (Liu, Carvalhais, Schenk, et al., 2017). The number of bacterial cells per gram of root tissues ranges from 10^4 to 10^8 per gram of root tissues, which is much less compared to the number of bacteria in rhizosphere and soil bulk which is 10^6–10^9 (Liu, Carvalhais, Crawford, et al., 2017). Thus, roots can act as habitat

filters and limit bacterial communities to narrower lineage as the environment deviates from soil to roots (Bulgarelli et al., 2012). The root endophytic bacterial community is dominated by Proteobacteria (about 50% relative abundance), Actinobacteria (about 10%), Firmicutes (~10%), and Bacteroidetes (~10%). Other bacterial communities present in roots as endophytes in smaller fraction includes Chloroflexi, Cyanobacteria, Armatimonadetes, Verrucomicrobia, Planctomycetes, and Nitrospirae (Liu, Carvalhais, Crawford, et al., 2017). Some bacteria that have a significant presence in bulk soil but are either absent or rare in the root endosphere include Archea, Acidobacteria, and Gemmatimonadetes (Sessitsch et al., 2012). The robust selection of the bacterial groups by the plant is also evident from many studies that shows that the plant root endosphere is dominated by few bacterial groups despite the abundance of diverse bacterial communities in soil bulk.

Growth promoting activities of endophytes

Many endophytic bacteria are known to have positive effects on the growth of groundnuts, lentils, wheat, red pepper, soybean, corn, and spinach (Cakmakci et al., 2007; Goswami et al., 2014; Joo et al., 2004; Midekssa et al., 2015; Mumtaz et al., 2017; Ramesh et al., 2014). The endophytic bacteria can increase the plant's shoot dry weight, root dry weight, root number, plant height, and nutrient content in the shoot and leaf. Some of the mechanisms employed by endophytic bacteria include phytostimulation, biofertilization and biocontrol.

Phytostimulation: Phytostimulation is the direct growth promotion of plant by endophytes by producing growth hormones (Bloemberg & Lugtenberg, 2001).

Azospirillum spp. has the ability to secrete phytohormones like auxins, cytokinins and gibberellins as well as the ability to fix the nitrogen (Steenhoudt & Vanderleyden, 2000). The most important phytohormone produced by *Azospirillum* is auxin indole-3-acetic acid (IAA). The changes in root morphology after *Azospirillum* inoculation is assumed to be caused by bacterial phytohormone production. The changes in root morphology may be related to enhanced mineral uptake (Jain & Patriquin, 1985). Some endophytes can remove heavy metals and protect plants from metal toxicity. *Mucor* sp. MHR-7 was able to lock down heavy metals in its mycelium thereby making them less available to plant roots and thus reducing toxicity in mustard. Besides bioremediation potential, the strain produced IAA, 1-aminocyclopropane-1-carboxylate (ACC), and solubilized phosphate (Zahoor et al., 2017). *Nostoc* spp. is shown to enhance several growth parameters such as fresh weight, dry weight, shoot length, root length of the crop plants (rice and wheat) and the cytokinin production was the tool used by *Nostoc* to colonize plant roots and promote its growth (Hussain et al., 2013).

Biofertilization: biofertilization is the promotion of plant growth by increasing the accessibility or supply of major nutrients to plants. Biological nitrogen fixation is a well-studied biofertilization mechanism which is the conversion of atmospheric nitrogen to ammonia (Bloemberg & Lugtenberg, 2001). The most studied and most efficient nitrogen fixers are endophytic bacteria that belong to the genera *Rhizobium, Sinorhizobium, Mesorhizobium*, *Bradyrhizobium*, *Azorhizobium,* and *Allorhizobium* (Bloemberg & Lugtenberg, 2001). Many endophytes are capable of phosphate solubilization which increases the availability of phosphorus to plants. The bacteria release organic acids into the soil which solubilize the phosphate complex and convert them into ortho-phosphate

which is available for plant uptake and utilization. Otieno et al. (2015) described *Pseudomonas* isolates that can produce gluconic acid (14–169 mM) and have moderate to high phosphate solubilization capacities (~400–1300 mg L−1). When these isolates were inoculated into Pea grown in soluble phosphate limiting conditions, the isolates displayed beneficial growth promotion effects. *Bacillus* sp. Isolate EB. 78 from banana exhibited P solubilization capacity when supplied with $Ca_3(PO_4)_2$ and soy lecithin as P source. The isolate significantly reduced the pH of the liquid medium and exhibited acid phosphatase activity (Matos et al., 2017). The application of biofertilizers is a promising technology for a sustainable farming system. Based on 171 peer-reviewed publications, Schütz et al. (2018) reported that biofertilizers were able to increase yield up to 20% in a dry climate, 14% in a tropical climate, and 10% in an oceanic climate. The combined application of P solubilizers and N fixers is better than their separate application, and a higher yield increase with combined application suggests an absence of competition and rather synergies between the two traits.

Biocontrol: endophytes help plants by controlling harmful pathogens. Endophytes inhabit plant tissues in a similar niche as phytopathogens and they compete with pathogens as a biocontrol agent (Berg et al., 2005). The mechanisms involved include host defense (induced systemic resistance, ISR), parasitism, competition, signal interference, production of inhibitory allelochemicals, detoxification and degradation of virulence factors, and competition for iron and production of siderophores (Compant, Duffy, et al., 2005). *Bacillus subtilis* strain E1R-j isolated from wheat root showed antifungal activity to *Gaeumannomyces graminis* var. *tritici* (Ggt). When wheat plants were inoculated with E1R-j, take all disease caused by Ggt was reduced by up to 70.7%

compared to uninoculated control (Liu et al., 2009). *Bacillus amyloliquefaciens* BZ6-1 isolate was shown to produce antimicrobial compounds and reduced the disease incidence of peanut bacterial wilt from 84.5% in control to 12.1% in inoculated plants (Wang & Liang, 2014).

Rationale of the study

Crop yield is suppressed by environmental stress and nutrient-poor soil. Roots are important for overall plant productivity and grain yield and the spatial deployment of the root system determines the ability of plants to capture the soil resources. Thus, it is necessary to identify the genetic control underlying the RSA to improve the crop yield under adverse conditions and maintain global food security. The root system architectural traits are controlled by the plant's genetic as well as environmental factors. There is considerable genetic variability in root system architecture in several crop species. The root system is impacted by environmental factors such as drought, nutrient levels in the soil as well as the presence of diverse microorganisms in root rhizosphere. Thus, a better understanding of the root system and its role for yield and adaptation in unstable climates requires a more extensive study into how the roots respond to these environmental factors. The specific objectives of this study were to (1) evaluate the potential of endophytic bacteria to enhance root and shoot growth under varying level of nitrogen fertilization, (2) evaluate the genetic variability of root system architectural traits in oats and identify candidate genes involved in root development in oats, (3) evaluate root and shoot traits in oats under drought stress and analyze root architectural components that contribute to drought tolerance in oats.

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CHAPTER 2

Effect of endophytic bacteria on oat (*Avena savita* L.) growth

Abstract

Endophytic bacteria are known to influence the vital activities of host plants. They can promote plant growth and defense response against pathogens, and act as remediators of abiotic stress. Use of endophytes in crop production has the potential to reduce the application of fertilizers and pesticides and thus improve the sustainability of crop production. In this study, we tested the effects of endophytic bacteria on oat (*Avena sativa* L) growth with a root vigor assay in a growth chamber and a greenhouse experiment. For root vigor assay, seeds of two cultivars (Gopher and Hayden) were treated with 16 endophytic bacteria and grown on a germination paper in Petri dishes for 6 days. Root length, root surface area, and root volume were determined with WhinRhizo. In the greenhouse experiment, endophytes treated seeds were grown in a sand perlite (60:40) mixture in cone containers. The experiment was conducted in a complete randomized design. There were 10 genotypes, two bacterial treatments along with control, and two levels of fertilization. Forty-two days after planting, chlorophyll content, shoot dry weight, root dry weight, root length, root surface area, and root volume were measured. In root vigor assay, *Bacillus licheniformis, Enterobacter kobei, Brevibacterium halotolerans, Bacillus cereus, Bacillus aryabhattai,* and *Lysinibacillus fusiformis* increased either root length, root area**,** or root volume in Gopher. In Hayden, a decrease in root length was observed with some isolates while others had no effect. In the greenhouse study, the effect of endophytic bacteria was significant for shoot dry weight, root dry weight, and chlorophyll content; however, the effect of bacteria was not

significant for root length, area, and volume. There was a significant interaction effect between genotype and bacteria for all traits. The magnitude and direction of endophyte effects on oat growth varied with nitrogen levels and differed between oat genotypes.

Introduction

Oats (*Avena sativa* L.) are annual grasses that belong to the tribe Avenae of the family Gramineae. Oat consumption presents many health benefits such as reducing LDL-cholesterol and the risk of cardiovascular diseases. Oats exhibit glucose-lowering effects and reduce the risk of type-2 diabetes (Martinez-Villaluenga & Penas, 2017). Most health benefits associated with oat consumption are attributed to the soluble fiber beta-glucan. Although oats are thought to require significantly lower nitrogen input and can perform well on lands less suitable for wheat production (Weightman et al., 2004), nitrogen fertilization up to 150 kg ha^{-1} can significantly increase the yield, milling quality, and grain compositional quality such as β-glucan, protein, and oil (Yan et al., 2017). Despite the fact that nitrogen fertilization can boost crop production, it is considered environmentally unfriendly (Rütting et al., 2018) and it is a costly input for producers. Many bacterial endophytes, known to be associated with the oat, are capable to fix nitrogen and produce indole acetic acid (Soares et al., 2006; Venieraki et al., 2011). Thus, employing the nitrogen-fixing endophytes may provide oat with additional nitrogen for better yield. Bacterial endophytes can also improve tolerance to NaCl by improving the biochemical and physiological status of oat seedlings (Sapre et al., 2018). Using endophytes in oat production could improve oat growth, reduce the reliance on chemical fertilizers, and improve the sustainability of oat production.

Bacterial endophytes are bacteria that invade the tissue of living plants and cause asymptomatic infections within plant tissue (Wilson, 1995a). Endophytic bacteria are ubiquitous in most plants and are either residing latently or actively colonizing plant tissue. Endophytes can enter and thrive on plants from various species and have multidimensional interactions with the host plant. Endophytic bacteria in plants can originate from the bacterial communities of the rhizosphere, phylloplane, or endophyte-infected seeds or vegetative materials like stem, tubers, and rhizomes (Hallmann et al., 1997). Endophytes are known to influence vital activities of host plants like promoting plant growth, promoting defense response against pathogens, and acting as remediators of abiotic stress (Khare et al., 2018).

Endophytic bacteria can promote plant growth either directly or indirectly. Endophytes can facilitate the acquisition of resources from the environment including nitrogen, phosphorus, and iron. They can modulate plant growth by providing or regulating plant hormones like auxin, cytokinin, or ethylene. Indirect plant growth promotion can occur when endophytic bacteria reduce infection by other pathogenic bacteria, fungi, and nematodes. Mechanisms include the production of antibiotics, cell wall degrading enzymes, lowering plant ethylene levels, induced systemic resistance, decreasing the amount of iron available to pathogens, and synthesis of pathogen inhibiting volatile compounds (Glick, 2015; Santoyo et al., 2016).

Since many endophytic bacteria help plants in nutrient acquisition and defense against pathogens, endophytes are considered an alternative to replace or reduce the use of fertilizers and pesticides. Nitrogen is one of the most important yield-limiting factors in agricultural crops. The excessive and imbalanced use of fertilizers for decades has

contributed to greenhouse gas emissions (N_2O) and underground water leaching. The nitrogen-fixing bacteria can provide an alternative to use of nitrogenous fertilizer. In leguminous crops, biological nitrogen fixation provides a substantial amount of nitrogen for the plant. Nitrogen-fixing bacteria can co-exist as an endophyte within non-legumes. Many non-leguminous crops like rice, sugarcane, wheat, and maize form an extended niche for various species of nitrogen-fixing bacteria (Bhattacharjee et al., 2008). When non-leguminous plants are inoculated with endophytic bacteria, the nitrogen accumulation in plants can be due to the results of biological nitrogen fixation or through increased nitrogen uptake from soil (Bhattacharjee et al., 2008). Some Brazilian sugarcane cultivars are capable of obtaining as much as 60% of nitrogen through biological nitrogen fixation and rice cultivars are capable of obtaining 30-60 kg N Ha^{-1} depending on the cultivar (Boddey et al., 1995). Some of the other benefits provided by endophytes to plants include osmotic adjustment, stomatal regulation, modification of root morphology, enhanced uptake of minerals and alteration of nitrogen accumulation and metabolism (Compant, Duffy, et al., 2005).

Endophytes are known to enhance root growth and root branching which further lead to an increase in plant growth. These positive effects of endophytes on root growth and branching are considered to be the consequence of the production of growth regulators by endophytes; however, enhanced nutrient acquisition by microbes may equally contribute to the enhanced plant growth (Compant et al., 2010; Irizarry & White, 2018; Kandel et al., 2017; White et al., 2019). Seed inoculation with endophytes (*Bacillus amyloliquefaciens*), isolated from non-cultivated plants growing in stressful environments, can promote growth, alter root architecture and alleviate salt stress in

cotton and okra seedlings (Irizarry & White, 2017). Bacterial isolates of *Bacillus* species have been shown to enhance root length, shoot length, and number of lateral roots of soybean seedlings (Wahyudi et al., 2011). Some *Bacillus* isolates were able to stimulate the primary root growth and lateral root developments while other isolates were able to promote lateral root formation in *Arabidopsis thaliana* (Gutiérrez-Luna et al., 2010). *Bacillus megaterium* can also alter the root system architecture of *Arabidopsis thaliana. B. megaterium* inoculation caused inhibition of primary root growth and an increase in lateral root number, lateral root growth, and root hair length. Reduction in cell elongation and reduction of cell proliferation in root meristem resulted in inhibition of primary root growth (López-Bucio et al., 2007).

Genotype specific effects of endophytes on plant growth have been reported. Significant genotype-by-endophyte infection interactions on rye grass growth and storage traits have been observed (Cheplick & Cho, 2003). Some genotypes showed enhanced tiller base mass while others showed decreased tiller base mass. The set of genotypes with decreased tiller base mass also showed decreased root area and root mass when infected with endophytes (Cheplick & Cho, 2003). Genotypic differences were also observed when wheat genotypes were inoculated with *Klebsiella pneumoniae* 342 (Iniguez et al., 2004) and *Herbaspirillum seropedicae* (Neiverth et al., 2014). The nitrogen difficieny symptoms were relieved in one cultivar but not in the other two evaluated when inoculated with a strain of *Klebsiella pneumoniae*. Inoculation of wheat plantlets with *Herbaspirillum seropedicae* resulted in an increase in root hairs and provided nitrogen in one wheat cultivar but not in others (Neiverth et al., 2014). Rice genotypes are also known to have a contrasting response to different endophytic bacteria. Two rice cultivars

responded differently for lateral root development and expression of ethylene receptors when inoculated with *Azospirillum brasilense* sp245 and *Burkholderia kururiensis* M130 (Vargas et al., 2012). Differential response of cultivars to endophyte infection has also been reported in maize. When seeds of two maize cultivars were inoculated with 15 diverse bacterial strains, a significant interaction between maize cultivars and inoculation treatment on dry root and shoot biomass was observed (Montañez et al., 2012). Maize cultivars were able to obtain a significant amount of nitrogen from biological nitrogen fixation, but this was dependent on the cultivar and the nitrogen fertilization level (Montañez et al., 2009). There is limited information available on the response of oats to endophytic bacteria. Identifying unique endophyte-oat relationships would help develop oat cultivars with a higher affinity for endophytic colonization and growth response to endophytic colonization. This could result in higher yield under organic management systems and reduce the application of chemical fertilizers in conventional systems. The objective of this study is to examine the potential of bacterial endophytes to enhance growth in oats and evaluate the response of various oat cultivars to endophytic inoculation under varying level of nitrogen fertilization.

Materials and methods

1. Root vigor assay

Surface sterilized seeds of oat cultivars Hayden and Gopher were treated with a suspension of 16 species of endophytic bacteria along with uninoculated control (Table 2.1). The sixteen endophytes used in this study were isolated from *Brassica carinata*. The bacterial isolates were tested for their ability to fix nitrogen and all isolates were able to

fix nitrogen (Peta, 2020). The seeds were first surface sterilized with a 5% solution of sodium hypochlorite. The surface sterilized seeds were placed in 15ml tubes and the bacterial suspension was added to the tube and shaken for about a minute to coat the seeds with bacteria. The seeds were treated at the rate of 2 µl of bacterial suspension (0.05 ocular density measured at 600nm wavelength) per seed. The bacteria inoculated seeds (15 per genotype) were placed in a line between four sheets of heavyweight germination paper with 50 mL of distilled water in a petri dish. The petri dishes were stacked randomly in a growth chamber maintained at 25^{o}C with a 16-hour photoperiod. The plates were kept in semi-vertical position. Roots were scanned after 6 days. To scan the roots, the top paper was removed, and the roots were pinched off from each seedling and scanned using an Epson flatbed scanner (Epson America, Inc. Los Alamitos, CA). The scanned images of the root were run through the WhinRhizo software (V5.0, Regent Instruments, Quebec, Canada) to measure the root length, root surface area, and root volume. The experiment was conducted twice using a complete randomized design. Data from the two repetitions were combined for data analysis. The experimental design was a factorial design with 17 × 2 treatments, in which two genotypes were evaluated with 16 bacterial isolates and an uninoculated control. Analysis of variance was conducted with R statistical program (R Core Team, 2020). Least significant difference (LSD) was conducted to test differences between treatments using agricolae package in R (Mendiburu, 2021)

Table 2.1 List of endophytic bacteria used for the root vigor assay.

SDSU name	Deposited ref
SDSU-BC-02-2013	*Bacillus licheniformis*
SDSU-BC-03-2013	*Enterobacter kobei*
SDSU-BC-04-2013	*Pantoea ananatis*
SDSU-BC-06-2015	*Enterobacter kobei*
SDSU-BC-07-2015	*Bacillus pumilus*
SDSU-BC-08-2015	*Pantoea agglomerans*
SDSU-BC-09-2015	*Brevibacterium halotolerans*
SDSU-BC-10-2015	*Bacillus toyonensis*
SDSU-BC-12-2015	*Bacillus pumilus*
SDSU-BC-13-2015	*Bacillus pumilus*
SDSU-BC-14-2015	*Bacillus thuringiensis*
SDSU-BC-15-2015	*Bacillus cereus*
SDSU-BC-16-2015	*Bacillus aryabhattai*
SDSU-BC-17-2015	*Lysinibacillus fusiformis*
SDSU-BC-19-2015	*Brevibacterium halotolerans*
SDSU-BC-20-2013	*Pseudomonas spp.*

2. Greenhouse study

Ten oat cultivars Deon, Goliath, Gopher, Hayden, Horsepower, Natty, Saddle, Shelby 427, Sumo and Warrior; and two endophytic bacteria, *Bacillus licheniformis* (BC02) and *Enterobacter kobei* (BC06) were used for this experiment.Seeds were surface sterilized by stirring them in a 200 ml of 5% solution of sodium hypochlorite for 10 minutes and then seeds were rinsed with sterile water. The surface sterilized seeds were inoculated with the bacterial suspension (with 0.05 ocular density measured at wavelength of 600nm) at a concentration of 2 µl per seed. A sand perlite (60:40) mixture was used to grow plants in cone containers. The experiment was conducted in complete randomized design with 7 replications per treatment. There were 10 genotypes, two bacterial treatments along with a control and two levels of fertilization. Seven plants were maintained per treatment resulting in a total of 420 plants. Two seeds were sown per cone

and thinned to one seedling per cone after germination. The plants were irrigated with Hoagland solution every other day and 50 ml of solution was given to each plant. Two sets of nutrient solutions were prepared to irrigate the plants with the two doses of nitrogen. One set of plants were irrigated with full strength Hoagland's solution to give 100% nitrogen application another set of plants were irrigated with half-strength Hoagland's solution that contained only 50% of the nitrogen based on Hoagland's solution recipe (Hoagland & Arnon, 1950). At 42 days after planting, chlorophyll content was measured using a SPAD 502 chlorophyll meter. The roots were cleaned with water and the cleaned roots were scanned with an Epson flatbed scanner. The scanned images were run through WinRhizo software to determine root length, root surface area, and root volume. The root and shoot were dried to determine dry root and dry shoot weight. Analysis of variance was conducted with R (R Core Team, 2020). Least significant difference (LSD) was conducted to test differences between treatments using agricolae package in R (Mendiburu, 2021).

Results

1. Effect of endophytes on root development in oat seedlings (root vigor assay)

To screen the growth promoting ability of a set of 16 endophytic bacteria on oats (Table 2.1), a root vigor assay was performed. The effect of endophytic treatment was evaluated on oat cultivars Hayden and Gopher by measuring root characteristics on 6-day-old seedlings. All factors including oat genotype, endophyte isolate, and their interaction had a significant effect on the total root length, root area, and root volume of oat seedlings (Supplementary Table 2.1).

After 6 days, cultivar Gopher had developed seedlings with significantly larger roots than Hayden whether the seeds were inoculated with endophytes or not. When compared to the non-inoculated checks, the total root length of Gopher seedlings was approximately 37% longer than those of Hayden, and the root area and root volume were approximately 16% larger than those of Hayden (Table 2.2).

Because of the significant interaction between genotype and endophyte isolates, data analysis was performed for each cultivar separately. For cultivar Gopher, seed inoculation with six of the sixteen endophyte isolates tested (BC02, BC03, BC09, BC15, BC16, and BC17), resulted in seedlings with significantly higher root length and root area in comparison to the non-inoculated control (Fig. 2.1). Three of those isolates (BC02, BC03, and BC09) also resulted in higher root volume when Gopher seeds were inoculated with them. Endophytic treatment with isolates BC02 and BC03 increased the root length of Gopher seedlings by 34 and 27%, respectively; the root area by 33 and 23%, respectively; and the root volume by 30 and 17%, respectively. Isolate BC12 significantly increased root length but had no effect on root area and root volume. The other nine bacterial isolates (BC04, BC06, BC07, BC8, BC10, BC13, BC14, BC19, and BC20) had no significant effect on root length, root area, and root volume.

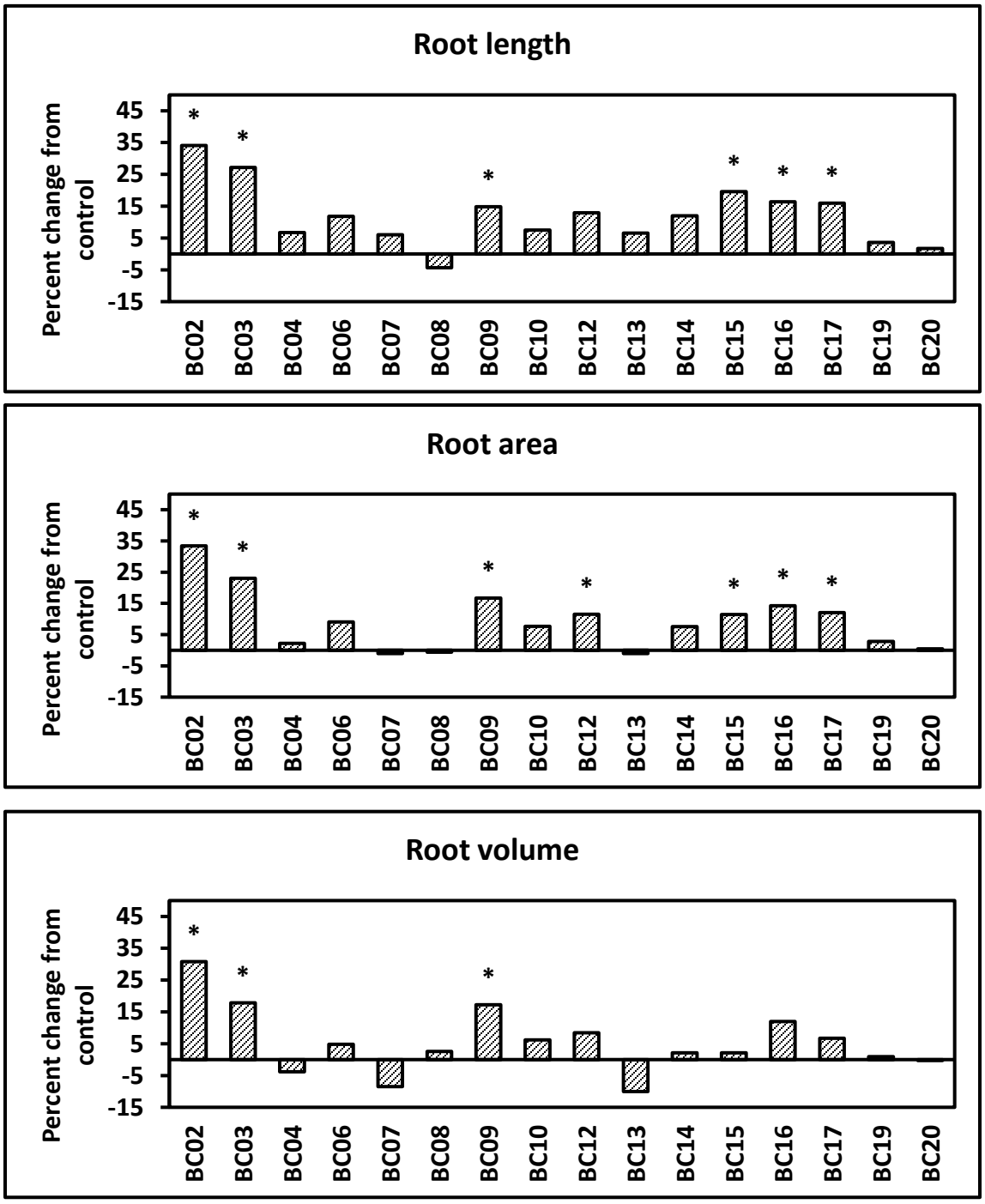

Figure 2.1 Percent change in root length, root area, and root volume when inoculated with endophytic bacteria compared to noninoculated control in Gopher

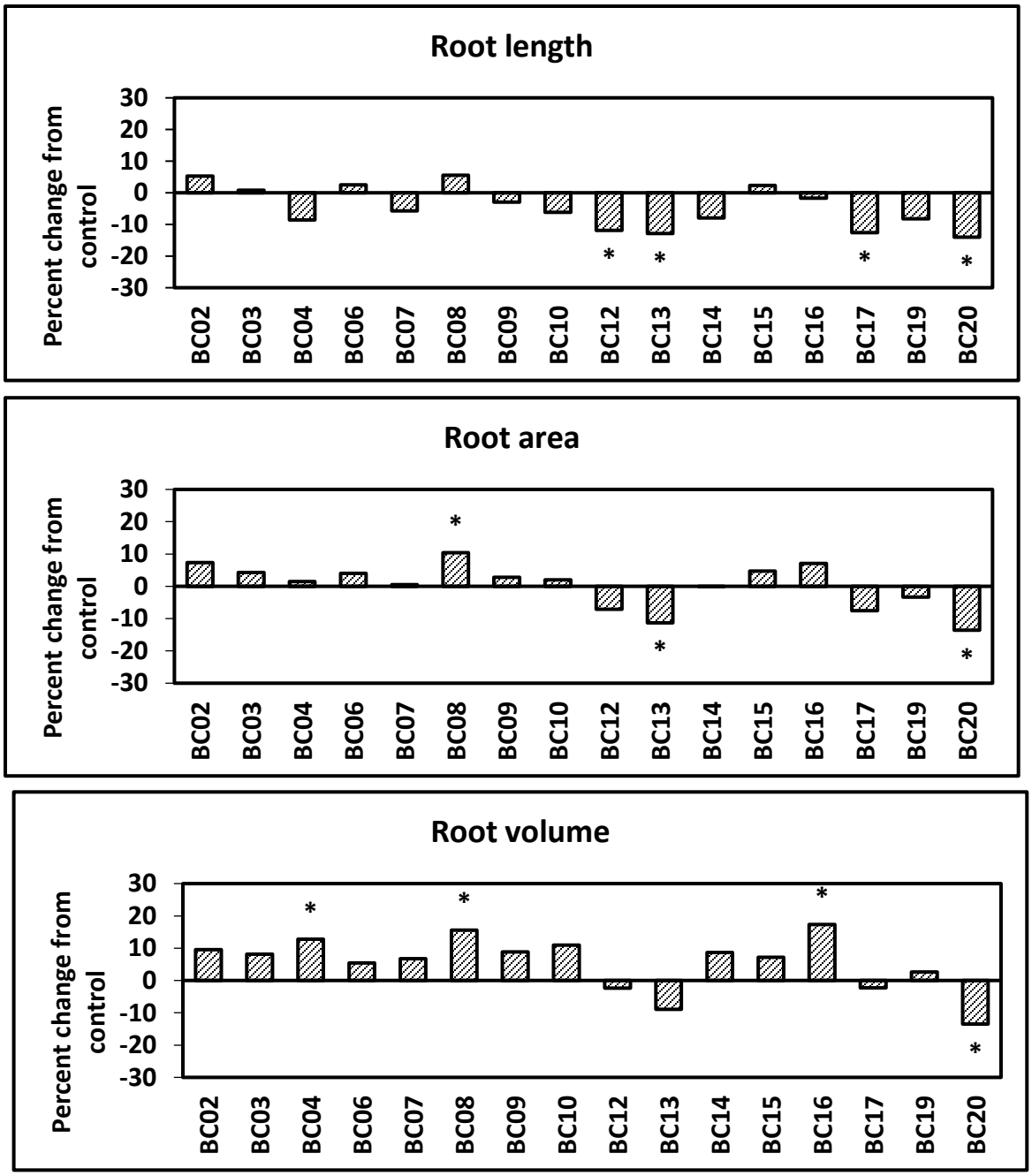

Figure 2.2 Percent change in root length, root area, and root volume when inoculated with endophytic bacteria compared to noninoculated control in Hayden.

For oat cultivar Hayden, however, with a few exceptions, inoculation with endophytic bacteria had no significant effect on root characteristics. Twelve out of sixteen isolates had no effect on root length while 4 isolates significantly reduced root length (Fig. 2.2). Only inoculation with isolate BC08 resulted in increased root area and root volume as compared to the non-inoculated Hayden control. Isolates BC16 and BC04 resulted in an increase in root area but had no significant effect on root length and root volume (Fig. 2.2). The response to endophytic inoculation on root growth was more pronounced for oat cultivar Gopher than for Hayden. Only isolate BC16 had some positive effects on root growth across both genotypes. In contrast, isolate BC20 had no effect on the root growth of Gopher seedlings but significantly inhibited root growth for Hayden.

Table 2.2 Average total length, area, and volume of roots of Gopher and Hayden seedlings in root vigor assay across bacterial treatment and noninoculated control.

Genotype	Total root length (cm)	Root area (cm^2)	Root volume (cm^3)
Gopher	38.50 a	7.34 a	0.1139 a
Hayden	28.21 b	5.84 b	0.0978 b

Values followed by different letters in a column are significantly different ($p<0.05$).

2. **Effect of endophytes on root and shoot growth of oat cultivars in the greenhouse.**

For this experiment, a larger set of oat cultivars was considered (ten). The effect on root and plant growth was evaluated at a later stage of plant development (panicle initiation stage with the first spikelet of inflorescence just visible). Two nitrogen levels were considered because the response to endophytic treatment is expected to be higher under

limited nutrient availability (Smith, 1992). To keep the number of experimental units to a manageable level, only two endophyte isolates were considered for this experiment, *Bacillus licheniformis* (BC02) and *Enterobacter kobei* (BC06). Overall mean, range, and standard deviation for each trait under 50% and 100% nitrogen application are shown in table 2.3.

Table 2.3 Mean, range, and standard deviation for biomass and root traits of oat plants grown at two nitrogen levels.

	50% Nitrogen application			100% Nitrogen application		
Traits	Mean	Range	Standard deviation	Mean	Range	Standard deviation
Shoot dry weight (mg)	736.3	163 -1437	208.6	906.6	293-1608	227.7
Root dry weight (mg)	200.5	68 -400	57.6	231.9	97-452	61.8
Chlorophyll content	52.4	35.1 -69.4	5.7	56.3	34-73.4	5.6
Root length (cm)	469.8	178.9 -836.2	116.5	517.7	156.2-859.1	117.7
Root area (cm^2)	118.3	53.2 -189.2	24.0	130.8	42.7-217.9	25.5
Root volume (cm^3)	2.45	1.02 -5.38	0.72	2.72	0.93-5.81	0.83

Considerable variation was observed among the oat genotypes for the traits evaluated without endophyte inoculation. Gopher had the largest root system (Table 2.4), while Deon, had the smallest root system. Natty had the highest shoot dry weight while Deon had the lowest shoot dry weight. Hayden has the highest root dry weight and Saddle had the lowest root dry weight. Chlorophyll content was higher for Sumo and lower for Horsepower (Table 2.4).

Table 2.4 Mean values of each trait for ten cultivars under noninoculated conditions.

Genotype	Shoot dry weight (mg)	Root dry weight (mg)	Chlorophyll content	Root length (cm^2)	Root area (cm^2)	Root volume (cm^3)
Deon	681.4 b	188.2 de	55.4 ab	407.8 d	104.9 f	2.17 de
Goliath	761.1 ab	211.4 cd	53.9 abcd	514.4 b	134.9 b	2.91 ab
Gopher	806.9 a	222.8 bc	50.4 ef	649.4 a	159.6 a	3.19 a
Hayden	769.8 ab	262.6 a	55.2 abc	486.8 bc	129.0 bc	2.93 ab
Horsepower	788.1 ab	198.0 cde	49.5 f	518.4 b	125.8 bcd	2.50 cd
Natty	866.9 a	243.3 ab	52.4 bcdef	483.8 bc	114.5 def	2.20 de
Saddle	770.6 ab	158.1 f	52.9 abcde	508.5 bc	121.4 cde	2.36 cde
Shelby427	681.6 b	177.6 ef	51.0 def	450.2 cd	109.5 ef	2.18 de
Sumo	810.0 a	175.4 ef	55.8 a	484.8 bc	110.2 ef	2.08 e
Warrior	807.7 a	201.4 cde	52.4 cdef	466.1 bcd	123.7 bcd	2.70 bc
Mean	774.4	203.9	52.9	497.0	127.18	2.52
CV	28.13	26.14	11.04	23.6	19.15	29.70

An analysis of variance was conducted to determine the effect of each factor. The effect of the cultivar and nitrogen treatments were significant on all traits (shoot dry weight, root dry weight, chlorophyll content, root length, root area, and root volume). The bacterial isolate had a significant effect on shoot dry weight, root dry weight, and chlorophyll content but not on root length, area, and volume. Significant interactions between the three main factors (genotype, nitrogen, and bacteria) were observed for all traits except chlorophyll content. The interaction between genotype and bacteria was also significant for all traits. The interaction between genotype and nitrogen was significant for root length and root area. The interaction between bacteria and nitrogen was significant for chlorophyll content. The response to endophyte inoculation varied depending on the trait considered, the oat genotype, the bacterial isolate, and the nitrogen fertilization level. Due to those complex interactions between factors, the effect of the endophyte treatments on oat root and shoot growth was analyzed at each fertilizer level and for each bacterial isolate.

Response of oat genotypes to endophyte inoculation under 100% N application

The response (as compared to the non-inoculated check) in shoot dry weight, root weight, chlorophyll content, and root length, area, and volume of the ten oat cultivars following bacterial inoculation are presented in Figures 2.3 and 2.4 for BC02 and BC06, respectively. Under full nitrogen rate, inoculation with BC02 isolate significantly increased the shoot dry weight in five cultivars (Deon, Hayden, Natty, Saddle, and Sumo) but significantly decreased shoot dry weight for cultivar Goliath (Fig 2.3). Inoculation with BC06 also significantly increased shoot dry weight in Deon, Gopher, and Saddle but significantly reduced shoot dry weight for Warrior (Fig 2.4). Only two cultivars (Deon and Saddle) showed an increase in shoot dry weight across bacterial treatments (BC02 and BC06).

Inoculation with bacterial isolate BC02 also resulted in an increase in root dry weight for four cultivars (Deon, Natty, Saddle, and Sumo) but significantly decreased root dry weight for cultivar Warrior (Fig. 2.3). Inoculation with BC06 significantly increased root dry weight in four cultivars (Natty, Saddle, Shelby, and Sumo). Three cultivars out of the ten evaluated showed an increase in root dry weight for both bacteria treatments.

Inoculation with BC02 significantly increased chlorophyll content in Horsepower, Natty, Sumo, and Warrior, while inoculation with BC06 significantly increased chlorophyll content in Horsepower and Saddle. Only Horsepower showed a consistent increase in chlorophyll content across bacterial treatments(Fig 2.3).

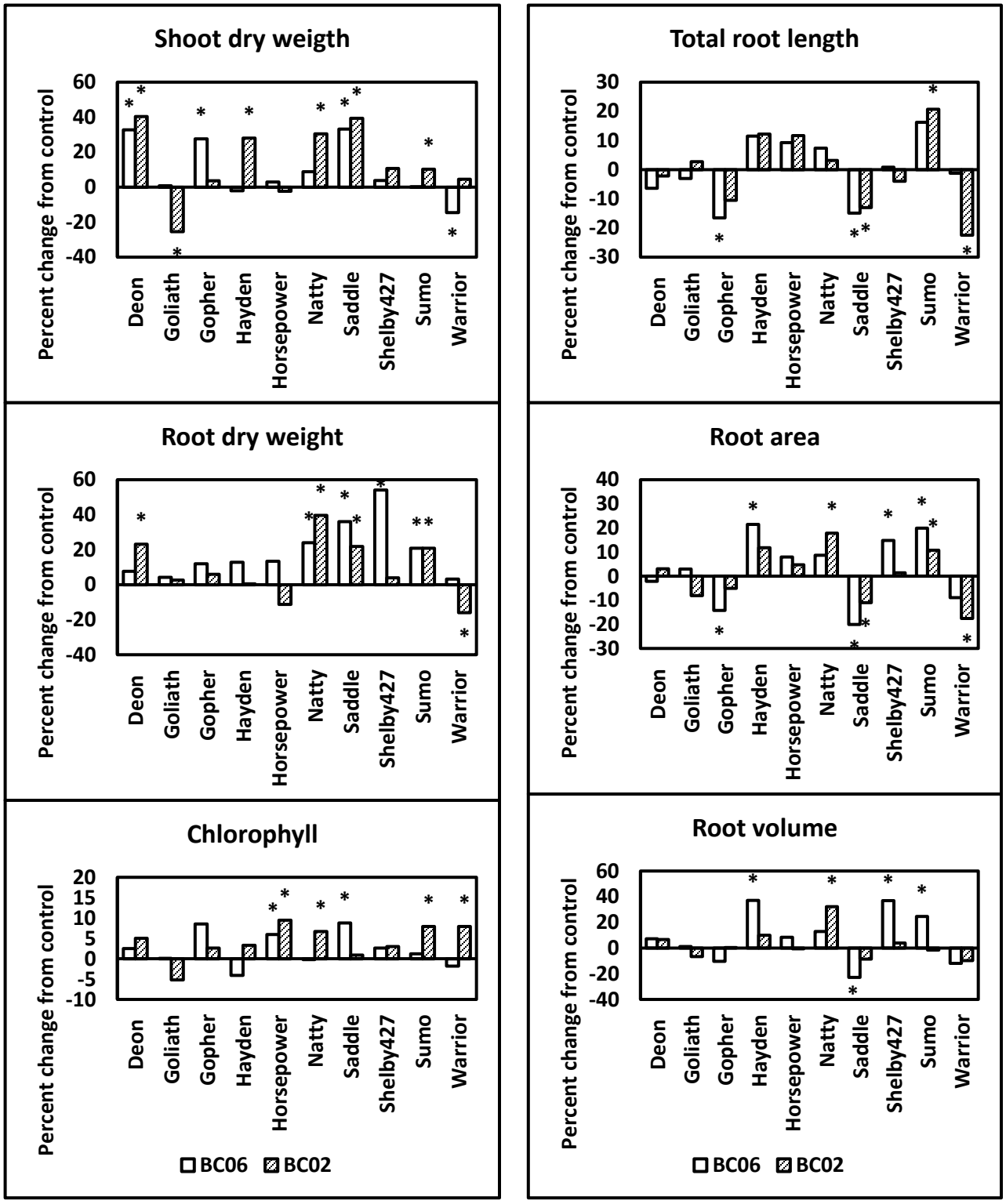

Figure 2.3 Percent change in shoot dry weight, root dry weight, chlorophyll content, root length, root area, and root volume when inoculated with BC02 and BC06 compared to noninoculated control under 100% nitrogen application

Inoculation with BC02 significantly increased root length in Sumo, but significantly decreased root length in Saddle and Warrior (Fig. 2.3). Inoculation with BC06 did not increase root length significantly in any of the cultivars, however significantly decreased root length in Gopher and Saddle. Interestingly, inoculation of cultivar Saddle with either bacterial isolate led to the development of plants with higher root dry weight but with lower total root length in comparison to the non-inoculated control.

Inoculation with BC02 significantly increased root area in Natty and Sumo, and significantly decreased root area in Saddle and Warrior (Fig. 2.3). Similarly, BC06 inoculation significantly increased root area in Hayden, Shelby427, and Sumo, and significantly decreased root area in Gopher and Saddle. The same response was observed across isolates for Saddle (decrease in root area) and Sumo (increase in root area).

Inoculation with BC02 resulted in an increase in root volume in only one cultivar, (Natty); the response to inoculation with bacterial endophyte was not significant for any of the other cultivars. Inoculation with BC06 significantly increased root volume in three cultivars (Hayden, Shelby427, and Sumo), and decreased root volume in Saddle.

Response of oat genotypes to endophyte inoculation under 50% N application

When inoculated seeds were grown under 50% nitrogen regime, the response in shoot dry weight, root weight, chlorophyll content, and root length, area, and volume of the ten oat cultivars differed from the full nitrogen regime. Inoculation with BC02 significantly increased shoot dry weight for half of the cultivars (Goliath, Natty, Saddle, Sumo, and

Warrior) (Fig. 2.4). Inoculation with BC06 significantly increased shoot dry weight for three genotypes (Goliath, Horsepower, and Saddle) but reduced shoot dry weight for two genotypes (Deon, and Sumo) (Fig 2.4). For Goliath and Saddle, seed inoculation with endophytes resulted in an increase in shoot dry weight, irrespective of the bacterial treatment (BC02 or BC06). For Sumo, the response to endophyte inoculation on shoot dry weight was in opposite direction depending on the bacterial isolate, when inoculated with BC02, Sumo showed a significant increase in shoot dry weight, but when it was inoculated with BC06, shoot dry weight was reduced in comparison to the non-inoculated Sumo. Root dry weight significantly increased for four cultivars (Deon, Gopher, Sumo, and Warrior) but significantly decreased for Hayden as a result of seed inoculation with BC02 (Fig 2.4). Inoculation with BC06 resulted in a significant increase in root dry weight for Goliath and Horsepower but resulted in reduced root dry weight for Hayden (Fig. 2.4). Inoculation of cultivar Hayden with endophytes resulted in a reduction in root dry weight for both isolates. Inoculation with BC02 increased the chlorophyll content in Deon and Hayden (Fig. 2.4). Inoculation with BC06 significantly increased chlorophyll content in five cultivars (Gopher, Hayden, Horsepower, Saddle, and Sumo) (Fig. 2.4). Hayden is the only cultivar that showed a significant increase in chlorophyll when inoculated with either isolate.

Inoculation with BC02 significantly increased root length in Deon and Warrior and decreased root length in Gopher (Fig. 2.4). Inoculation with BC06 resulted in significantly higher total root length for Natty but reduced total root length for Gopher and Hayden (Fig. 2.4). Gopher showed a decrease in root length with both BC02 and BC06 inoculation under 50% nitrogen regime.

Inoculation with BC02 resulted in an increase in root area for four of the cultivars (Deon, Goliath, Sumo, and Warrior) but a decrease in root area for two of the cultivars (Gopher and Hayden) (Fig. 2.4). Inoculation with BC06 resulted in a significant increase in root area for Deon and Natty but a decrease in root area for Gopher (Fig. 2.4). Seed inoculation with endophytes resulted in a reduction in root area for oat cultivar Gopher irrespectively of the isolate used for inoculation (BC02 or BC06).

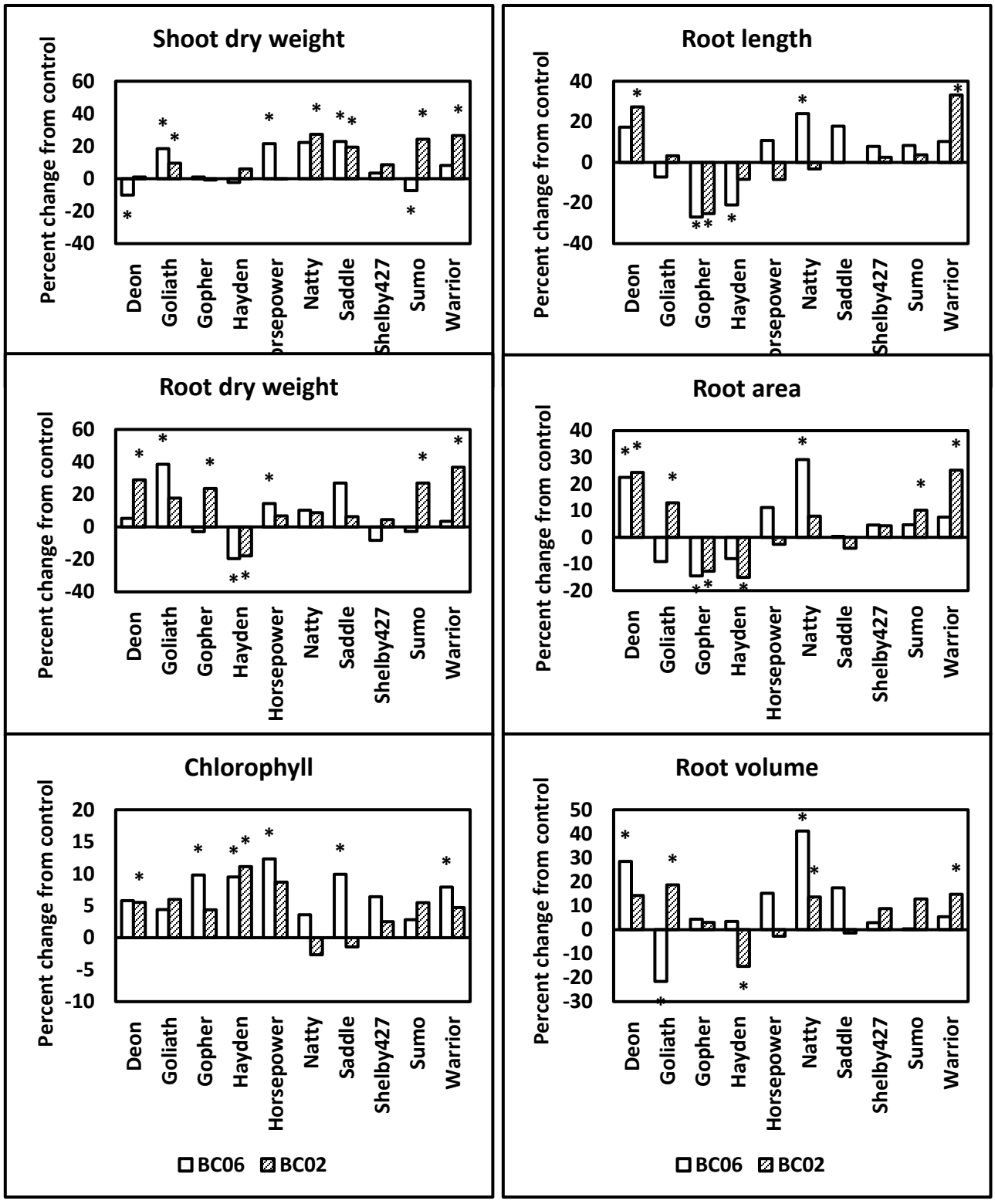

Figure 2.4 Percent change in shoot dry weight, root dry weight, chlorophyll content, root length, root area, and root volume when inoculated with BC02 and BC06 compared to noninoculated control under 50% nitrogen application

Inoculation with BC02 resulted in an increase in root volume for Goliath, Natty, and Warrior, however, the opposite effect (a reduction in root volume) was observed for Hayden. Inoculation with BC06 resulted in an increase in root volume for Deon and Natty, but in a smaller root volume for Goliath. The root volume of Goliath was affected in opposite directions (increase or reduction in volume) depending on the bacterial isolate used for inoculation.

3. Relationships among traits measured:

Significant positive correlations were observed between the traits measured (Fig. 2.5). Shoot dry weight has significant positive correlation with all traits except root volume. Root dry weight and root length were positively correlated with all other traits measured. As expected, the strongest correlations were observed between root area and root volume (r= 0.8) and between root length and root area (r=0.68) (Fig. 2.5).Genotype by trait biplot is shown in Figure 2.6. The biplots usually provide information about the relation between traits measured and the traits profile for the genotypes. Traits with positive correlation have an acute angle between their trait vectors. All the root traits have an acute angle between them (Fig. 2.6). The angle between the vector for a genotype and a vector for a trait indicates the relative level of the genotype for that trait. An acute angle indicates that the genotype is above average for that trait; an obtuse angle indicates that the genotype is below average for that trait and right angle indicate that the genotype is average for the trait; vector length of traits indicate how well a trait is represented in the biplot (Yan & Frégeau-Reid, 2018). First principal component (PC1) accounted for 50.5% of the variation and second principal component (PC2) accounted for 19.6% of the

variation. The goodness of fit of the biplot (Fig. 2.6) is 70%. Based on the vector length of each trait, the variation of shoot dry weight and other root traits are well represented in the biplot. The shorter vector length of chlorophyll content shows that variation of chlorophyll across genotypes is relatively small. The genotypes are scattered on the biplot and do not form distinct group indicating each genotype has a different trait profile. Gopher has higher root area; root length, and root volume and lower chlorophyll content while Warrior has the opposite trait profile .

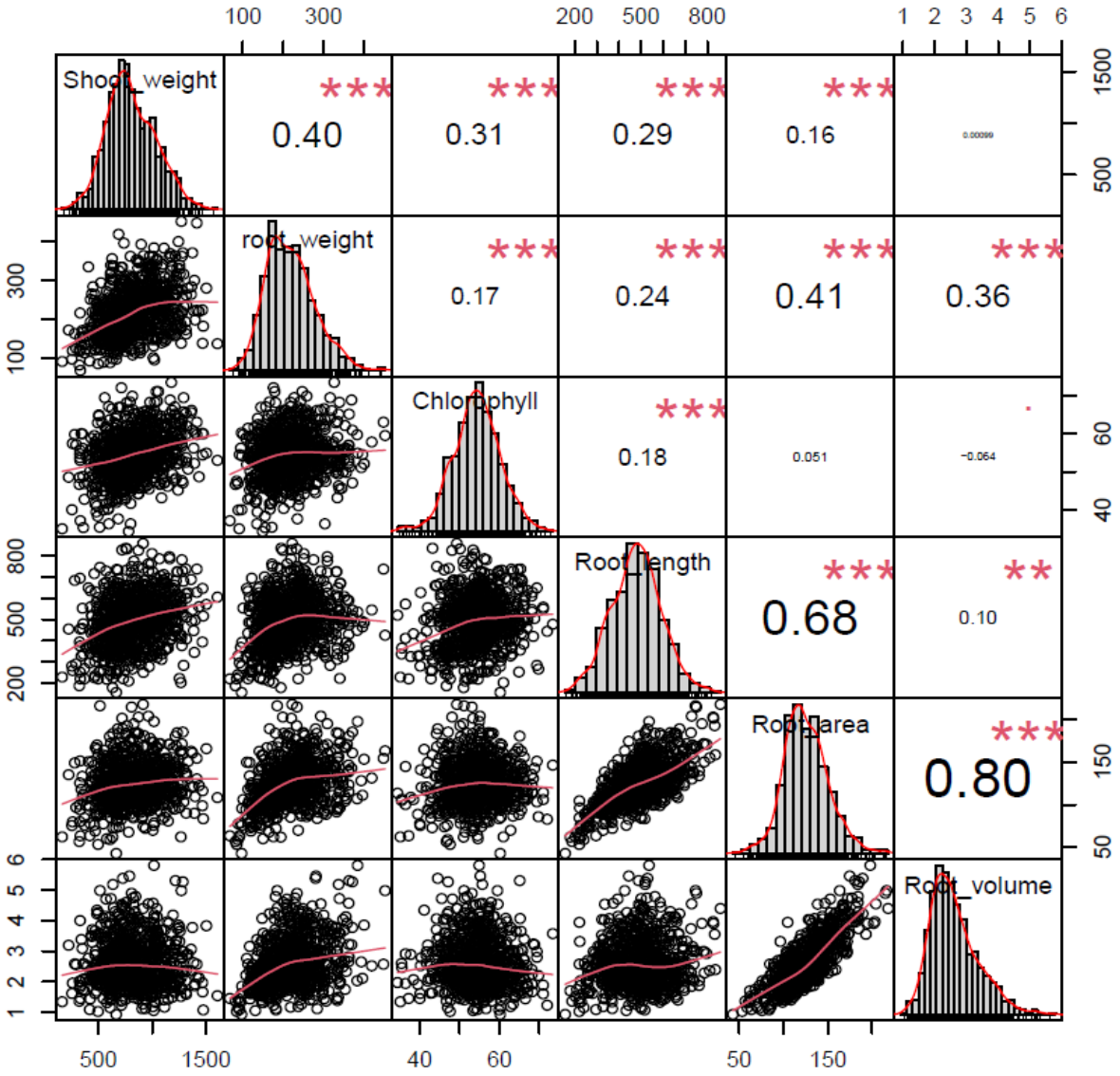

Figure 2.5 Correlation matrix of different traits. Scatter plots are shown in the lower left quadrant, and values in the upper right quadrant are Pearson's correlation coefficients.

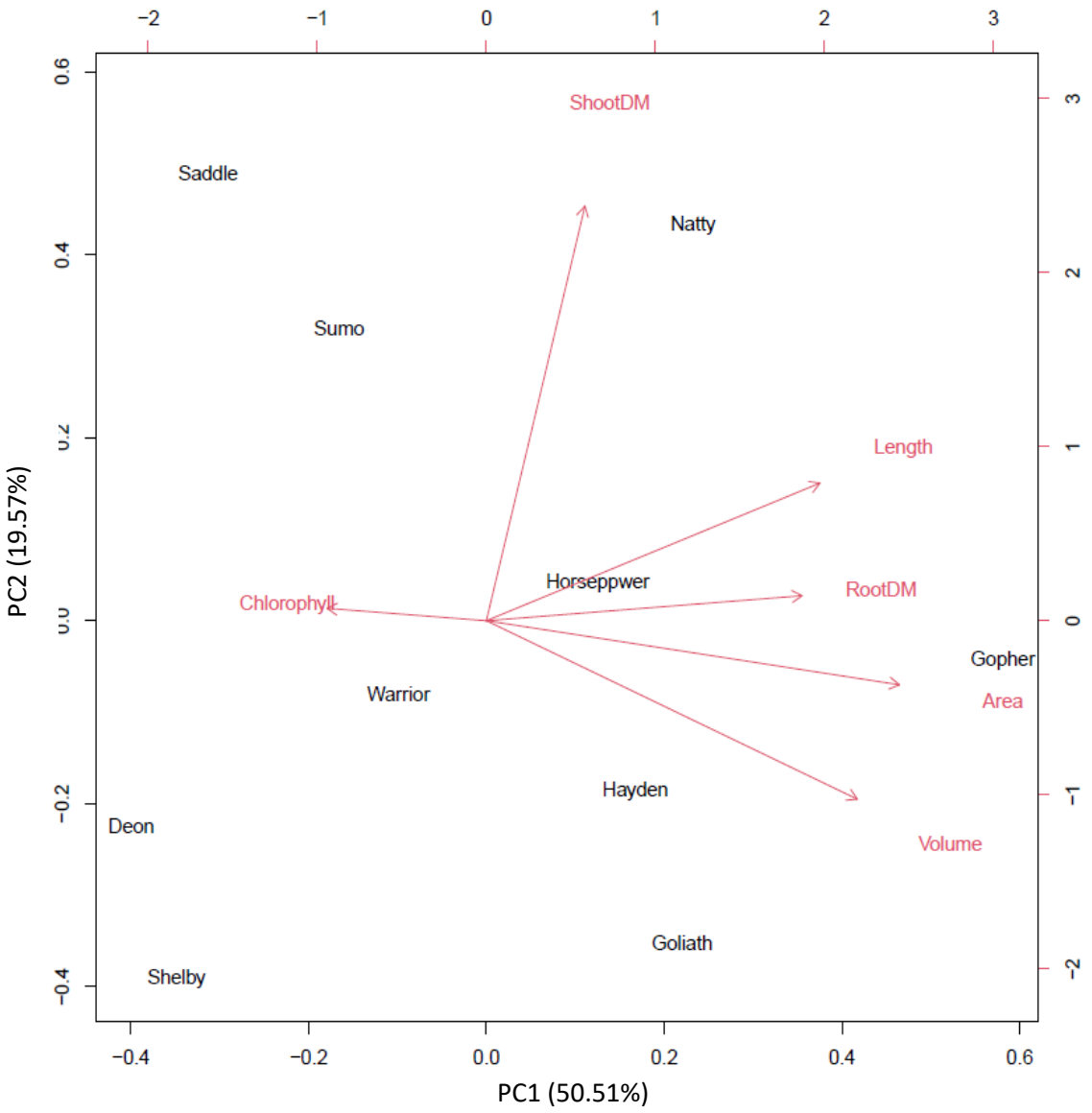

Figure 2.6 Genotype by traits biplot.

Discussion

In this study, we examined the potential of endophytic bacteria for plant growth promotion. Results from the root vigor assay indicate that the endophytes inoculation can have positive effects on total root length, root area, and root volume depending on the bacterial isolate and oat genotype. There was significant interaction between endophytic bacteria and oat cultivar, thus, changes in plant and root development associated with plant colonization by bacterial endophyte depend on the specific combination of the bacterial isolate and the plant genotype. In our root vigor study, endophytes isolates that significantly increased either total root length, root area, or root volume belong to the following species *Bacillus licheniformis, Enterobacter kobei, Brevibacterium halotolerans, Bacillus cereus, Bacillus aryabhattai.* These endophytes are known to have positive effects on growth of Groundnut, Lentil, Wheat, Red pepper, Soybean, Corn, and Spinach (Cakmakci et al., 2007; Goswami et al., 2014; Joo et al., 2004; Midekssa et al., 2015; Mumtaz et al., 2017; Ramesh et al., 2014). In our root vigor study, seed inoculation with *Bacillus cereus* (BC15) enhanced total root length and root area in comparison to non-inoculated control, similar results have been reported by Cakmakci et al. (2007) where inoculation with *Bacillus cereus* resulted in significantly higher shoot fresh weight, total root number, and shoot dry weight, and plant height compared to non-inoculated control.

Seed inoculation with *Bacillus aryabhattai* (BC16) promoted significantly higher root area and root volume compared to non-inoculated control. This growth promoting effect of *Bacillus aryabhattai* has also been observed in soybean and wheat (Ramesh et al., 2014).. Many endophytic bacteria are known to produce growth-promoting hormones

such as gibberellins and indole acetic acid (Joo et al., 2004; Khan et al., 2014). Joo et al. (2004) reported that the growth of red pepper seedlings was increased by all three tested bacteria (*Bacillus cereus* MJ-1, *B. macrolides* CJ-29, and *B. pumilus* CJ-69). The bacteria were able to produce GAs, thus the growth of red pepper may be promoted by the hormones produced by the bacteria. Tomato plants inoculated with gibberellins and indole acetic acid producing *Sphingomonas,* significantly increased growth parameters like shoot length, chlorophyll contents, shoot, and root dry weights compared to the control (Khan et al., 2014).

The oat cultivars evaluated in our study responded differently to endophytic treatment. In the root vigor assay, Gopher showed an increase in root length, root area and/or root volume with seven endophytic bacterial treatments, however, Hayden showed a decrease in root length with four endophytes and an increase in root area and/or root volume with three endophytic bacteria. Gopher is a selection from Sixty Day (Coffman, 1977) and was released by the University of Minnesota in 1923 (GRIN-Global, NPGS). Sixty Day was introduced from Russia in 1901 (Coffman, 1977). Hayden is a modern oat cultivar released by the South Dakota Experimental Station in 2014 (Caffe-Treml et al., 2017). The genetic background and breeding procedure is different for these two cultivars. Many researchers have reported genotype specific effects of endophytes in wheat, maize, and rice (Iniguez et al., 2004; Montañez et al., 2009; Neiverth et al., 2014; Vargas et al., 2012). Thus, oat cultivars can show specific response to endophytic bacteria with some cultivars being more responsive than others.

In our greenhouse study, the magnitude and direction of endophyte effects on oat growth varied with nitrogen levels and differed between oat genotypes. Plant growth response

was variable and dependent on growth parameter evaluated. Similar results have been reported in previous studies where variable response to endophytes inoculation were observed dependent on the inoculant strain, plant species (northern oat grass, wheat, and spinach), and growth parameter evaluated (Buckley et al., 2019; Cakmakci et al., 2007; Hughes et al., 2020). Some cultivars are more sensitive to nitrogen application than others and the nitrogen application level can influence the response of the oat genotype to endophyte inoculation. While the response of oat cultivars to endophyte inoculation was different for different traits, a similar response was seen for the traits that were highly correlated. For most cultivars, they showed similar responses for total root length, root area, and root volume. While the plant growth response to endophytic treatments can be due to phenomenon like nutrient acquisition, and synthesizing plant hormones; these phenomena are plant-genotype interaction specific (Khare et al., 2018). Since we observed an increase in shoot dry weight in some cultivars while other cultivars showed a decrease in shoot dry weight or no significant change, one of the reasons for these discrepancies in response may be because the oat cultivars may have different capabilities to support nitrogen fixation by endophytes. Maize cultivars are known to differ in their capabilities to support nitrogen fixation and their capacity to fix nitrogen was affected by nitrogen fertilization level (Montañez et al., 2009).

Inoculation of plants with auxin producing bacteria can enhance the root growth. The stimulation of root growth by rhizobacteria is considered to be associated with their capacity to synthesize indole acetic acid (IAA) (Spaepen & Vanderleyden, 2011). IAA is the most common naturally occurring plant hormone of auxin class and one of the best-known effects of auxin in the stimulation of rhizogenesis. Thus, the promotion of root

traits in this study may be due to IAA production by endophytes. The ability of plant roots to exude flavonoids and IAA can impact the plant colonization by endophytes and thus impact the overall plants' response to endophyte inoculation. Different oat cultivars may have different flavonoids profile and different abilities to exude those flavonoids and IAA. This could explain the difference in oat cultivar's response to endophyte inoculation for different root traits like root length, root area, and root volume. IAA production by endophytes is important for increases in root growth however auxin may affect root development based on dose-dependent capacity (Arteca & Arteca, 2008). When endophytes produce auxin in moderate concentration, there is stimulation of root branching without inhibition of root elongation (Kudoyarova et al., 2019). If the endophytes produce auxins in higher concentrations there is inhibition of root elongation especially in dicotyledonous plants (Kudoyarova et al., 2019). The root surface area of wheat seedlings was decreased by 13%–38% when inoculated with IAA deficient mutant of salt-tolerant *Pseudomomas moraviensis* compared to wild type strain (Ul Hassan & Bano, 2019). Endophytes and their interactions with plant genotypes influence the level of plant hormones and this interaction is most critical and consistent factor in influencing host growth and physiological outcome (Morse et al., 2007). The endophyte inoculation in this study may as well be responding in a similar manner. The different oat genotypes may experience different plant hormones levels and thus are showing different outcomes for the different traits evaluated. We observed some specific combinations of oat genotypes and bacterial strain where endophyte inoculation provided benefits on plant growth at both 50% and 100% nitrogen application rate. Many studies have reported beneficial effects of endophytes in both low and high nutrient conditions. Cheplick et al.

(1989) reported beneficial effect of endophytic infection on ryegrass when soil nutrient was not limited. Ravel et al. (1997) and Lewis (2004) reported the advantage of endophyte infection over uninfected plants at low nitrogen rate.

Some endophytes may use additional soil nitrogen for the production of alkaloids rather than that nitrogen being used for plant growth (Buckley et al., 2019). Endophytes with a high alkaloid synthesis capacity are thought to consume the majority, if not all, of the nitrogen they stimulate, as well as additional nitrogen from the soil (Faeth & Fagan, 2002). This may explain why some genotypes showed a negative response to endophytic treatments under high nitrogen application. Warrior showed negative response for shoot dry weight, root dry weight, root length, and root area. A negative response under 100% nitrogen was also observed with Goliath for shoot dry weight, and with Saddle for root length and root area. We also observed a negative response or decrease in growth parameters with endophyte inoculation under low nitrogen application. One of the hypotheses for this might be the metabolic cost of harboring endophytes. In nitrogen fixing interaction between host and endophyte, the host plant plays an important role by supplying the carbon and energy source for bacterial growth and nitrogen fixation (Rosenblueth et al., 2018). Because of the limited quantity of accessible photosynthate, there may be a metabolic cost to the host in resource-limited conditions (Cheplick et al., 1989).

In nitrogen fixing host-endophyte interactions, if the nitrogen fixed by the endophyte is used by the endophyte for its growth, multiplication, and production of secondary metabolites instead of that fixed nitrogen being assimilated to host plant, such interaction may not be beneficial to the host plant. The nitrogen fixing capabilities in oat cultivars

may be specific to specific endophyte-oat genotype combination and it may be affected by the amount of nitrogen application. Cultivar specific nitrogen fixation was observed in wheat. Wheat cultivar Trenton showed relief to nitrogen deficiency symptoms when inoculated with *Klebsiella pneumoniae* 342, however, cultivar Russ or Stoa exhibited no relief of nitrogen deficiency symptoms when inoculated with *Klebsiella pneumoniae* 342 (Iniguez et al., 2004).

Nitrogen fertilization have the ability to modify the composition and abundance of root exudates and to subsequently affect the rhizosphere microbial communities (Zhu et al., 2016). Since different oat cultivars might have different root exudates owing to their genotypic differences, we might expect them to behave differently when inoculated with endophytes under different nitrogen levels. This might also explain why the response to endophytic inoculation are not consistent for the genotype across nitrogen level. When maize was supplied with increasing amounts of nitrogen, roots secreted more sugars, sugar alcohol, and phenolics which altered soil microbial community. High nitrogen can increase the activity of ammonia-oxidizing and denitrification bacteria leading to a decrease in nitrogen-use-efficiency (Zhu et al., 2016). Since root exudates can harbor microorganisms on rhizosphere, it may be possible to select cultivars that can secrete reduced root exudates even at high nitrogen application and increase nitrogen efficiency.

Our results suggest that identifying growth promoting strains of endophyte can be challenging given variation in direction and magnitude of endophyte effects on oat genotypes. Since the response of oat genotypes to endophyte inoculation was cultivar specific and dependent on the growth parameters evaluated, inoculation by multiple endophytes may be more effective in enhancing overall plant growth. Several studies

have shown that inoculation with multiple endophytes has a greater influence on plant growth promotion than single strain inoculation (Govindarajan et al., 2008; Knoth et al., 2014; Oliveira et al., 2002). Oliveira et al. (2002) used seven different combinations of inoculum using five endophytic species (*Gluconacetobacter, diazotrophicus, Herbaspirillum seropedicae, Herbaspirillum rubrisubalbicans, Azospirillum amazonense,* and *Burkholderia* sp.) to evaluate the effect of inoculating endophytic N_2-fixing bacteria on sugarcane. The analysis of the BNF contribution using the 15N-isotope dilution technique showed that inoculation promoted some increase in the BNF contribution to the plant tissues and the best treatment was a mixture of all five strains, followed by the treatment with a mixture of *Herbaspirillum* spp. Knoth et al. (2014) conducted a greenhouse trial with single-strain endophyte and consortia inoculation in poplar clones and they reported that endophyte inoculation contributed to an increase in biomass over nonincubated control with this growth promotion being more pronounced with multi-strain consortia than single strain inoculum.

Our results show that different oat cultivars respond differently to endophyte inoculation. The cultivar and endophytic interaction are important, and this interaction can be influenced by the amount of nitrogen applied. A better understanding of oat genotype-endophyte interaction is needed to identify endophytes with the potential to enhance oat growth. In addition, field studies should be carried out to determine the potential agronomic benefits of endophytes on oat production.

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CHAPTER 3.

Genome-wide association studies of root architectural traits of oat (*Avena sativa* L.) seedlings

Abstract

Roots play an important role in plant production as they help with the acquisition of essential plant nutrients and water. Roots are the first organ that can sense and respond to drought stress. With the increase in frequency and severity of droughts around the world and the increase in global food demand, developing crops adapted to drought and low soil fertility is necessary. Thus, breeding for efficient roots is a high priority to achieve yield improvement and drought resistance. In this study, we performed a genome-wide association study on oat root traits using 285 diverse oat genotypes. The seeds were imbibed on wet germination paper for two days and were grown between blue blotter germination paper for 9 days. The roots images were taken with a digital camera and the images were analyzed with RootNav. We found considerable variability in root traits among genotypes for different root traits and low to moderate heritability ranging from 0.17 to 0.59. We identified 82 significant marker-trait associations using the mixed linear model approach. With many makers associated with multiple traits, there were 22 unique makers associated with different root traits (total length, convex hull area, maximum depth of the root system, maximum width of the root system, length of the primary root, average length of primary roots, primary root number, and lateral root density). The markers significantly associated with the root traits explained from 7.6 to 19.9 % of the phenotypic variation. We found several likely candidate genes in close proximity to the markers. Many genes close to the markers have a role in root development.

Introduction

Roots play an important role in plant productivity as they provide an interface between plant and complex soil environments. The root system provides anchorage, helps in water and nutrient uptake from the soil, and is the site of synthesis of many metabolites such as

cytokinins and auxins, which play an important role in the growth and developmental processes (Ortíz-Castro et al., 2009). Root system architecture (RSA) refers to the spatial configuration of the root system in the soil and describes the shape and structure of the root system, and the geometric deployment of root axes (Lynch, 1995). RSA is controlled by both plant genetic composition and environmental cues (Tian et al., 2014). Availability of water and soil nutrient elements like nitrogen and phosphorous that are critical to growth and yield can strongly change RSA on diverse crops (Desnos, 2008; Vidal et al., 2010). With an increase in global food demand, significant improvement in crop yield and the development of crops adapted to water stress and low soil fertility is necessary (Lynch, 2007; Tracey & Anne, 2008). Breeding for efficient roots is becoming a high priority target to achieve yield improvements (Araus & Cairns, 2014). The RSA is vital for plant productivity because the soil resources are heterogeneously distributed in the soil and the spatial deployment of the roots will substantially determine the ability of plants to secure edaphic resources.

Understanding the genetic basis of these RSA traits is important so that researchers can breed crops with an efficient root system. Root system architectural traits are sensitive to environmental stimuli and show considerable plasticity. Both monocotyledons and dicotyledons have an abundance of natural variation in RSA. Variations in RSA traits have been reported in lentils, rice, barley, maize, sorghum, and wheat (Gahoonia et al., 2006; Henry et al., 2011; Jia et al., 2019; Li et al., 2015; Mace et al., 2012; Manschadi et al., 2008; Richard et al., 2015). Genes have been characterized and genetic control of RSA has been reported in many crops like rice, corn, wheat, and soybean. Multiple root architecture quantitative trait loci (QTLs) reported in maize control root architecture and

yield stability across multiple genetic backgrounds and water regimes. Major QTLs in maize that control root length, number, dry weight, and root length/area is known to co-localize with grain yield (Cai et al., 2012).

Genome-wide association is an efficient and reliable tool for deciphering the molecular basis of complex traits. The genome-wide association analysis is meant for detecting variants at genomic loci that are associated with complex traits in the population (Isidro-Sánchez, Akdemir, & Montilla-Bascón, 2017). Statistically, a causal mutation occurs when $\mathrm{Cov}(Y,X) \neq 0$ where Y is the value of the phenotypes and X is the value of the genotypes. Genome-wide association studies take advantage of a large number of historical and evolutional recombination events and link these events with phenotype. A large number of diverse lines are used in genome-wide studies and thus phenotyping a large number of plants for root traits can be a challenge for genome-wide studies.

Field phenotyping of root traits is very difficult and time-consuming (A. P. Wasson et al., 2012). Since genome-wide association studies rely on a large population, field phenotyping of root traits for GWAS may not be feasible unless a large amount of time and resources are provided. Many researchers have successfully used a germination paper-based phenotyping approach to identify QTLs associated with root traits in many crops such as wheat, oat, barley, and corn (Atkinson et al., 2015; Huang et al., 2020; Reinert et al., 2016; Sanchez et al., 2018). A germination paper-based approach can also measure many root traits that cannot be determined in traditional root excavation methods.

Quantification of the RSA trait is often done by image analysis of the root system captured by digital cameras or scanners. In recent years improvements have been made in techniques to image the root system and image analysis tools to generate multiple quantifiable root traits, thus interest in root studies has increased (Atkinson et al., 2019). Successful GWAS have been conducted with a germination paper-based approach that allowed quantification of many root traits (Beyer et al., 2019; Pace et al., 2015), and these genome-wide studies have identified SNPs within or near (<1kb) gene models and identified candidate genes involved in root development at the seedling stage (Pace et al., 2015).

Given the importance of root systems in capturing soil resources and their importance on overall plant productivity, understanding the genetic basis of root system architectural traits will help in developing cultivars with an efficient root system. The information on oat seedling QTLs for root traits is lacking as there are not many studies on the genetics of root traits in oat. Huang et al. (2020) conducted a GWAS on oat seed vigor and found several SNPs associated with root traits and they reported ten SNPs identified which were close to previously reported plant height QTLs. Due to a lack of information on root QTLs, and a positive correlation between the root size and plant height, Huang et al. (2020) focused on plant height QTLs in the literature to provide further support for their QTLs. This highlighted the need for more studies related to the genetics of root traits in oat.

This study aimed to study the genetic variation in root system architectural traits in oat seedlings and identify the molecular markers and candidate genes associated with various root traits.

Materials and methods

Plant material

A set of 285 diverse spring oat genotypes were used for this study. The oat genotypes used in the study were oat cultivars or breeding lines developed by South Dakota State University, North Dakota State University, University of Illinois, University of Minnesota, University of Wisconsin, Purdue University, and Agriculture and Agri-Food Canada. The genotypes were selected based on genotypic data (SNP data obtained by genotyping-by-sequencing) for 721 oat genotypes available on the T3 oat (https://triticeaetoolbox.org/oat/). A cluster analysis was done on genotype data to select genotypes with diverse genetic backgrounds. The list of genotypes used in this study is provided in supplementary Table 1.

Phenotyping

Root phenotyping was done with a germination paper-based approach coupled with image analysis. The seeds were first imbibed on wet germination paper for 48 hours. To germinate the seeds, a wet germination paper was placed on the plastic box and the imbibed seeds were placed on the paper. The seeds were covered with another germination paper and the lid of the box was closed and placed in a growth chamber in a semi-vertical position.

Uniformly germinated were grown in a growth pouch that consisted of a sheet of blue blotter paper (Anchor Paper Company, St Paul, MN, USA) and two polythene sheets held together with the help of two paper clips. The growth pouches design was similar to the one used for wheat (Atkinson et al., 2015). A single seedling was placed on the blotter

paper, centered 2 cm from the top edge of the paper. The growth pouches were suspended in a bucket and placed into the growth chamber with a 16 hour photoperiod and a temperature of 20^{o}C.

A completely randomized design was used and fifteen seedlings per genotype were grown. After 9 days, an image of the roots of each seedling was taken with a digital camera (Nikon7200). The polythene film covering each pouch was carefully removed leaving roots fixed to the blotter paper for taking images. The experiment included multiple batches with each batch having 24 genotypes. To ensure consistency of experimental procedure and environmental conditions, two common checks were included in all batches. Each batch included 22 genotypes plus 2 checks (Gopher and Hayden). The experiment was repeated twice.

Image analysis

Root images were processed using RootNav software (Pound et al., 2013). RootNav is a software that allows semiautomated quantification of complex root system architectures in a range of plant species. The user specifies the source of the root system and the tips of the primary and lateral roots and the software quantifies total root length, average length of all roots, average length of primary roots, average length of lateral roots, lateral root count, primary root count, the convex hull, maximum width of the root system, maximum depth of root system, and the width to depth ratio. Other root traits such as total length of lateral roots, total length of primary roots, and lateral root density were calculated based on the root traits obtained from RootNav. The total length of the lateral root and primary root was determined as the product of the average length of roots by the number of roots.

The lateral root density was calculated as the number of lateral roots divided by the total length of the primary root.

Genotype data

The genotypic data for this study was obtained from the T3 oat toolbox (https://triticeaetoolbox.org/oat/). The genotype data for this study was obtained from four genotyping projects (SDSU_2015, SDSU_2017, UMN_2017, and UPON_2015). Genotyping for all four projects was carried out by the USDA-ARS genotyping facility in Fargo, ND, using genotyping-by-sequencing. Bioinformatics, including SNP calling was done using Haplotag (Tinker et al., 2016). To retain only high-quality SNPs, SNP markers with minor allele frequency (MAF) at <5%, and missing data at >10% were excluded when downloading the SNP data from the T3 toolbox.

Linkage disequilibrium

Linkage disequilibrium (LD) was calculated on a sliding window of 100 adjacent markers using TASSEL v.5.0 (Bradbury et al., 2007). Results from TASSEL were plotted using the ggplot2 package in R. The distribution and extent of LD was displayed in a plot where marker R-squares were plotted against the distance and the locally-weighted polynomial regression (LOESS) curve was fitted (Cleveland, 1979).

Statistical analysis

Descriptive statistics for root traits along with correlation analysis were done in the R programming language (R Core Team, 2020). The linear mixed model approach was used

to analyze the data using the lmer function in the lme4 package (Bates et al., 2007). Best linear unbiased predictions (BLUPs) of root traits for each genotype were calculated based on the linear mixed model. Broad-sense heritability (h^2) was estimated as $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2)$, where σ_g^2 is genotypic variance component, σ_e^2 is residual variance component.

Genome-wide association analysis

Marker traits associated analysis was conducted in TASSEL v.5.0 (Bradbury et al., 2007). The mixed linear model (MLM) was used to conduct the association analysis. Including both random and fixed effects enables MLM the ability to incorporate information about the relationship among the individuals (Zhang et al., 2010). To account for population structure, a population structure matrix was built with the first five principal components of genotypic data using TASSEL v.5.0 (Bradbury et al., 2007). To quantify genetic relatedness among individuals, kinship matrices were generated using the Centered-IBS approach in TASSEL v.5.0. A mixed linear model (MLM) with principal components and kinship was used to perform the GWAS. A significant threshold for the association was set at a false discovery rate of 5% (Storey & Tibshirani, 2003). The Manhattan plots and Q-Q plots were drawn using qqman package in R (Turner, 2014).

Candidate Gene Analysis

All the potential candidate genes within 100 kb of the detected SNPs were identified. Gene annotation information from the oat genome browser from GrainGenes was used to

identify the high confidence protein-coding genes (Kamal et al., 2022) (https://wheat.pw.usda.gov/jb/?data=/ggds/oat-sang).

Results

Oat genotypes (285 elite breeding lines) were evaluated for seedling root traits using a germination paper-based approach. The mean, range, standard deviation, coefficient of variation, and broad-sense heritability for each of the traits are shown in Table 3.1. Considerable variation was observed among the genotypes for the traits evaluated. The heritability estimates were moderate to low ranging from 17% to 59%.

Correlation analysis between traits showed that most root traits were positively correlated (Fig 3.1). The average length of lateral roots, lateral root count, lateral root density, and total length of lateral roots were strongly positively correlated (r=0.54 to 0.93). Similarly, a strong positive correlation was found between total root length, total length of primary root, maximum depth, maximum width, and convex hull area (r=0.65-0.91). The average length of all roots showed a strong negative correlation with lateral root number, lateral root length, and lateral root density (r=-0.62 to -0.85).

Table 3.1 Mean, range, standard deviation, coefficient of variation, and broad-sense heritability for the root traits.

Traits	Mean	Range	Standard deviation	Coefficient of variation	Heritability

Average primary emergence angle	34.6	11.7-62.8	7.5	21.6	22.7
Average length of all roots (cm)	6.8	1.5-23.7	3.4	50.1	44.9
Average length of lateral roots (cm)	0.7	0-1.6	0.2	38.7	38.5
Average length of primary roots (cm)	17.8	3.5-27.2	3.3	18.7	58.9
Number of lateral roots	8.0	0-32.0	6.1	77.1	46.2
Number of primary roots	3.6	3-6	0.5	16.0	46.6
Lateral root density	0.1	0-0.65	0.1	74.1	51.1
Total length of lateral roots (cm)	5.7	0-32.1	5.6	91.3	53.7
Total length of primary roots (cm)	59.5	17.0-96.5	9.9	16.7	59.0
Total root length (cm)	65.8	21.1-111.2	13.1	19.9	55.6
Maximum depth of root (cm)	30.0	9.5-38.8	3.9	13.0	51.2
Maximum width of root (cm)	20.3	3.2	29.4	20.4	40.2
Convex hull area (cm^2)	296.1	5.0-517.1	82.7	27.9	51.2
Width to depth ratio	0.67	0.2-1.1	0.13	20.1	17.2

A trait by genotype biplot was obtained with 71.5% goodness of fit (Fig 3.2). The first principal component accounted for 45.5% and the second for 26% of the variation. The biplot provides a mean to visualize the trait profile of the genotypes and the relationship between the traits. An acute angle between the vectors of the traits indicates that the traits have a positive correlation. The genotypes are scattered in the plots thus indicating there is considerable variation in the genotypes for the different traits and they have diverse root trait profiles. Consistent with results from correlation analysis, the vectors corresponding to the average length of all roots, lateral root count, lateral root density,

lateral root length, and total root length formed obtuse angles, thus indicating a negative correlation with those traits.

A set of 12,454 filtered SNPs with minor allele frequencies >0.05 were used for GWAS. Linkage disequilibrium for the marker pair showed quick decaying over a few Megabases (Mb). Similar linkage decay behavior was observed in oat germplasm from Federal University of Rio Grande do Sul (UFRGS) Oat Breeding Program (Zimmer et al., 2020). For all the traits measured, 82 significant marker traits associations were discovered, and these were located on chromosomes 1A, 1D, 2C, 2D, 3A, 3C, 4A, 4D, 5A, 5C, 5D, 6C, 6D, 7A, and 7C (Table 3.2). Since many traits were highly correlated with one another, markers showed association with multiple traits that were highly correlated. Twenty-two unique markers were found to be associated with different root traits. Chromosome 7C has the highest (four) significant markers, chromosomes 2C, 2D, and 6D each have three significant markers, and chromosomes 1A, 3C, 4A, 4D, 5A, 5C, 5D, and 6D each have one significant marker.

Table 3.2 . List of markers significantly associated with root traits.

Trait	Markers	Chr	Position	Marker R^2	-log(p)_
Total length of the root (cm)	avgbs_cluster_18623.1.43	4A	111652422	0.09309	5.55
	avgbs_79552.1.20	6C	498339130	0.07733	4.64
Primary root length (cm)	avgbs2_159517.1.51	1D	118491673	0.09245	5.46
	avgbs_511953.1.35	2D	374197735	0.10695	6.28
	avgbs_cluster_9240.1.63	2D	12999237	0.08507	5.05
	avgbs_cluster_18623.1.43	4A	111652422	0.13206	7.66
	avgbs_cluster_7121.1.63	4A	432106453	0.08902	5.27
	avgbs_502505.1.47	4D	253198958	0.07754	4.62
	avgbs_79552.1.20	6C	498339130	0.1277	7.42
	avgbs_36707.1.7	7C	45781425	0.10876	6.38
	avgbs_cluster_29357.1.31	7C	442715656	0.10969	6.43
Primary root number	avgbs_62666.1.21	2D	377369685	0.07907	4.68
	avgbs_14605.1.37	5C	49221746	0.07898	4.67
Maximum width of the root system (cm)	avgbs2_159517.1.51	1D	118491673	0.11907	6.92
	avgbs_239249.1.10	2C	116161892	0.09042	5.33
	avgbs_73002.1.62	2C	364998306	0.08689	5.13
	avgbs_511953.1.35	2D	374197735	0.08019	4.75
	avgbs_cluster_18623.1.43	4A	111652422	0.1802	10.19
	avgbs_cluster_7121.1.63	4A	432106453	0.07722	4.58
	avgbs_502505.1.47	4D	253198958	0.11962	6.95
	avgbs2_120048.1.27	5A	442229316	0.09061	5.34
	avgbs_79552.1.20	6C	498339130	0.08832	5.21
	avgbs_457381.1.22	6D	218666226	0.08809	5.20
	avgbs_53126.1.60	6D	235099530	0.07822	4.63
	avgbs_206020.1.46	7A	455721009	0.09748	5.72
	avgbs_36707.1.7	7C	45781425	0.08082	4.78
	avgbs_cluster_2187.1.35	7C	174716343	0.08973	5.29
	avgbs_cluster_29357.1.31	7C	442715656	0.08179	4.84
	avgbs2_94229.1.50	7C	590202422	0.08155	4.82
Maximum depth of the root system (cm)	avgbs2_159517.1.51	1D	118491673	0.13584	7.85
	avgbs_73002.1.62	2C	364998306	0.08909	5.27
	avgbs_511953.1.35	2D	374197735	0.10362	6.08
	avgbs_cluster_18623.1.43	4A	111652422	0.19929	11.20
	avgbs_cluster_7121.1.63	4A	432106453	0.0923	5.45
	avgbs_502505.1.47	4D	253198958	0.12325	7.17
	avgbs2_120048.1.27	5A	442229316	0.07851	4.66
	avgbs_79552.1.20	6C	498339130	0.08286	4.91
	avgbs_206020.1.46	7A	455721009	0.09972	5.86
	avgbs_36707.1.7	7C	45781425	0.10544	6.18
	avgbs_cluster_29357.1.31	7C	442715656	0.10413	6.11
	avgbs2_94229.1.50	7C	590202422	0.08868	5.24
Lateral root density	avgbs_511953.1.35	2D	374197735	0.11505	6.71
	avgbs_36707.1.7	7C	45781425	0.11612	6.77
	avgbs_cluster_29357.1.31	7C	442715656	0.11443	6.68

Convex hull area (cm^2)	avgbs_49689.1.53	1A	320817143	0.079	4.66
	avgbs2_159517.1.51	1D	118491673	0.12624	7.28
	avgbs_239249.1.10	2C	116161892	0.07859	4.64
	avgbs_73002.1.62	2C	364998306	0.09979	5.83
	avgbs_cluster_33489.1.16	2C	150449292	0.08097	4.77
	avgbs_511953.1.35	2D	374197735	0.15157	8.64
	avgbs_cluster_9240.1.63	2D	12999237	0.10202	5.95
	avgbs2_139585.1.38	3A	393592291	0.0886	5.20
	avgbs_cluster_18623.1.43	4A	111652422	0.1909	10.70
	avgbs_cluster_7121.1.63	4A	432106453	0.13904	7.97
	avgbs_502505.1.47	4D	253198958	0.10422	6.08
	avgbs2_120048.1.27	5A	442229316	0.08133	4.79
	avgbs_79552.1.20	6C	498339130	0.13493	7.75
	avgbs_457381.1.22	6D	218666226	0.07804	4.61
	avgbs_206020.1.46	7A	455721009	0.1015	5.92
	avgbs_36707.1.7	7C	45781425	0.15263	8.70
	avgbs_cluster_2187.1.35	7C	174716343	0.08012	4.72
	avgbs_cluster_29357.1.31	7C	442715656	0.15172	8.65
	avgbs2_94229.1.50	7C	590202422	0.08568	5.04
Average length of primary roots (cm)	avgbs2_159517.1.51	1D	118491673	0.08578	5.02
	avgbs_73002.1.62	2C	364998306	0.09102	5.31
	avgbs_511953.1.35	2D	374197735	0.12789	7.34
	avgbs_cluster_9240.1.63	2D	12999237	0.07924	4.65
	avgbs2_139585.1.38	3A	393592291	0.08657	5.06
	avgbs_cluster_18623.1.43	4A	111652422	0.14451	8.23
	avgbs_cluster_7121.1.63	4A	432106453	0.11515	6.65
	avgbs_502505.1.47	4D	253198958	0.07789	4.58
	avgbs_79552.1.20	6C	498339130	0.11307	6.53
	avgbs_457381.1.22	6D	218666226	0.08376	4.91
	avgbs_cluster_18376.1.27	6D	199328735	0.08379	4.91
	avgbs_36707.1.7	7C	45781425	0.13027	7.47
	avgbs_cluster_2187.1.35	7C	174716343	0.07806	4.59
	avgbs_cluster_29357.1.31	7C	442715656	0.12722	7.30
Average length of lateral roots (cm)	avgbs_492934.1.64	3C	577800817	0.07966	4.76
	avgbs2_27280.1.27	5D	19584016	0.07975	4.76

A total of two markers were associated with the total length of the root (Fig. 3.4), nineteen markers were associated with the convex hull area (Fig. 3.5), twelve markers were associated with the maximum depth of the root system (Fig. 3.6), sixteen markers were associated with the maximum width of the root system (Fig. 3.7), nine markers were

associated with primary root length (Fig. 3.8), thirteen markers were associated with the average length of primary roots (Fig. 3.9), two markers were associated with the primary root number (Fig. 3.10), three markers were associated with lateral root density (Fig. 3.11), and two markers were associated with the average length of lateral roots (Fig. 3.12). Markers significantly associated with root traits explained from 7.6 to 19.9 % of the phenotypic variation.

Many studies have chosen a variable window ranging from 100kbs to 500kbs to identify candidate genes (Brodie et al., 2016; Guo et al., 2009; Schoof et al., 2011). Sometimes the affected gene is up to 2Mbs away from the associated SNP (Brodie et al., 2016). We identified genes within 100kb upstream and downstream of the significant makers as potential genes. This resulted in 39 genes within 100kb distance of 14 unique SNP markers. The candidate genes, their position, and distance from the associated SNP markers are listed in Table 3.3.

Table 3. 3 Candidate genes identified near significant SNPs marker.

Marker	Chr	Gene name	Distance	Position	Description

avgbs_49689.1.53	1A	AVESA.00010b.r2.1AG0048980	13kb	chr1A:320841247..320845019 (+ strand)	RNA-binding KH domain-containing protein
avgbs_49689.1.53	1A	AVESA.00010b.r2.1AG0048990	-21kb	chr1A:320795600..320803642 (- strand)	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 1
avgbs_49689.1.53	1A	AVESA.00010b.r2.1AG0049000	-24kb	chr1A:320792548..320795300 (+ strand)	heat-inducible transcription repressor
avgbs_49689.1.53	1A	AVESA.00010b.r2.1AG0048970	41kb	chr1A:320859134..320862042 (+ strand)	B3 domain-containing protein
avgbs_49689.1.53	1A	AVESA.00010b.r2.1AG0048960	55kb	chr1A:320872218..320874257 (- strand)	Magnesium and cobalt efflux protein CorC
avgbs_cluster_33489.1.16	2C	AVESA.00010b.r2.2CG0279120	-5kb	chr2C:150444061..150447625 (- strand)	BTB/POZ domain-containing protein
avgbs_239249.1.10	2C	AVESA.00010b.r2.2CG0276460	35kb	chr2C:116197646..116200847 (+ strand)	Protein DETOXIFICATION
avgbs_239249.1.10	2C	AVESA.00010b.r2.2CG0276470	39kb	chr2C:116200909..116204248 (+ strand)	Protein DETOXIFICATION
avgbs_73002.1.62	2C	AVESA.00010b.r2.2CG0294470	85kb	chr2C:365083565..365088258 (- strand)	Hydroxyproline-rich glycoprotein-like
avgbs_cluster_9240.1.63	2D	AVESA.00010b.r2.2DG0401450	80kb	chr2D:13079649..13083413 (+ strand)	E2F-DP transcription factor
avgbs_cluster_9240.1.63	2D	AVESA.00010b.r2.2DG0401440	84kb	chr2D:13083915..13085679 (- strand)	B12D protein
avgbs_cluster_7121.1.63	4A	AVESA.00010b.r2.4AG0647010	-7kb	chr4A:432099277..432100255 (+ strand)	Eukaryotic aspartyl protease family protein
avgbs_cluster_7121.1.63	4A	AVESA.00010b.r2.4AG0647000	-10kb	chr4A:432095980..432097013 (- strand)	Retrotransposon protein, putative, Ty1-copia subclass
avgbs_cluster_7121.1.63	4A	AVESA.00010b.r2.4AG0647020.1	69kb	chr4A:432175799..432177100 (- strand)	Eukaryotic aspartyl protease family protein
avgbs_502505.1.47	4D	AVESA.00010b.r2.4DG0743890	-30kb	chr4D:253167994..253176555 (- strand)	Homeobox protein knotted-1-like 1

avgbs_502505.1.47	4D	AVESA.00010b.r2.4DG0743900	50kb	chr4D:253249817..253255559 (- strand)	Homeobox protein knotted-1-like 1
avgbs_502505.1.47	4D	AVESA.00010b.r2.4DG0743880	-65kb	chr4D:253133179..253136306 (+ strand)	Endo-1,4-beta-xylanase, putative, expressed
avgbs_502505.1.47	4D	AVESA.00010b.r2.4DG0743920	101kb	chr4D:253300910..253308327 (- strand)	Early-responsive to dehydration stress protein (ERD4)
avgbs2_120048.1.27	5A	AVESA.00010b.r2.5AG0851400	0kb	chr5A:442228785..442234555 (- strand)	Auxin efflux carrier family protein
avgbs2_120048.1.27	5A	AVESA.00010b.r2.5AG0851390	-3kb	chr5A:442222055..442226330 (- strand)	Auxin efflux carrier family protein
avgbs2_120048.1.27	5A	AVESA.00010b.r2.5AG0851380	-6kb	chr5A:442213613..442222589 (+ strand)	NEP-interacting protein, putative (DUF239)
avgbs2_120048.1.27	5A	AVESA.00010b.r2.5AG0851410	40kb	chr5A:442269479..442271464 (+ strand)	Splicing factor U2af small subunit A
avgbs2_120048.1.27	5A	AVESA.00010b.r2.5AG0851420	43kb	chr5A:442272993..442276367 (- strand)	Heat shock protein 70
avgbs2_120048.1.27	5A	AVESA.00010b.r2.5AG0851370	-50kb	chr5A:442174862..442178501 (+ strand)	Carboxyl-terminal peptidase, putative (DUF239)
avgbs_14605.1.37	5C	AVESA.00010b.r2.5CG0927810	-13kb	chr5C:49205745..49208206 (- strand)	MYB transcription factor
avgbs_14605.1.37	5C	AVESA.00010b.r2.5CG0927820	-107kb	chr5C:49111397..49113782 (- strand)	Cytochrome P450 family 71 polypeptide
avgbs_457381.1.22	6D	AVESA.00010b.r2.6DG1167980	10kb	chr6D:218672961..218676220 (- strand)	Receptor-like protein 12
avgbs_457381.1.22	6D	AVESA.00010b.r2.6DG1167990	-18kb	chr6D:218645914..218648442 (- strand)	tRNA pseudouridine synthase family protein
avgbs_457381.1.22	6D	AVESA.00010b.r2.6DG1168000	-21kb	chr6D:218643577..218644826 (+ strand)	Cellular retinaldehyde-binding/triple function, C-terminal
avgbs_457381.1.22	6D	AVESA.00010b.r2.6DG1167970	59kb	chr6D:218725461..218726495 (+ strand)	Geranylgeranyl pyrophosphate synthase, chloroplastic

avgbs_457381.1.22	6D	AVESA.00010b.r2.6DG1167950	76kb	chr6D:218738281..218742939 (- strand)	Mechanosensitive ion channel family protein
avgbs_cluster_2187.1.35	7C	AVESA.00010b.r2.7CG0681780	0kb	chr7C:174716232..174728824 (+ strand)	DNA-directed RNA polymerases I, II, and III subunit RPABC1
avgbs_367077.1.7	7C	AVESA.00010b.r2.7CG0702550	-4kb	chr7C:45774698..45777077 (- strand)	C-8 sterol isomerase
avgbs_367077.1.7	7C	AVESA.00010b.r2.7CG0702560	-8kb	chr7C:45767062..45773302 (+ strand)	Myb/SANT-like DNA-binding domain protein
avgbs_cluster_2187.1.35	7C	AVESA.00010b.r2.7CG0681770	12kb	chr7C:174729111..174730489 (- strand)	Glycosyltransferase
avgbs_cluster_2187.1.35	7C	AVESA.00010b.r2.7CG0681760	27kb	chr7C:174744006..174747202 (+ strand)	Ubiquitin-like protein 5
avgbs_367077.1.7	7C	AVESA.00010b.r2.7CG0702540	55kb	chr7C:45835693..45836767 (+ strand)	DUF1677 family protein (DUF1677)
avgbs_367077.1.7	7C	AVESA.00010b.r2.7CG0702570	-77lb	chr7C:45700305..45703739 (- strand)	Tryptophan RNA-binding attenuator protein-like protein

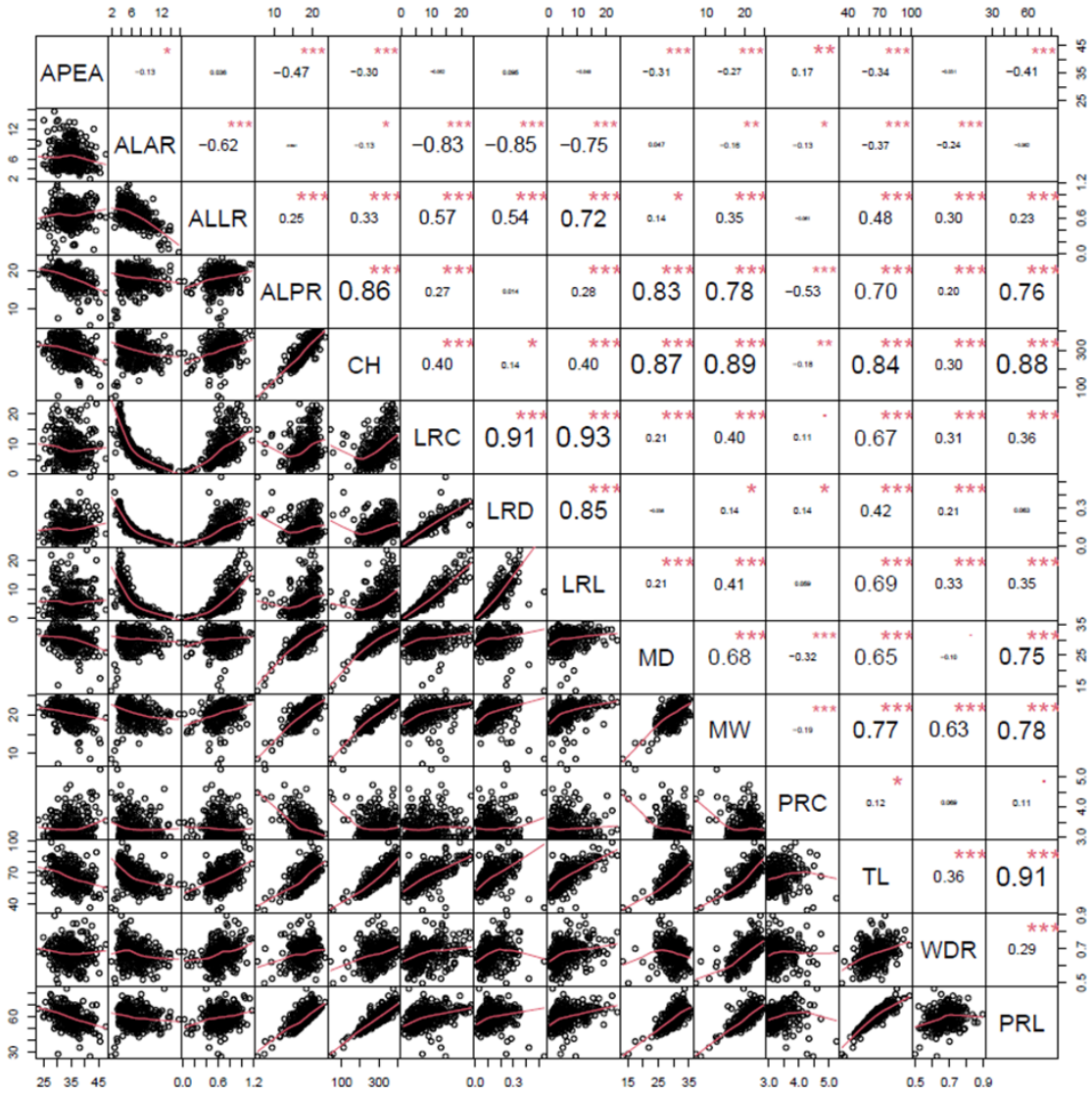

Figure 3.1 Correlation matrix of different root traits. Scatter plots are shown in the lower left quadrant, and values in the upper right quadrant are Pearson's correlation coefficients.

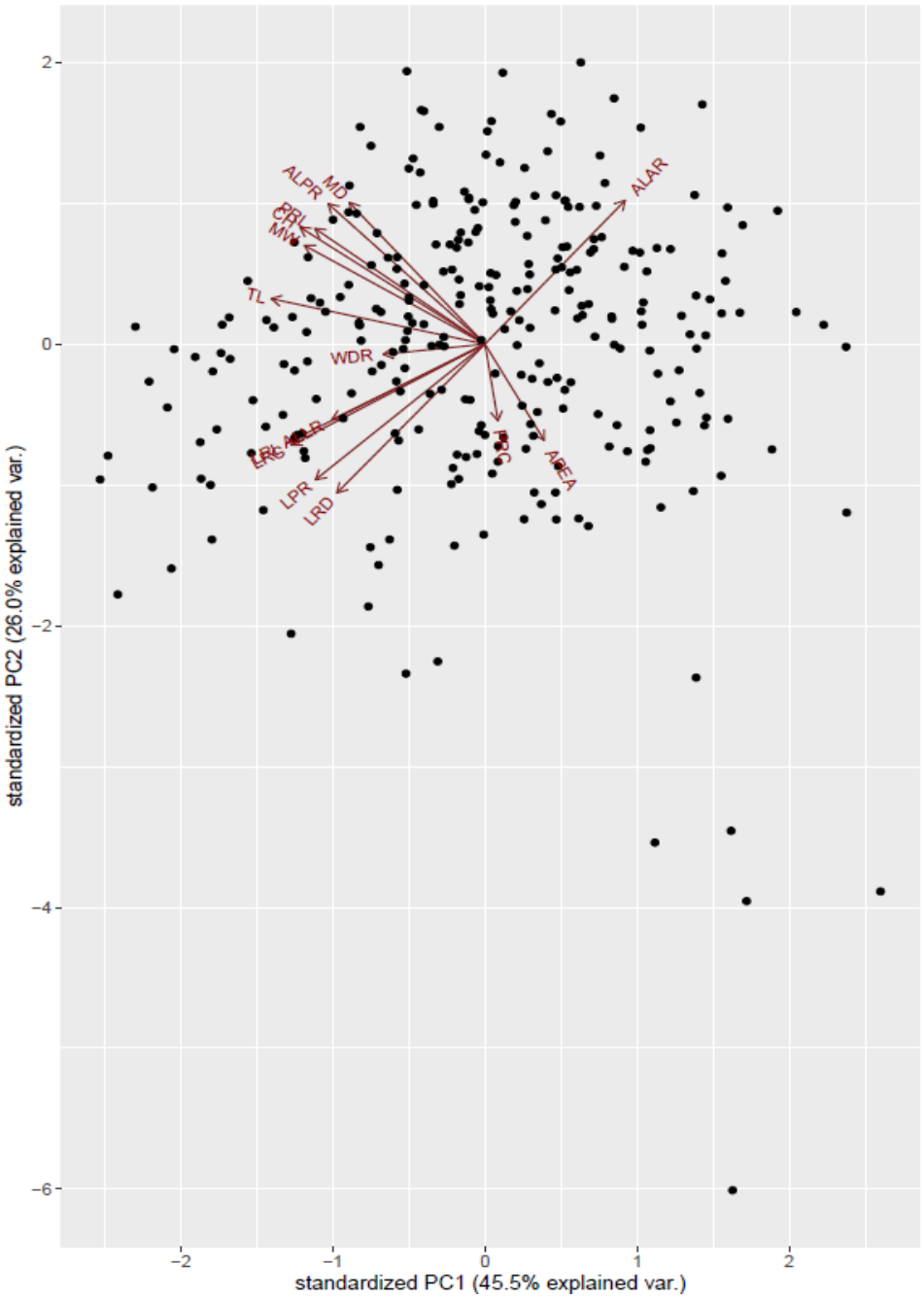

Figure 3.2 Genotype by trait biplot.

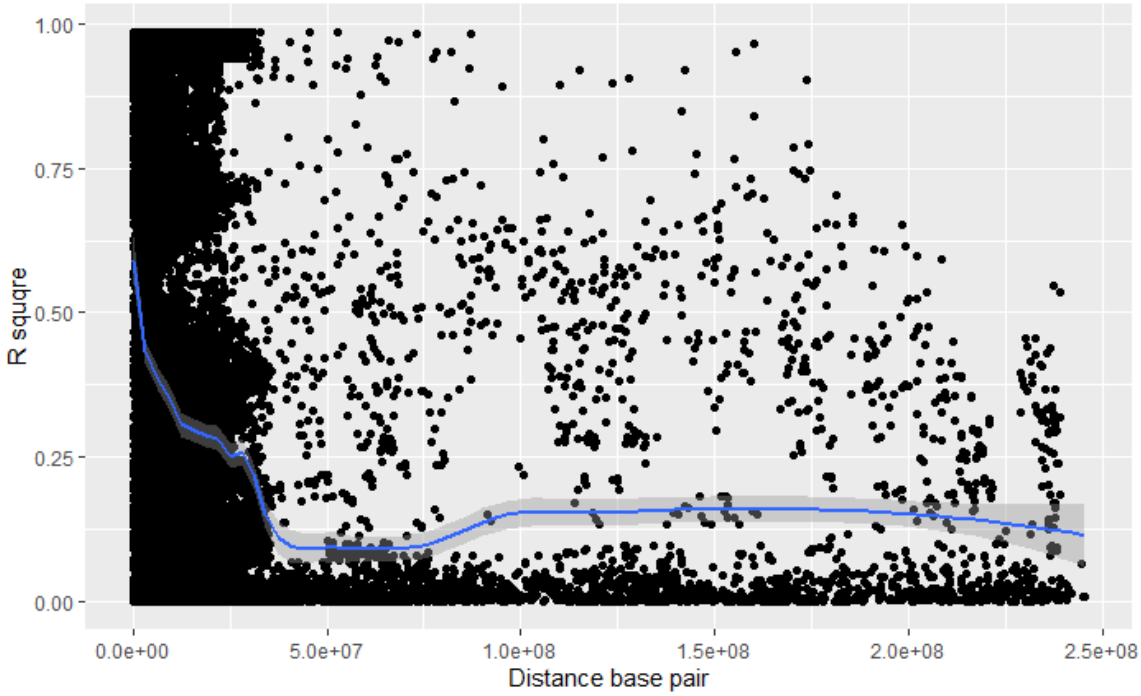

Figure 3.3 Linkage decay curve with Pairwise LD (r2) values plotted against the physical distance

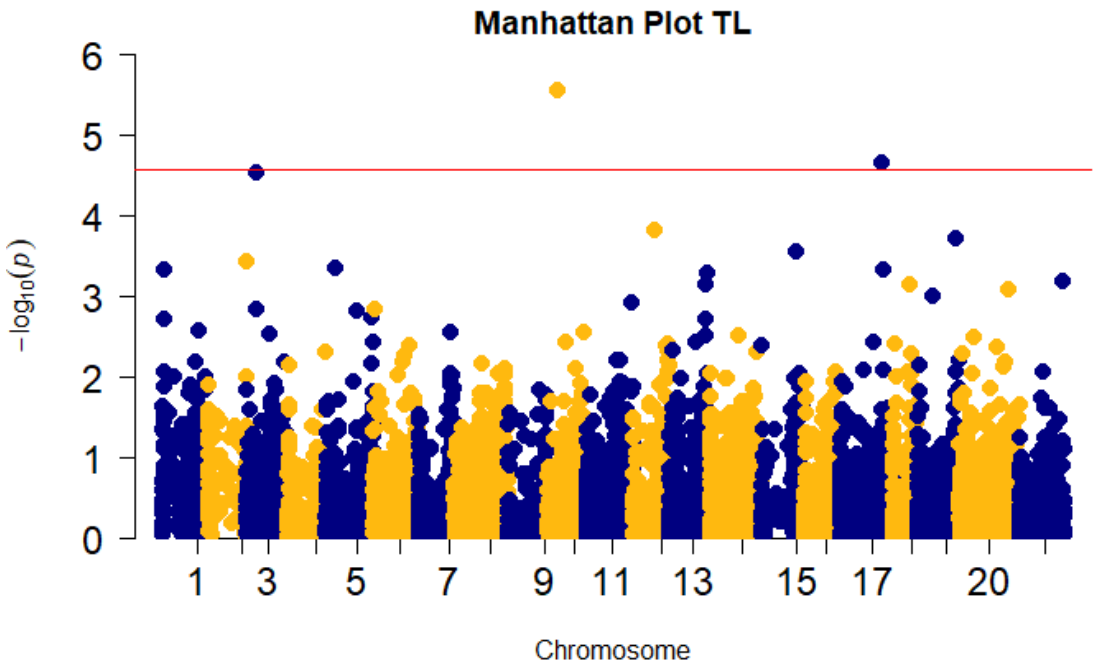

Figure 3.4 Manhattan plots for total root length with chromosome on the x-axis and −log10P on the y-axis. Each dot represents an SNP. Red line indicates the threshold of significance.

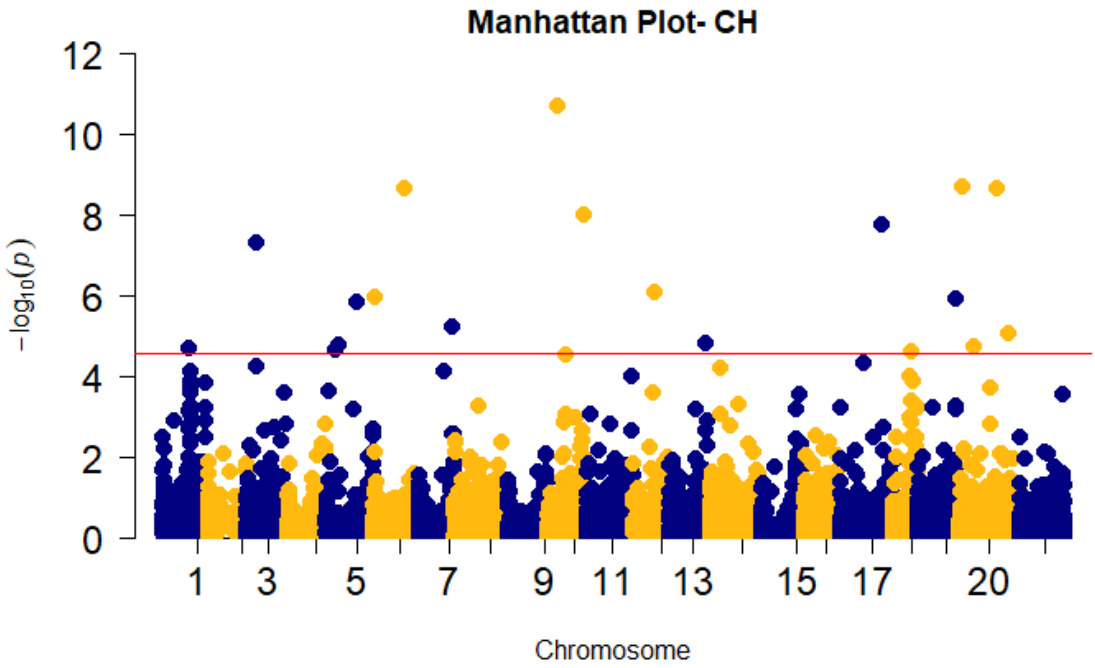

Figure 3.5 Manhattan plots for convex hull area with chromosome on the x-axis and −log10P on the y-axis. Each dot represents an SNP. Red line indicates the threshold of significance.

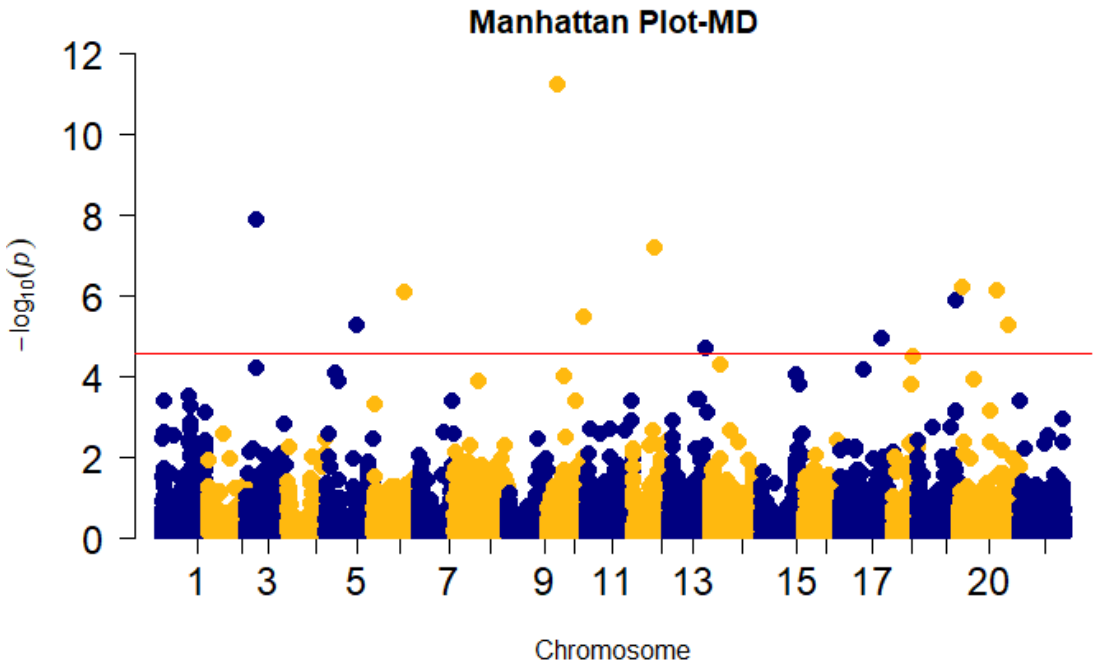

Figure 3.6 Manhattan plots for maximum depth with chromosome on the x-axis and −log10P on the y-axis. Each dot represents an SNP. Red line indicates the threshold of significance.

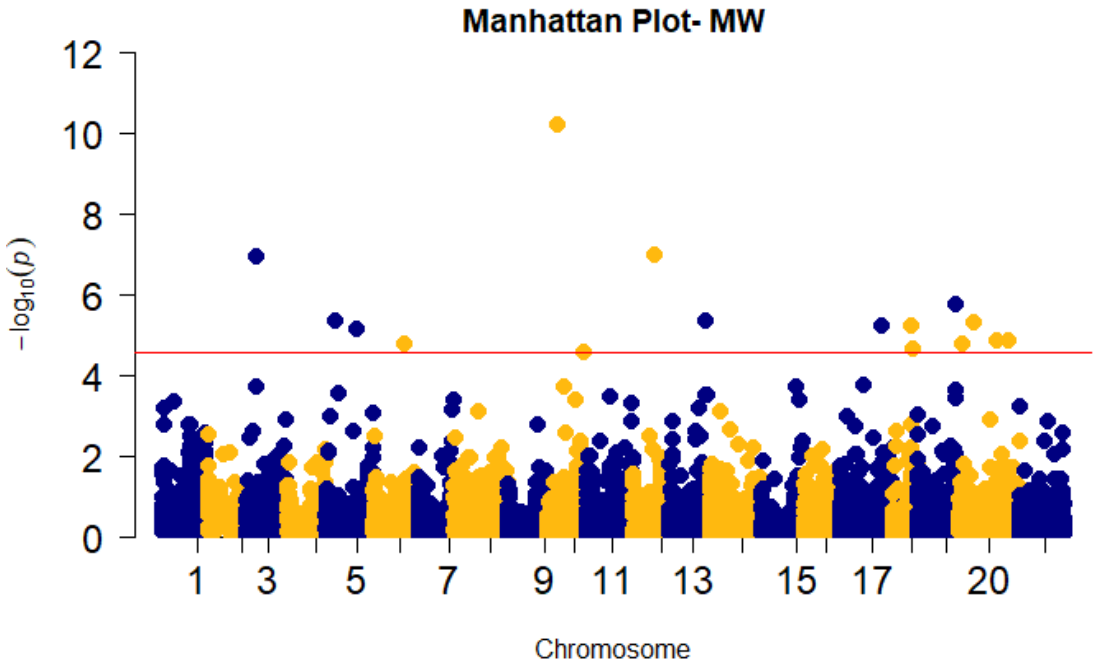

Figure 3.7 Manhattan plots for maximum width with chromosome on the x-axis and −log10P on the y-axis. Each dot represents an SNP. Red line indicates the threshold of significance.

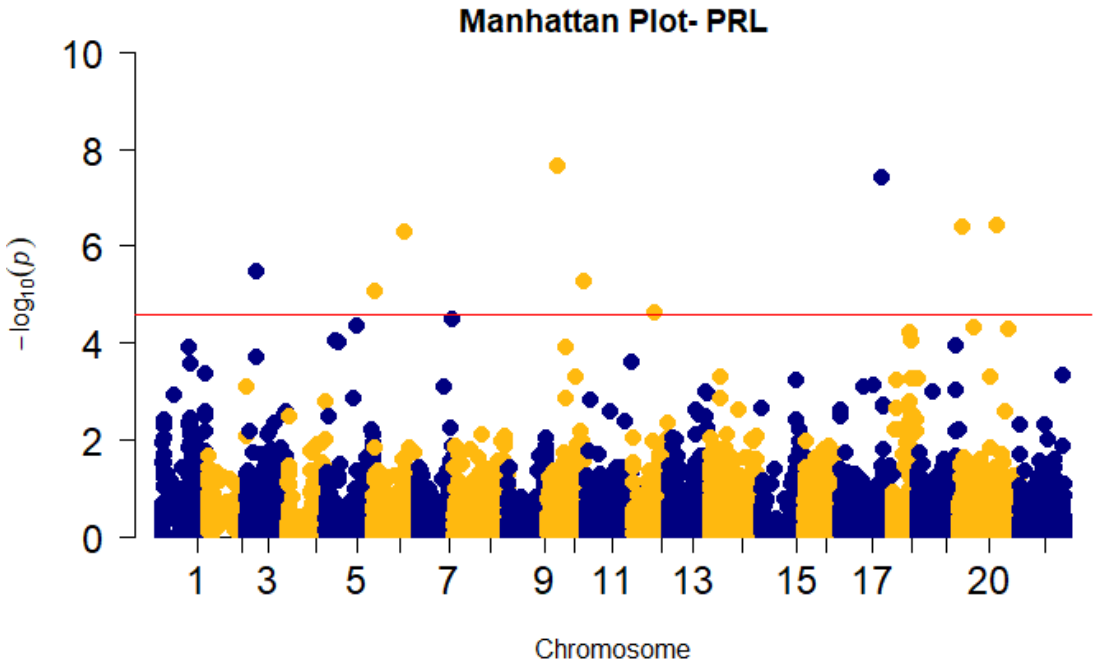

Figure 3.8 Manhattan plots for primary root length with chromosome on the x-axis and −log10P on the y-axis. Each dot represents an SNP. Red line indicates the threshold of significance.

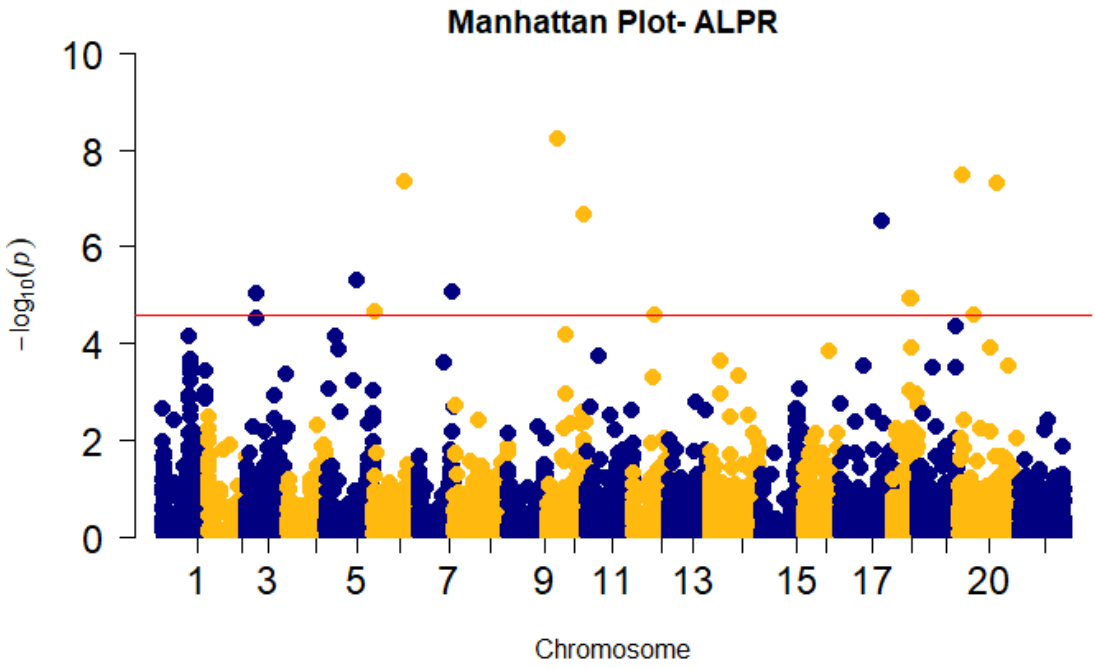

Figure 3.9 Manhattan plots for the average length of primary root with chromosome on the x-axis and −log10P on the y-axis. Each dot represents an SNP. Red line indicates the threshold of significance.

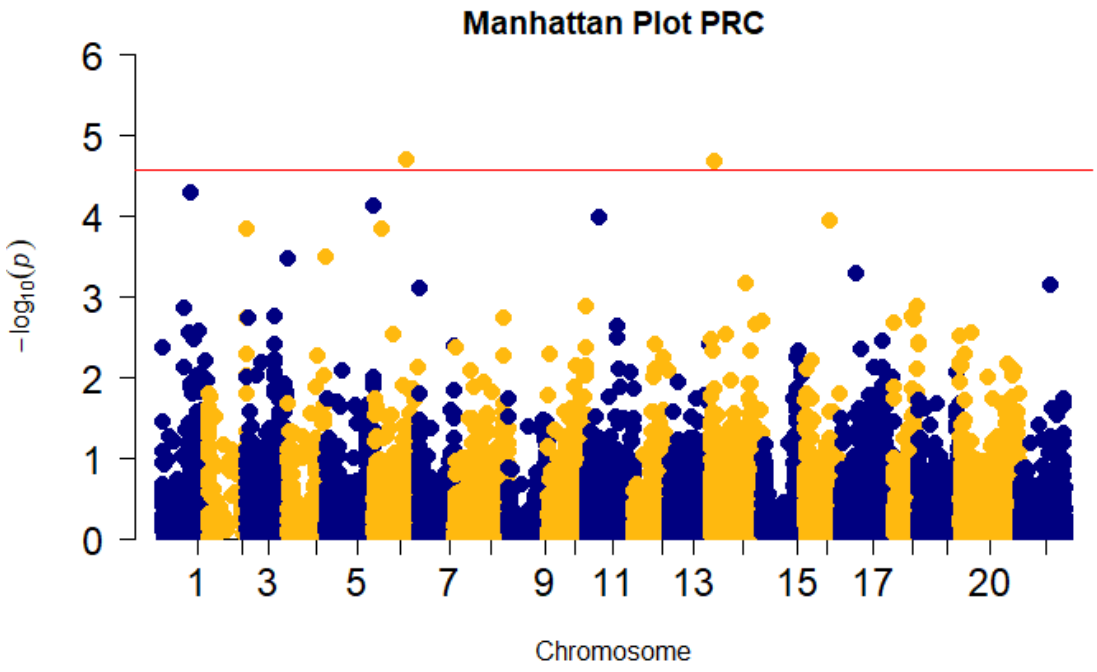

Figure 3.10 Manhattan plots for primary root number with chromosome on the x-axis and −log10P on the y-axis. Each dot represents an SNP. Red line indicates the threshold of significance.

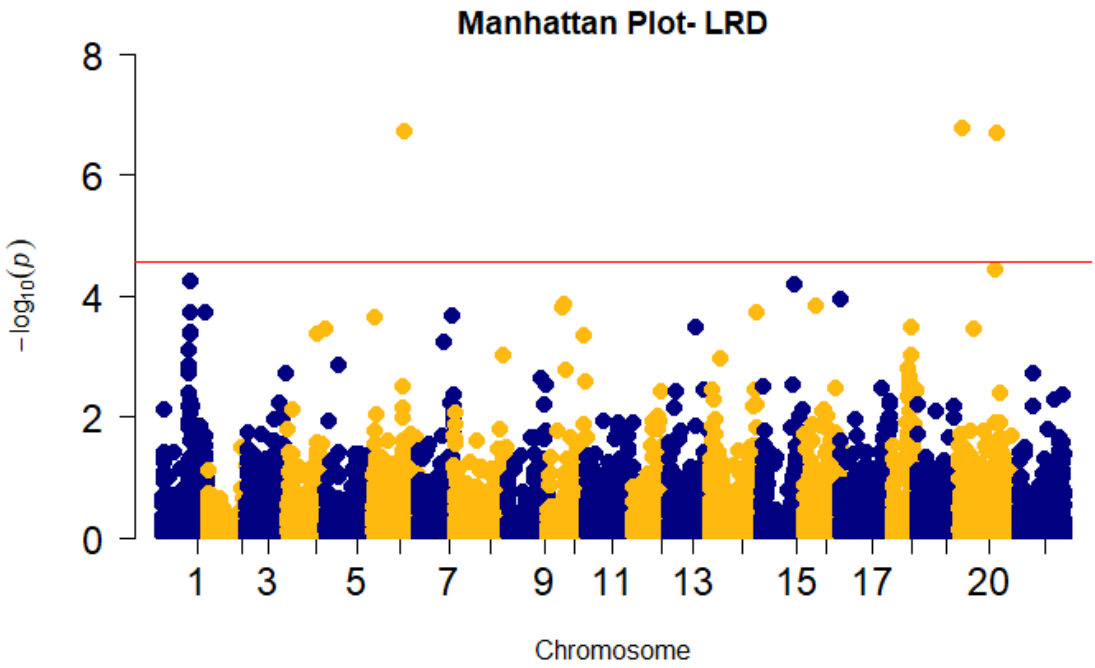

Figure 3.11 Manhattan plots for lateral root density with chromosome on the x-axis and −log10P on the y-axis. Each dot represents an SNP. Red line indicates the threshold of significance.

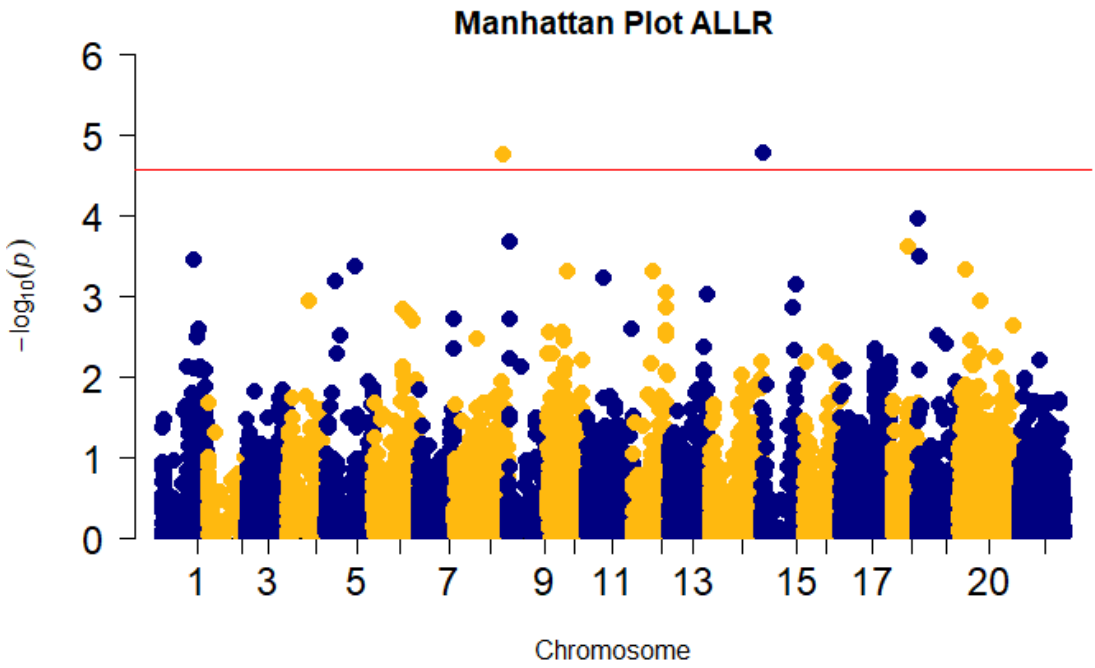

Figure 3.12 Manhattan plots for the average length of lateral root with chromosome on the x-axis and −log10P on the y-axis. Each dot represents an SNP. Red line indicates the threshold of significance.

Discussion

In this study, we utilized a high-throughput phenotyping method to measure root traits in oat seedlings and revealed many SNPs associated with different root traits and potential candidate genes affecting those traits. The phenotyping system used in this study allows for rapid phenotyping of a large number of plants, and quantification of several root system architectural traits in a relatively short time (Atkinson et al., 2015). Genome-wide association analysis is a powerful tool to study the association between a genome and phenotype and to identify causal loci or genes, however, for obtaining meaningful results relatively large numbers of individuals are needed. Measuring root traits in field conditions is difficult and time-consuming, thus the phenotyping protocol utilized in this study is effective for phenotyping a larger number of individuals for root traits for genome-wide association studies. Testing and selection for root traits in a laboratory setting are subjected to criticism. While it is assumed that trait estimates in a laboratory setting are carried over to the soil (primary) environment, there are not enough studies to confirm this. However, phenotyping in field conditions through root excavation also has its limitations, like low heritability, and loss of roots (distorted root architecture) behind in soil depending upon the size of the soil core or dimension of the excavation device.

Although optimization of the root system has been proposed for improvement in yield, genetic dissection and improvement of roots are rarely attempted (Lynch & Wojciechowski, 2015). Root architecture is an important plant trait that varies greatly amongst genotypes. A wide range of variability for the traits evaluated in this study is seen in Table 1. Phenotypic variability is an important part of association analysis, and traits with moderate to high heritability estimates can be considered for GWAS since

heritability is an indicator of how much genetic variance contributes to the phenotype (Alqudah et al., 2020). The heritability estimates for our root traits ranged from 0.17 to 0.59, similar results have been reported for root traits in maize, rice, and wheat (Cane et al., 2014; Pace et al., 2015; Phung et al., 2016).

In this study, we were able to identify significant SNP and root trait associations and were able to identify candidate genes located in proximity to those markers. Candidate genes with various roles in overall plant growth and development and with role in root development were explored. We found multiple traits associated to the same SNP locus because the traits were highly correlated. The same SNP locus has been associated to multiple correlated root traits in maize as well (Wu et al., 2022). The genes controlling the oat root system may have multiple effects. The SNP marker avgbs2_120048.1.27 was associated with maximum width of the root system, maximum depth of the root system, and convex hull area. This marker was found within a gene that encodes for an Auxin efflux carrier family protein. This is an auxin efflux transporter and helps in root-specific auxin transport and mediates root gravitropism. Many proteins related to auxin efflux are involved in the root development of *Arabidopsis* (Garay-Arroyo et al., 2013). This gene may be involved in the root development process in oats. In *Arabidopsis*, epidermal expression of a sterol biosynthesis gene drives root growth through a non-cellular autonomous method. We found a gene coding C-8 sterol isomerase within 4kb downstream of a marker avgbs_36707.1.7 in chromosome 7C. The *Arabidopsis* HYDRA1 (HYD1) gene encodes sterol 8-7 isomerase, and although hyd1 seedlings are deficient in radial patterning throughout numerous tissues, HYD1 gene is most robustly expressed in the root epidermis (Short et al., 2018). In the mutant, the seedling usually

produces a very short root, a short hypocotyl with roots that are defective in the apical meristem with aberrant patterning of surrounding cells (Short et al., 2018). A gene coding for Glycosyltransferase was found within 12kb upstream of avgbs_cluster_2187.1.35. Glycosyltransferases play an important role in cellular metabolism as they modify the activities of structural and regulatory metabolites. *Arabidopsis* Glycosyltransferase Mutant ray1 mutants show about 19% smaller primary roots compared to wildtypes (Gille et al., 2013). *Arabidopsis thaliana* plants expressing PsUGT1, a UDP-glucuronosyltransferase encoding gene from *Pisum sativum,* show an altered root morphology where the root does not respond to gravity (Woo et al., 2003; Woo et al., 2007). Another gene close to one of the significant markers is MYB transcription factor which is 13kb downstream of the marker avgbs_14605.1.37 on chromosome 5C. MYB proteins are important components of regulatory networks that control development, metabolism, and biotic and abiotic stress responses (Dubos et al., 2010). Many subgroups within the MYB gene family including AtMYB068 and AtMYB059 are involved in root development and root elongation (Feng et al., 2004; Mu et al., 2009). Huang et al. (2020) conducted a GWAS to study seed vigor in oat and identified many SNPs associated with different root traits (root surface area, root growth rate, root relative growth rate, average root surface area) measured at day 3, 4 and 5 days after sowing on germination paper. Although none of the markers identified in our study were the same as the ones identified by Huang et al. 2020, one of the markers (avgbs_62666.1.21) we identified was significantly associated with plant height in oat based on GWAS results from T3 oat.

Although the root system architectural traits play a vital role in capturing heterogeneously distributed soil resources, crop breeders tend to focus on above-ground traits because of

the difficulty in phenotyping root traits. Many image analysis methods have recently been developed to quantify a variety of root traits, and these tools are still evolving to evaluate more complicated root traits. Many recent genome-wide studies of root traits were done in the seedling stage using non-soil growth platforms. Although root phenotyping at the seedling stage in a laboratory setting has limitations, many studies have successfully performed genome-wide studies by root phenotyping root traits at the seedling stage in various growth platforms and identified QTLs associated with those root traits (Atkinson et al., 2015; Courtois et al., 2013; Pace et al., 2015; Sanchez et al., 2018; Tuberosa et al., 2002). While there are not many genome-wide studies for roots in which root phenotyping is done on adult plants in a field setting, there are studies that found that QTLs for RSA traits can overlap with QTLs for yield (Cai et al., 2012; Steele et al., 2007; Tuberosa et al., 2002). In some cases, there may not be significant SNPs associated with both the root traits and the agronomic and yield traits, however, the candidate genes identified for the roots and the yield traits can be common (Wu et al., 2022).

Overall, we successfully phenotyped oat seedling roots using a germination paper-based growth system, image analysis, and conducted an association analysis on 285 oat genotypes. We found 82 significant maker trait associations and many SNPs were significantly associated with more than one trait that were highly correlated. We also found 39 candidate genes that are close to 16 unique SNP markers. We explored the potential role of the genes in controlling oat seedling root traits. Some genes identified in this study with a potential role in root development are MYB transcription factor, C-8 sterol isomerase, Glycosyltransferase, Ubiquitin-like protein 5, and Auxin efflux carrier family protein. While we explored the function of genes close to the significant SNPs, the

SNP trait association can be the result of more distant genes, especially in the case of enhancers and repressors, thus mapping SNPs to the nearest gene may lead to false SNP-gene mapping (Brodie et al., 2016). Thus, further exploration of genes near the SNP markers and understanding their function is necessary.

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CHAPTER 4

Evaluation of morpho-physiological traits of oats (*Avena sativa* L.) under drought stress

Abstract

Drought is the major cause of agricultural production losses globally. Drought can reduce yield, decrease crop quality, and as a result impact global food security. The increase in intensity and frequency of drought, due to global climate change, has raised the urgency of developing crop cultivars suitable for dry environments. Drought tolerance involves numerous plant physiological, biochemical, and morphological responses at the root and shoot levels. While it is known that oat genotypes vary in their ability to cope with drought, the role of root system morphology in drought tolerance has not been fully investigated in oats. In this study, we measured the morpho-physiological response of ten oat genotypes to drought stress to improve our understanding of the role of the root system in drought tolerance in oats. Twenty-one day old seedlings were subjected to drought stress by withholding water for two weeks. Following the drought treatment, we examined chlorophyll content, relative water content, stomatal conductance, stomata number, shoot dry weight, root dry weight, root length, root area, and root volume. We also measured the seed yield by continuing the drought treatment with a drying and rewatering cycle every 15 days until physiological maturity. An analysis of variance showed a significant impact of water regime on all traits evaluated. The cultivar that showed the lowest decrease in yield under drought (Hayden) also showed a relatively smaller decrease in relative water content, chlorophyll content, and a sharp decrease in stomata number. Thus, maintaining relative water content, chlorophyll content, and

reducing stomata number under drought may help oat plants cope with drought stress by better regulating the plant water status and maintaining photosynthesis level. Our results also suggest that a larger root length, root area, and root volume may not always contribute to higher yield under drought stress, however, additional studies are needed to improve our understanding of the importance of root mass distribution into soil layers for drought adaptation in oats.

Introduction

Water deficit is a major crop production constraint that reduces crop quality and productivity, and compromises economic output and global food security (Farooq et al., 2009). According to the FAO, drought has been determined as the single greatest reason for agricultural production loss. Over 34% of the losses in crop and livestock production in the least developed countries and low to middle-income countries from 2008 to 2018 was due to drought and amounted to a loss of USD 37 billion (FAO, 2018). A meta-analysis of drought and heat stress combination on crop yield revealed that crops subjected to drought displayed a 48% yield reduction, while the crops subjected to a combination of drought and heat stress resulted in a 65% reduction in yield (Cohen et al., 2021).

With global climate change, the frequency and severity of drought have increased. The average impact of drought and heatwave on crop production has tripled over the last fifty years in Europe (Brás et al., 2021). In 2021, drought has significantly reduced grain yield for oats produced in North America. The USDA estimated the oat production in 2021 around 41.3 million bushels which is lowest on records since 1866 (Michael & Carey, 2021). Similarly, Canada's oat harvest in 2021 was around 268.7 million bushels which is

about 15% lower than the previous year (Michael & Carey, 2021). As the climate becomes hotter, and drought becomes more frequent and severe, there is an urgent need to develop high-yielding varieties that uses water more efficiently (Gupta et al., 2020). Difference in drought tolerance among different varieties have been reported in many crops. The drought tolerance associated traits are controlled by quantitative traits thus, many genes with small effects are involved in drought tolerance (Chloupek et al., 2010). Genetic variability in drought tolerance in oat genotypes has been reported based on their performance under rainfed and irrigated conditions (Akcura & Ceri, 2011; Zaheri & Bahraminejad, 2012).

Many traits that control overall plant water relations such as relative water content (RWC), leaf water potential, and transpiration rate are significantly affected by drought. A reduction in RWC in response to drought has been reported in many crops (Ahmad et al., 2018; Canales et al., 2021; Meher et al., 2018; Swapna & Shylaraj, 2017). Higher RWC is considered as an indicator of drought tolerance and tolerant varieties may have an active accumulation of solutes for osmoregulation under drought conditions (Ahmed et al., 2020). Cultivars with higher RWC and chlorophyll content under drought may be more resistant to drought stress and yield stability (Keyvan, 2010).

Stomata play a central role in controlling leaf gas exchange and the stomatal closure can be initiated by many environmental cues such as elevated CO_2, elevated leaf to air vapor pressure deficit, soil water deficits, and abscisic acid (ABA) (Li et al., 2020). Plants can optimize their water use in various environments by regulating their stomatal features like stomatal size, stomatal density, and stomatal aperture to control the rate of water vapor loss and CO_2 intake (McAdam & Brodribb, 2012). Stomatal closure will not only reduce

transpirational water loss, but also limits CO_2 absorption and thus impacts photosynthesis and growth. The stomatal morphological features are plastic to abiotic stress. In rice, fewer stomata are associated with drought tolerance. When rice cultivar IR64 was engineered to produce fewer stomata, it showed improved tolerance to drought (Caine et al., 2019). In wheat, drought tolerance is regulated by stomatal characteristics through a reduction in transpiration rate. Drought tolerant wheat cultivar ‘Changhan 58’ showed lower stomatal density and higher stomatal area per unit organ (leaf, glume, lemma, and palea) area when compared to susceptible ‘Xinong 9871’ (Li et al., 2017). Plants produce ABA in roots in response to drought which induces stomatal closure (Brodribb & McAdam, 2013). The sensitivity of stomata to ABA plays a critical role in controlling transpiration and water use efficiency (Ghimire et al., 2021).

Drought tolerance is a highly complex process involving physiological, biochemical, and morphological traits both below and above ground levels (Canales et al., 2019). However, research efforts have primarily focused on the effect of drought on shoot development parameters. Root parameters on the other hand have not been investigated as often. Roots are the first organ to sense drying soil and to initiate a signaling cascade that leads to the overall plant's response to drought stress (Schachtman & Goodger, 2008).

The root system size and distribution determine the plant's access to water and thus sets the limit on the function of the plant shoot system. A deeper root system is shown to be effective for greater water uptake from soil and improving yield under drought conditions in wheat (A. Wasson et al., 2012). Accessing stored groundwater through deep roots can maintain more open stomata, have a cooler canopy and higher NDVI and thus maintain better plant morphology and photosynthetic capacity in wheat (Li et al., 2019). The size

of the root system is an important factor in the acquisition of soil resources but only when considered with whole-plant size (Comas et al., 2013). Dry root mass can change in response to drought, but may not capture all variations in root morphology, architecture, and physiology (Boot & Mensink, 1990; Comas et al., 2013). Root dry mass can remain constant while total root length, root area, root diameter, and proportion of coarse to fine roots may change dramatically in response to drought stress (Comas et al., 2013). In oats, drought tolerant genotypes showed increased root length, and higher branching rate, root surface area, and length of fine roots in comparison to drought susceptible genotypes (Canales et al., 2019). Evaluating diverse oat genotypes for root traits under drought stress may further explains the role of root system in drought tolerance.

The objective of this study is to evaluate the morphological and physiological traits of oats under drought and to analyze the root architectural component that contributes to the ability of oats to cope with drought.

Materials and methods

Plant materials

Ten oat cultivars (Clintford, Checota, Deon, Hayden, Goliath, Gopher, MN Pearl, Kame, Saddle, and SD140327) were used in this study. These cultivars were selected from among 285 oat genotypes based on seedling root characteristics (see Chapter 3). A cluster analysis of seedling root traits was conducted to select genotypes with diverse root system architectural traits. The cultivar Checota was selected as a drought tolerant check based on previous reports (Benlioglu & Ozkan, 2021).

Seeds were first pregerminated for two days on germination paper and a single plant was planted in each 4 × 14″ tall tree pots filled with topsoil (Stew and Sons, Inc., Tangent, Oregon, 97389). The experiment was conducted in completely randomized design in a greenhouse maintained at 24^{o}C temperature. The experiment was conducted twice with five replications each time (five plants per genotype-water regime treatment). The plants were grown for 21 days in well-watered conditions by watering with a nutrient solution (Peters Professional 20:20:20 at 0.2 g L^{-1}) in all pots every three days. After 21 days, the well-watered (control) plants were watered every third day and the drought treatment plants were not watered. The drought treatment was continued for 15 days, after which, shoot and roots were harvested. The shoot was dried at 60^{o}C for 72 hours before collecting the dry weight. The roots were cleaned from soil and scanned before they were dried for root dry weight determination. To determine the yield of plants under drought stress, the drought treatment was continued on another set of plants with five plants per treatments with a drying and rewatering cycle every 15 days until physiological maturity.

Relative water content (RWC)

Relative water content was determined on the mid-leaf section of the youngest mature leaf. A leaf sample was collected from every plant from all treatments. A leaf section (approximately 5 cm) was cut and weighed immediately to determine the fresh weight (W). After being hydrated in deionized water for 24 hours in a closed petri dish, the leaf samples were well dried of any surface moisture using a paper towel and weighed to determine the turgid weight (TW). The samples were then oven-dried and weighed to determine the dry weight (DW).

$$\text{RWC}(\%) = \frac{W-DW}{TW-DW} \times 100$$

W – Sample fresh weight

TW – Sample turgid weight

DW – Sample dry weight.

Stomata number

Stomata numbers were determined for each plant by the leaf imprints technique on the youngest mature leaf. A thin layer of nail polish was applied to the leaf surface. The nail polish was allowed to dry and the thin film of nail polish on the leaf was peeled off using a clear scotch tape. The nail polish film with the imprint of the leaf was mounted on a microscope slide and observed under a light microscope (ATC 2000 Leica, Buffalo Grove, IL). Stomata were counted at three random spots in each leaf imprint. The area of the field of view was determined using a stage micrometer and the stomata were counted in a field of view with an area of 2.01 mm^2.

Stomatal conductance

The stomatal conductance was measured using a portable SC-1 leaf porometer (Decagon Devices, Pullman, WA). The youngest fully matured leaf from each plant was chosen for measuring stomatal conductance. One measurement was made on every plant in each treatment.

Chlorophyll content

CCM-200 plus Chlorophyll Content Meter (Opti-Sciences, Inc., Hudson, NH) was used to measure chlorophyll content. The youngest fully matured leaf from each plant was

chosen for measuring stomatal conductance. One measurement was made on every plant in each treatment.

Root morphology

The roots were cleaned and scanned with an Epson flatbed scanner (Epson America, Inc. Los Alamitos, CA). The scanned root images were run through WhinRhizo to measure root length, root area, and root volume.

Statistical analysis

Statistical analysis was done with the R programing language (R Core Team, 2020). Multiple comparisons between treatment means were done with the least significant difference using agricolae package in R (de Mendiburu & de Mendiburu, 2019).

Results

To evaluate the morpho-physiological response of oat genotypes to drought stress, ten oat genotypes were subjected to drought stress by withholding watering on 21 day old plants for 15 days. After 15 days of drought treatment, various morpho-physiological traits such as shoot dry weight, root dry weight, root length, root area, root volume, RWC, chlorophyll content, stomatal conductance, stomata number, and grain yield per plant were evaluated. An analysis of variance showed that water regime had a significant impact on all traits evaluated. The genotype had a significant effect on all traits except for stomatal conductance. A significant genotype by water regime interaction was observed for RWC, chlorophyll content, stomatal conductance, grain yield, and stomata number. There was no significant interaction between the genotype and water regime for shoot dry

weight and root traits (root dry weight, root to shoot ratio, root length, root area, and root volume).

Significant difference was observed among the oat genotypes for shoot dry weight. Saddle along with Kame and Hayden produced the highest shoot dry weight (Fig 4.1A) under both well-watered and drought treatments. All ten genotypes exhibited significantly lower shoot dry weight under drought stress (Fig. 4.1A). The reduction in shoot dry weight from drought treatment ranged from 24 to 35 % depending on the genotype (Fig. 4.1B). There was no significant difference among genotypes for their response to drought stress expressed as a percent change in shoot dry weight between the two treatments (Fig 4.1B).

The chlorophyll content was highest for Clintford under well-watered conditions, and for Saddle, Kame and Clintford under drought treatment (Fig 4.2A). The lowest chlorophyll content was observed in Gopher and SD140327 under drought stress. A significant difference was observed in the response of genotypes to drought stress (Fig 4.2B). Drought treatment caused a significant reduction (close to 25%) in chlorophyll content in four genotypes (Checota, Clintford, Gopher, and SD140327). The reduction in chlorophyll content under drought stress was not significant in Deon, Goliath, Hayden, Kame, MN Pearl, and Saddle. Checota, a drought tolerant cultivar showed a significant decrease (25%) in chlorophyll content and the %change in chlorophyll content in Checota was significantly higher than Deon, Hayden, Kame, MN Pearl and Saddle.

The relative water content was 95-96% for all genotypes under well-watered conditions, and there was no significant difference in RWC between oat genotypes under well-watered conditions. A significant decrease in the RWC in response to drought stress was

observed in all genotypes except for SD140327 (Fig 4.3A). The strongest decrease in RWC was observed in Saddle (23%) followed by Kame (16.7%) and Gopher (14.2%) (Fig 4.3B). The %decrease in relative water content in Checota was significantly lower compared to Saddle.

Stomatal conductance ranged from 548-704 mmol m^{-2} s^{-1} for all genotypes and under well-watered conditions. MN Pearl and SD140327 showed significantly smaller stomatal conductance compared to other genotypes (except Gopher) under well-watered conditions (Fig 4.4A). Under drought conditions, SD140327 showed significantly higher stomatal conductance compared to Hayden, Checota, and Saddle (Fig 4.4A). All genotypes showed a significant decrease in stomatal conductance in response to drought. The decrease in stomatal conductance was highest in Saddle (82%) followed by Hayden (76.8%) and Checota (74.9%), and the lowest decrease in stomatal conductance was observed in SD140327 (55.7%) (Fig 4.4B).

Stomata numbers varied greatly under well-watered conditions with the highest stomata number for Hayden, Checota, Goliath, and MN Pearl, and the lowest stomata number for Deon, Clintford, and Kame (Fig 4.5A). A significant decrease in stomata number was observed in seven genotypes (Checota, Deon, Hayden, Kame, MN Pearl, Saddle, and SD140327). The decrease in stomata number was however not significant in Clintford, Goliath, and Gopher. Hayden showed the greatest decrease in stomata number with 33% followed by Saddle (24.5%) and Checota (22.6%) (Fig 4.5B). Checota showed a significantly higher %decrease in stomata number compared to Clintford, Goliath, and Gopher,

Grain yield per plant varied greatly among genotypes ranging from 2.4 - 4.6 g/plant under well-watered conditions, with the highest yield for Goliath, Deon and Hayden, and the lowest yield for Gopher and Clintford (Fig 4.6A). All genotypes showed a significant decrease in yield under drought stress. There was a significant difference among the genotypes for the percent change in yield under drought stress. The highest decrease in yield was observed in Saddle (45.2%), Goliath (45.2%) and Deon (42.3%) and the lowest decrease in yield was observed in Hayden (22.2%) (Fig. 4.6B). The % change in grain yield per plant was intermediate in Checota compared to other cultivars. And it was not significantly different form another cultivar.

Significant difference was observed for root dry weight among genotypes under well-watered conditions with Saddle showing significantly higher root dry weight compared to Deon, Checota, Clintford, Gopher, and SD140327 (Fig 4.7A). A significant decrease in root dry weight was observed in Clintford, Deon, Goliath, Hayden, Kame, MN Pearl, Saddle, and SD140327. No significant difference was observed in % change in root dry weight among the genotypes (Fig 4.7B).

There was little variation in root to shoot ratio among genotypes under well-watered conditions. MN Pearl showed a significantly higher root to shoot ratio compared to Gopher, Kame, Saddle, and SD140327 under well-watered conditions. Under drought conditions, Checota showed a significantly higher root to shoot ratio compared to Clintford, Deon, Gopher, Hayden, Kame, and SD140327 (Fig 4.8A). Checota and Gopher were the only cultivars that showed a significant increase in the root to shoot ratio under drought conditions. The increase in root to shoot ratio was highest in Gopher (21%) followed by Checota (19%) (Fig 4.8B).

Total root length ranged from 2506-4387 cm among genotypes under well-watered conditions with MN Pearl showing significantly higher root length compared to Deon, Gopher, Checota, Clintford, Hayden, and SD140327 (Fig 4.9A). Under drought conditions, Saddle showed significantly higher root length compared to Clintford and SD140327. A significant decrease in root length was observed in all genotypes, and no significant difference was observed among oat genotypes for the % change in root length under drought stress.

Root area ranged from 316 – 545 cm^2 and significant difference was observed among the oat genotypes under well-watered conditions. MN Pearl showed a significantly higher root area compared to Checota, Clintford, Gopher, Hayden, Kame, and SD140327. Under drought conditions, MN Pearl showed a significantly higher root area compared to Checota, Clintford, Hayden, Kame, and SD140327 (Fig 4.10A). A significant decrease in root area was observed in all genotypes except SD140327. A significant difference in % change in root area was observed with Saddle (31.7%) showing a significantly larger increase in root area compared to SD140327 (14%) (Fig. 4.10B).

Root volume ranged from 3.2-5.4 cm^3 under well-watered conditions and MN Pearl showed significantly higher root volume compared to Checota, Clintford, Gopher, Kame, and SD140327 under well-watered conditions (Fig 4.11A). A significant decrease in root volume was observed in all genotypes except SD140327. The decrease in root volume was highest in Saddle (35.7%) followed by Hayden (30.3%) and Deon (24.8%) (Fig. 4.11B). The % change in root volume was higher in Saddle compared to Checota, Goliath, Gopher and SD140327 (Fig 4.11B).

There were strong positive correlations between many traits when evaluated under both well-watered and drought-stressed conditions. The strongest correlation was observed between root dry weight and shoot dry weight (r=0.85) and among root length, root area, and root volume (r= 0.70 -0.94) (Fig 4.12). When we evaluated the correlation between traits under well-watered and drought conditions separately, different correlation patterns were observed. In well-watered conditions, a strong correlation was observed between root and shoot dry weight (r=0.85), and among root traits (root length, area, and volume (r=0.71-0.94). Shoot dry weight was also strongly correlated with root length, root area, and root volume (r=0.42-0.53) (Fig. 4.13). Correlation analysis under drought conditions also revealed a strong correlation between shoot dry weight with root dry weight and chlorophyll content (r=0.61 and 0.51). But we also observed a strong negative correlation between relative water content and shoot dry weight and root dry weight (r= -0.68 and -0.59) (Fig 4.14).

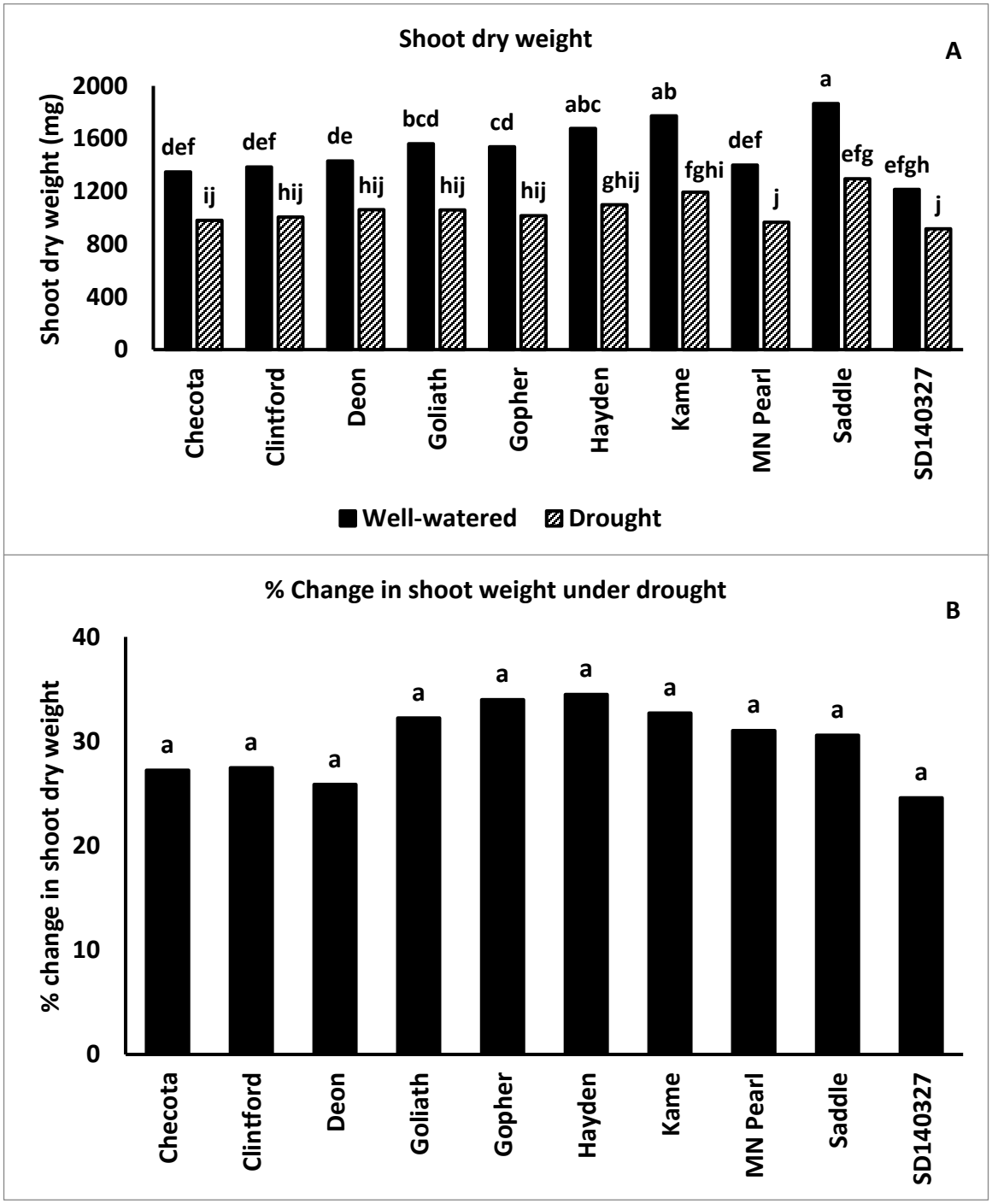

Figure 4.1 (A) Shoot dry weight of ten oat genotypes under well-watered and drought conditions, (B) Percent change in shoot biomass in response to drought stress. Different letters indicate a significant difference between treatments ($p<0.05$).

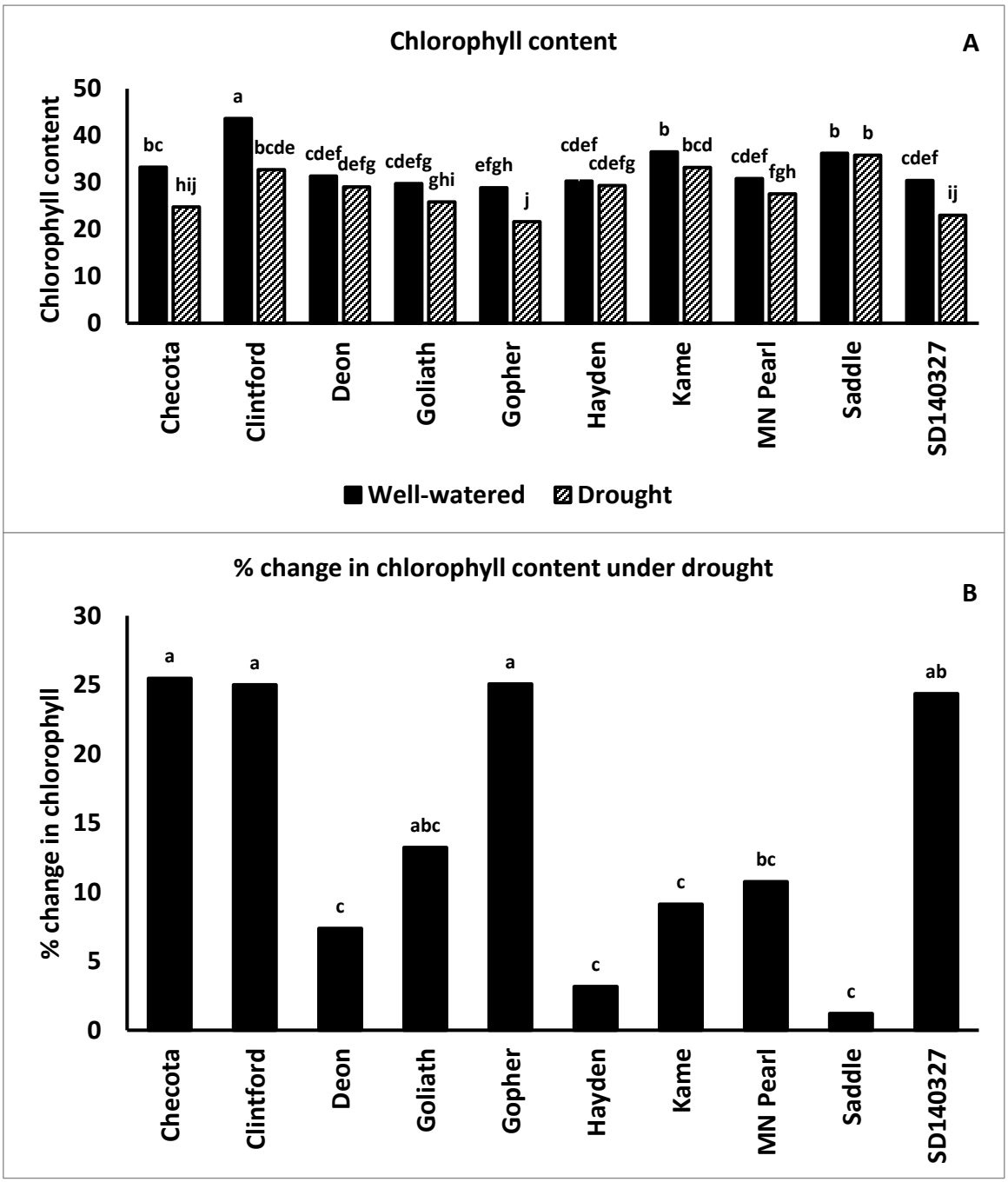

Figure 4.2 (A) Chlorophyll content of ten oat genotypes under well-watered and drought conditions, (B) Percent change in chlorophyll content in response to drought stress. Different letters indicate a significant difference ($p<0.05$).

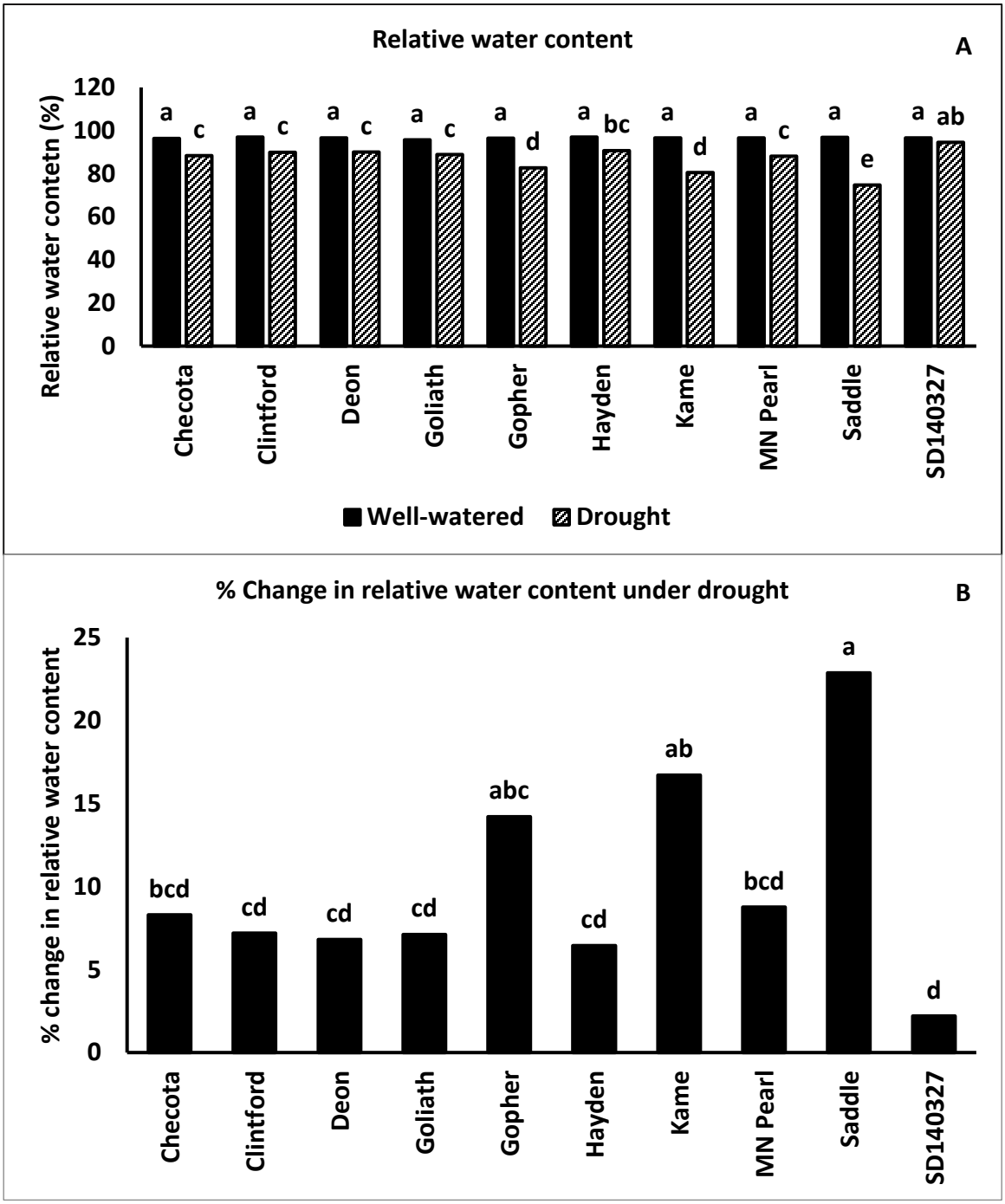

Figure 4.3 (A) Relative water content of ten oat genotypes under well-watered and drought conditions, (B) Percent change in relative water content in response to drought stress. Different letters indicate a significant difference ($p<0.05$).

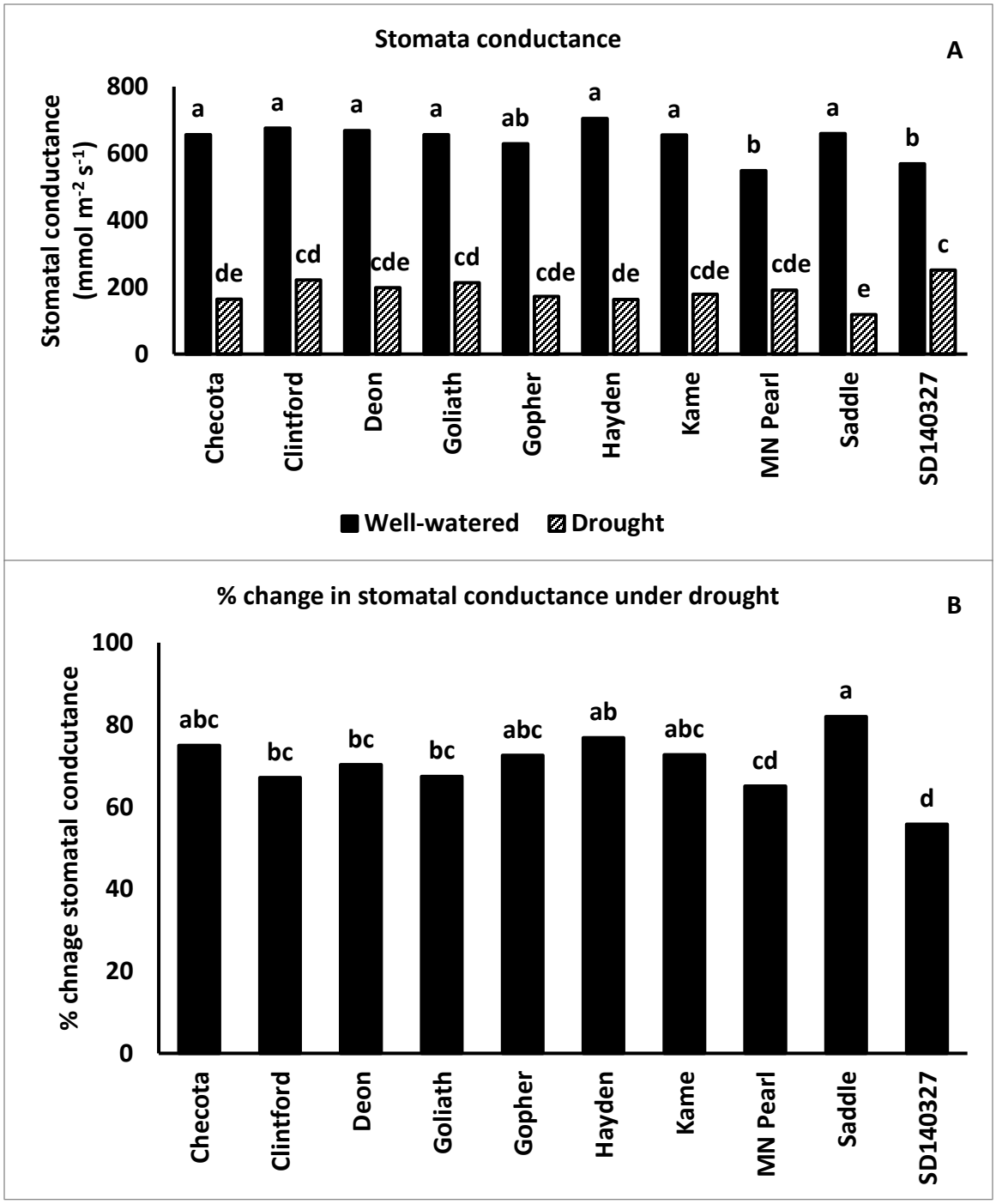

Figure 4.4 (A) Stomatal conductance of ten oat genotypes under well-watered and drought conditions, (B) Percent change in stomatal conductance in response to drought stress. Different letters indicate a significant difference ($p<0.05$).

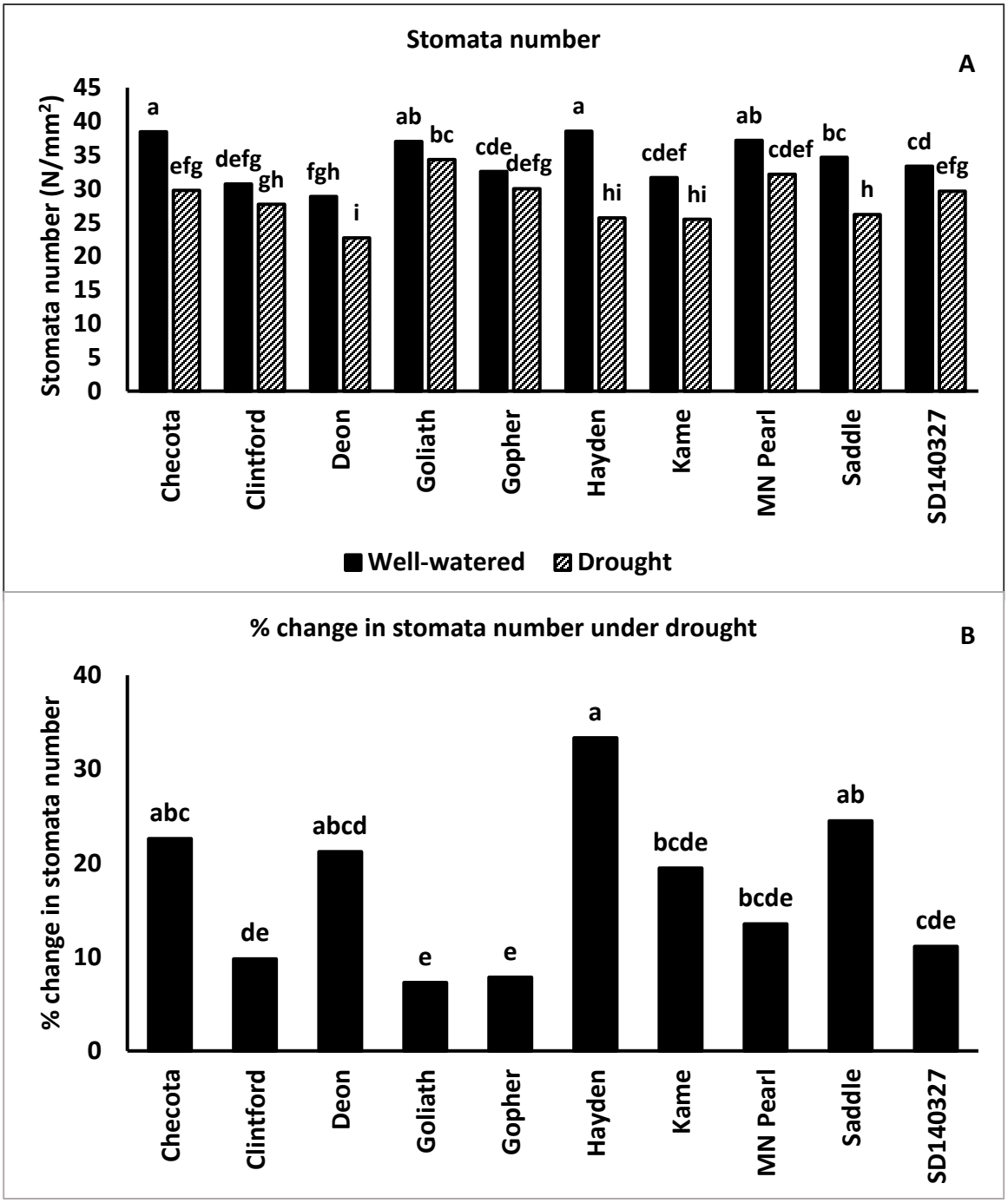

Figure 4.5 (A) Stomata number of ten oat genotypes under well-watered and drought conditions, (B) Percent change in stomata number in response to drought stress. Different letters indicate a significant difference ($p<0.05$).

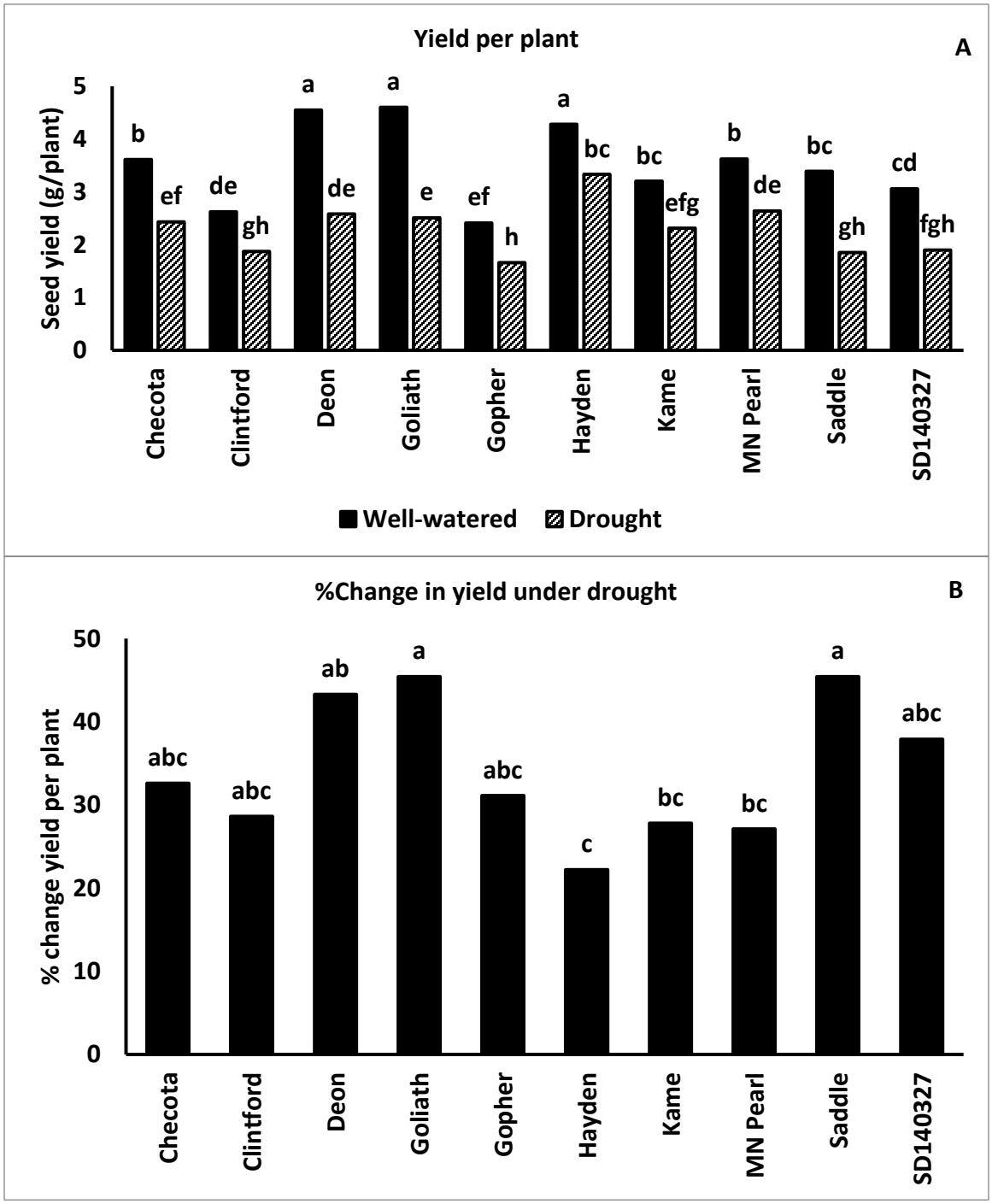

Figure 4.6 (A) Yield per plant of ten oat genotypes under well-watered and drought conditions, (B) Percent change in yield per plant in response to drought stress. Different letters indicate a significant difference ($p<0.05$).

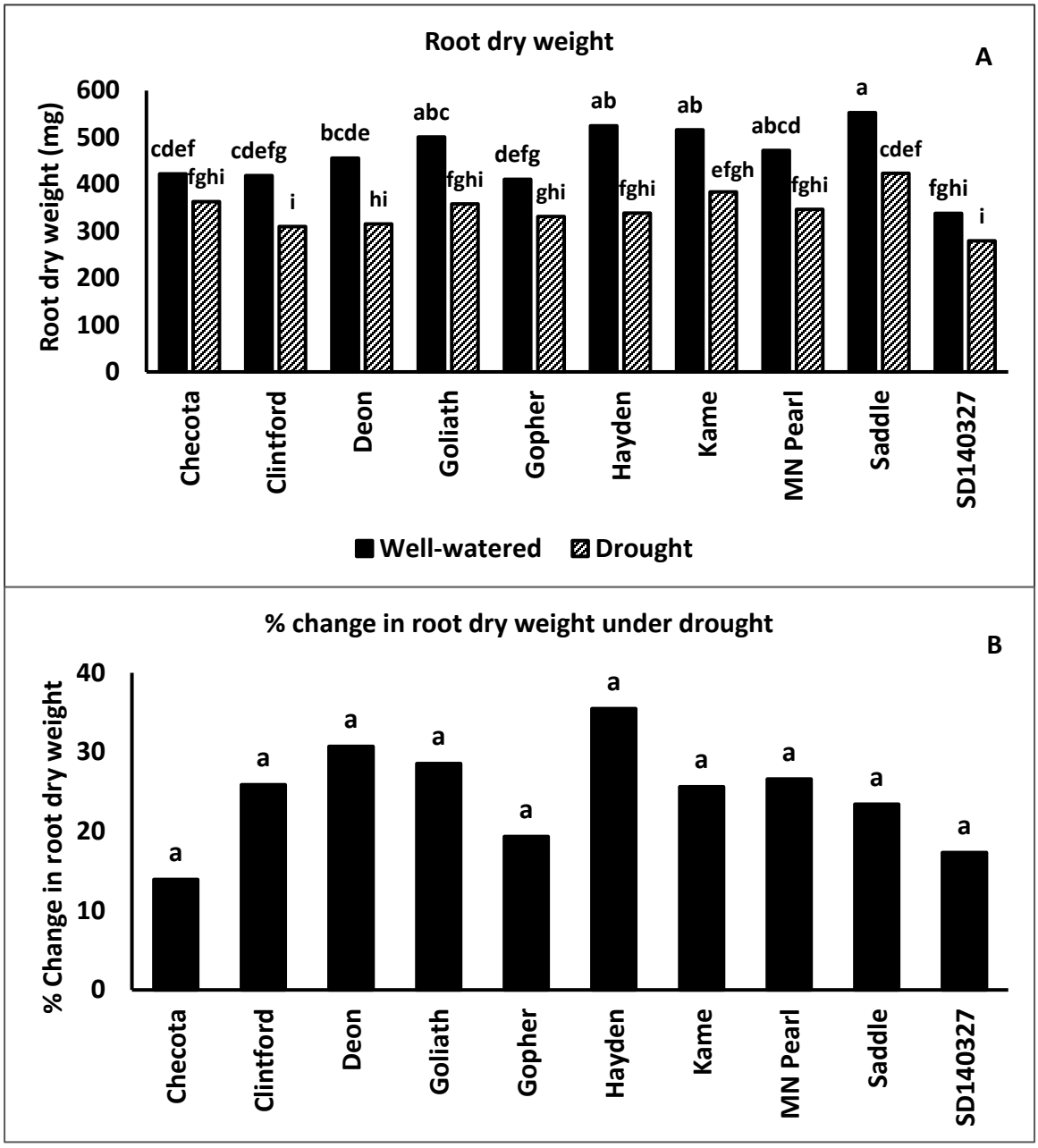

Figure 4.7 (A) Root dry weight of ten oat genotypes under well-watered and drought conditions, (B) Percent change in root dry weight in response to drought stress. Different letters indicate a significant difference ($p<0.05$)

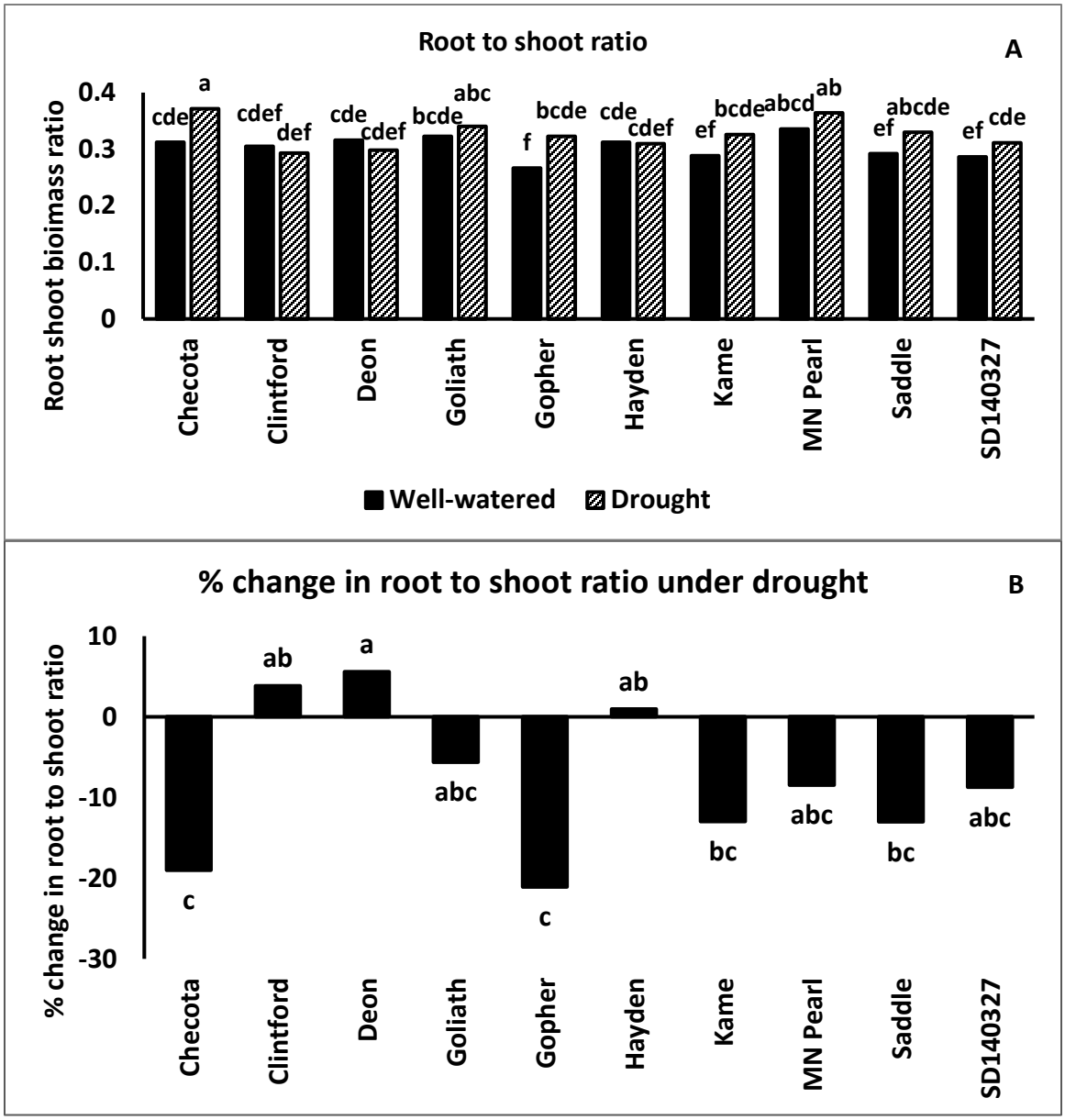

Figure 4.8 (A) Root to shoot biomass ratio of ten oat genotypes under well-watered and drought conditions, (B) Percent change in root to shoot ratio in response to drought stress. Different letters indicate a significant difference ($p<0.05$).

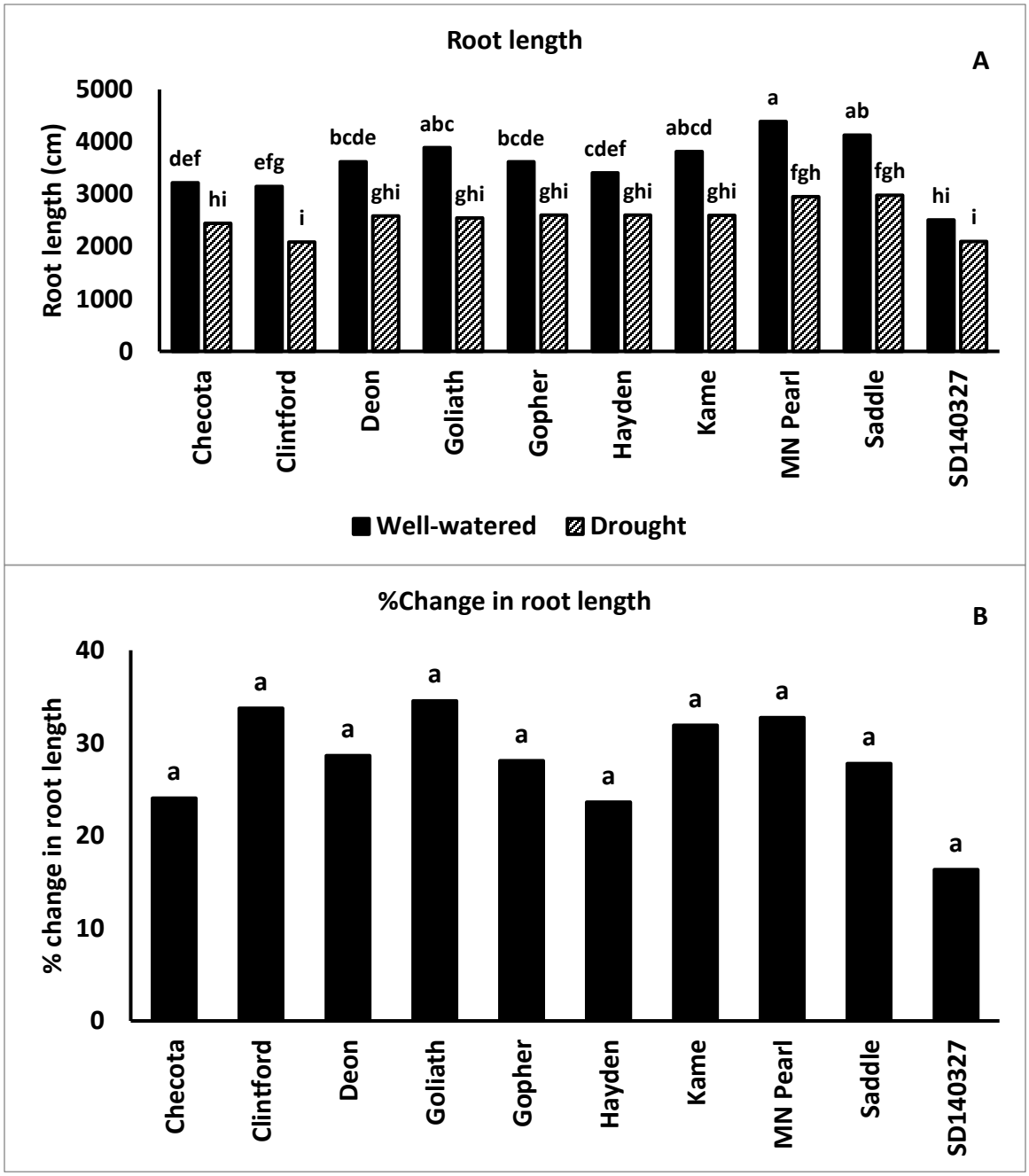

Figure 4.9 (A) Root length of ten oat genotypes under well-watered and drought conditions, (B) Percent change in root length in response to drought stress. Different letters indicate a significant difference ($p<0.05$)

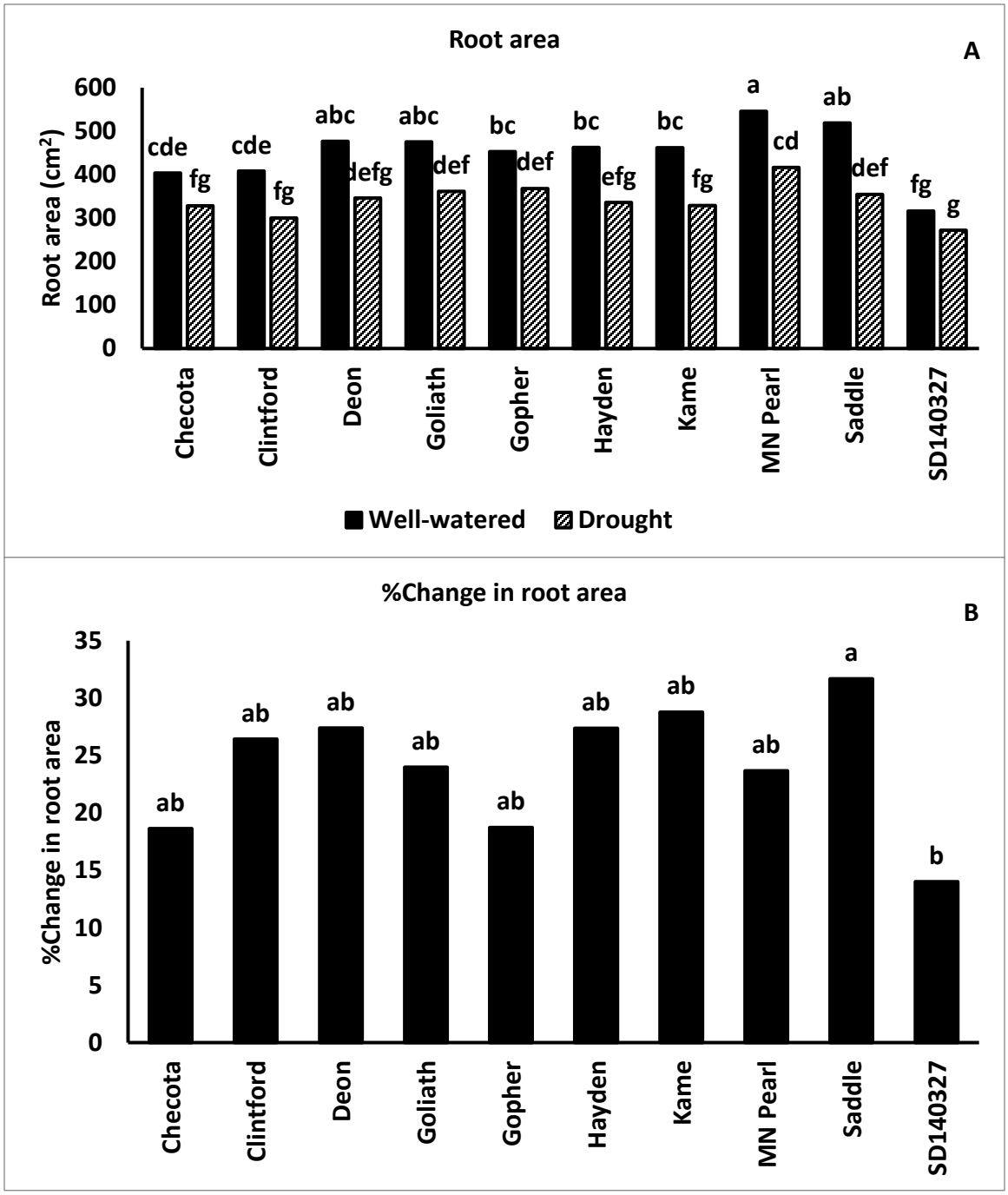

Figure 4.10 . (A) Root area of ten oat genotypes under well-watered and drought conditions, (B) Percent change in root area in response to drought stress. Different letters indicate a significant difference ($p<0.05$).

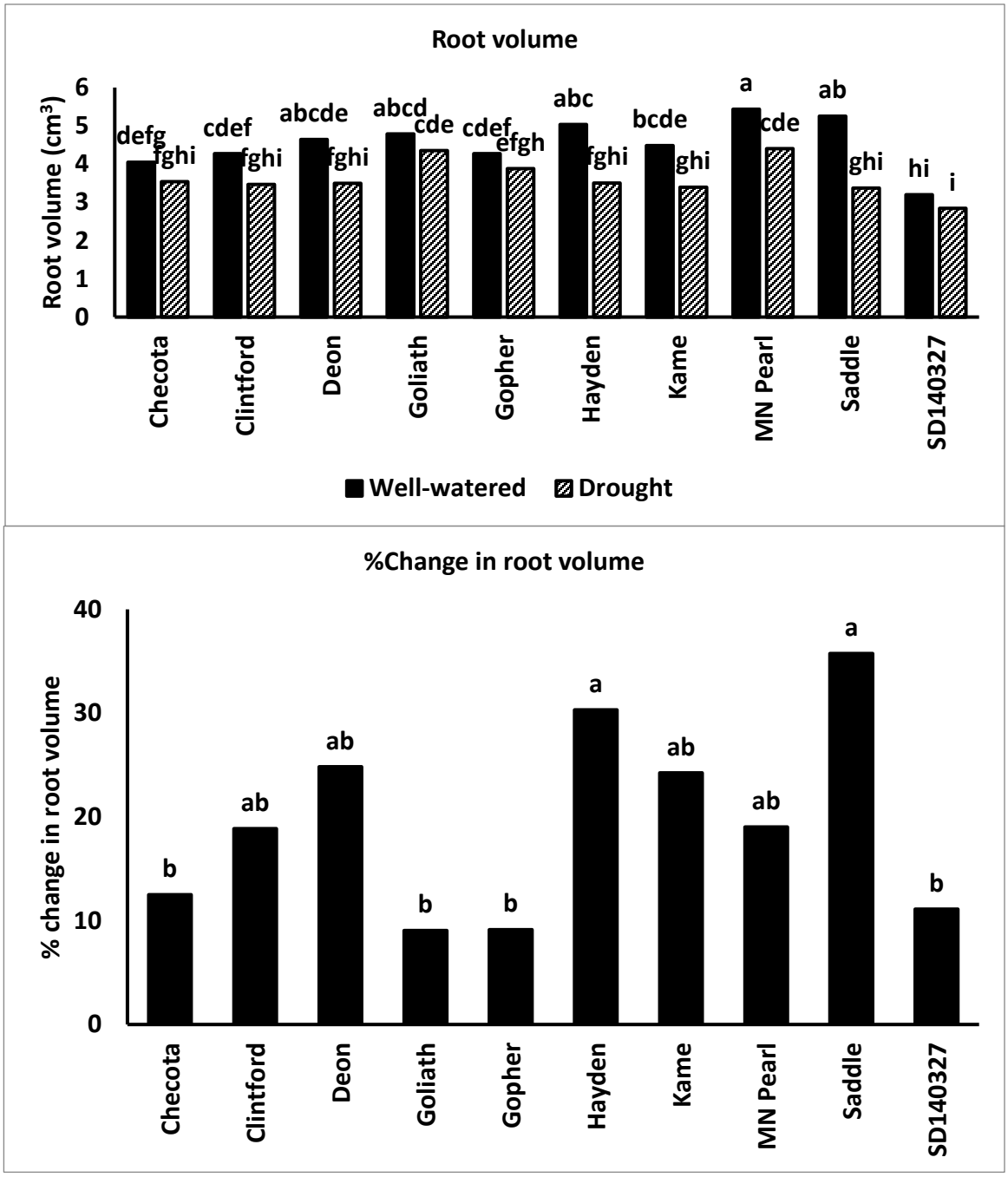

Figure 4.11 (A) Root volume of ten oat genotypes under well-watered and drought conditions, (B) Percent change in root volume in response to drought stress. Different letters indicate a significant difference ($p<0.05$).

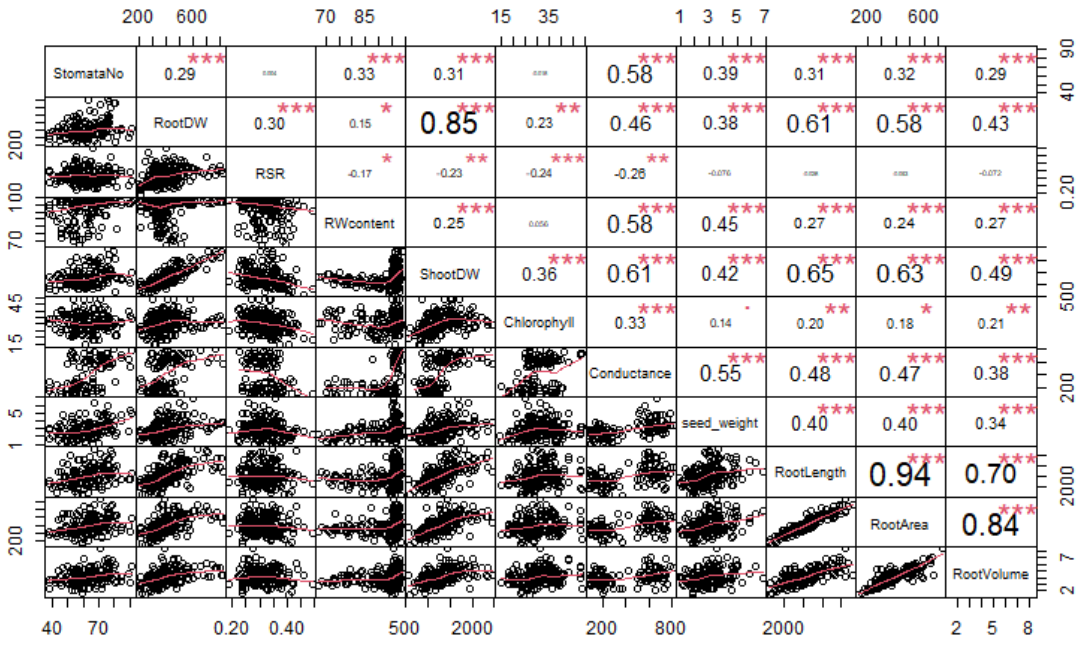

Figure 4.12 Correlation matrix of different root and shoot traits of ten oat genotypes under well-watered and drought conditions.

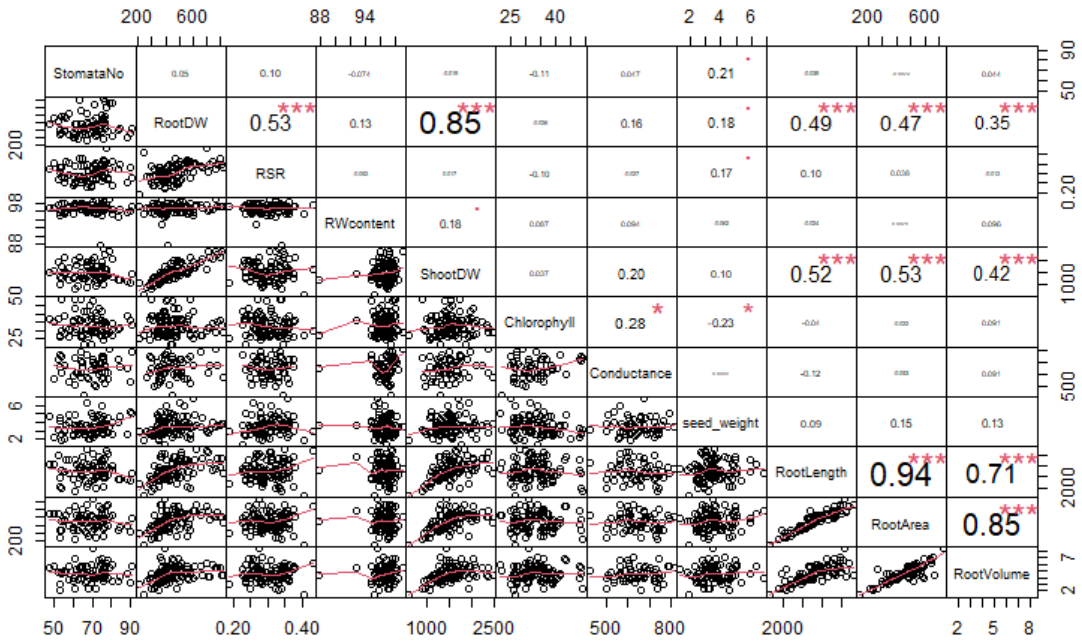

Figure 4.13 Correlation matrix of different root and shoot traits of ten oat genotypes under well-watered conditions.

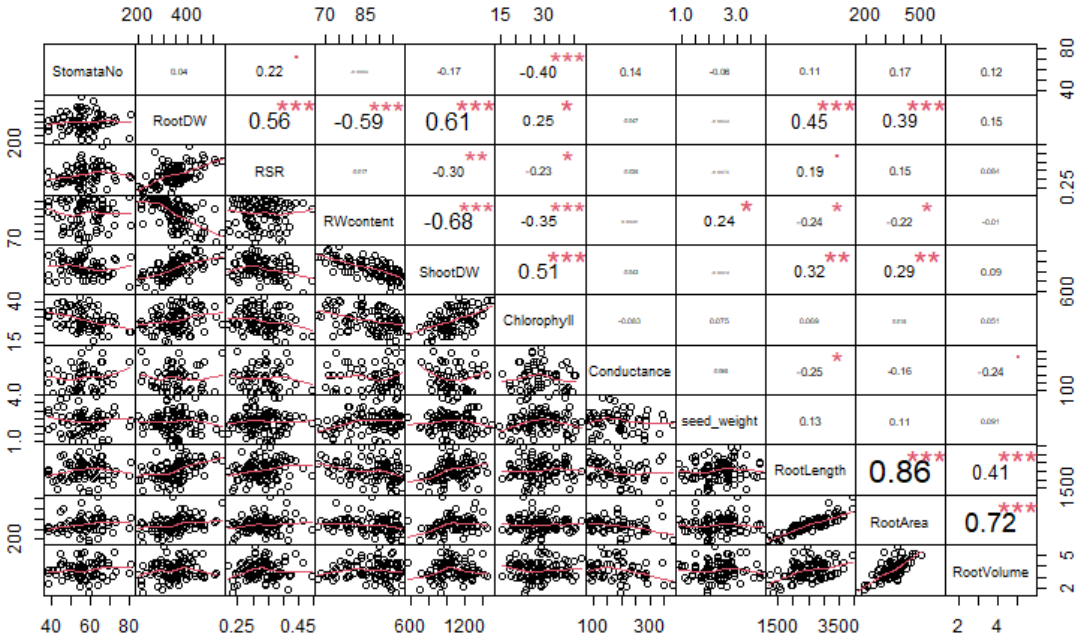

Figure 4.14 Correlation matrix of different root and shoot traits of ten oat genotypes under drought conditions

Discussion

In this study, we investigated the impact of drought stress on physiological and morphological parameters of ten oat genotypes. With an increase in the occurrence of droughts throughout the world, improving drought tolerance in crops is an urgent need, however, drought tolerance is a complex quantitative trait controlled by several small effects genes and confounded by different plant phenology (Barnabás et al., 2008; Fleury et al., 2010). Many traits controlled by several small effects genes can be affected by drought and thus evaluating diverse morphological and physiological root and shoot traits under drought stress is necessary to understand plant response to drought.

All oat genotypes tested in this study showed a significant reduction in shoot dry weight in response to drought treatment. Reduction in shoot dry weight is common for plants facing drought stress. The reduction in shoot dry weight under drought may be due to a reduced growth rate as a result of a reduction in photosynthetic capacity (Chaves et al., 2003). A decrease in photosynthesis can be due to the biochemical decline of the photosynthetic process or due to stomatal closure which reduces the CO_2 entry into the leaf (Flexas & Medrano, 2002). Drought tolerant cultivars typically show a smaller reduction in shoot dry weight compared to susceptible cultivars (Ahmed et al., 2019; Ghimire et al., 2021). In our study, the decrease in shoot dry weight ranged from 25 to 34% but the genotypes did not show a significant difference in their response.

Drought has an impact on leaf chlorophyll content, and we observed a significant effect of oat genotype, water regime, and their interaction on chlorophyll content. Similar results were reported in wheat and maize (Ahmad et al., 2022; Khayatnezhad & Gholamin, 2012). Although Saddle showed no reduction in chlorophyll content under

drought compared to control, it showed the highest reduction in yield. The reduction of yield in Saddle might be due to the decrease in photosynthesis which could be related to rapid stomatal closure and not to biochemical decline in photosynthesis. This is supported by the sharpest decline in stomatal conductance in Saddle. A rapid increase in abscisic acid leading to rapid reduction in stomatal conductance was reported in drought susceptible oat cultivars (Canales et al., 2021).

A decrease in plant photosynthesis will also impact root growth and allocation of photosynthates to the root. We observed a significant decrease in root dry weight in seven out of ten cultivars. Both a decrease (Almaghrabi, 2012) and an increase (Lozano et al., 2020) in root dry weight have been reported under drought in the literature. Fang et al. (2017) summarized the contrasting arguments about the importance of the root system for grain yield under drought. One argument is that a relatively large root system is essential for a crop to absorb more soil water and relieve drought stress. The alternative view is that reducing root biomass increase the availability of photosynthate for above ground parts including grain yield. Root to shoot biomass ratio can account for the size of root system relative to plant size. The size of the root system is an important factor in the acquisition of soil resources but only when considered with whole-plant size (Comas et al., 2013). In our study, two genotypes showed significant increase in root to shoot biomass ratio and eigth genotypes did not show significant difference in root to shoot ratio under drought conditions. This indicates that different genotypes may have different strategies in allocating photosynthates to root and shoot. A preferred mechanism to cope with drought in Mediterranean populations of *D. glomerata* is increasing the root to shoot

ratio and lowering the shoot transpiration requirements, rather than foraging deeper underground (Bristiel et al., 2019).

In our study, Saddle and MN Pearl showed a relatively larger root system (higher root dry weight, root area, root volume, and root length) compared to other genotypes. While all cultivars except SD140327 showed a decrease in root length, no significant difference was observed for % change in root length among cultivars. A reduction in root length, root area and root dry weight in response to drought have been reported in oats and drought tolerant oat cultivars have been reported to exhibit smaller reduction in root length, root area and root weight compared to susceptible cultivars (Canales et al., 2019). However, in our study, we did not observe significant difference in % change in root dry weight and root length among oat genotypes, suggesting that genetic difference in drought tolerance among oat genotypes may be associated with other traits.

A decrease in root length under drought has been reported in both winter and spring wheat. Drought tolerance in winter wheat is associated with a deeper root system and in spring wheat with a well-branched shallow root system (Djanaguiraman et al., 2019). Our results indicate that a having larger root length, root area and root volume may not necessarily contribute to higher yield under drought, further investigation into the distribution of roots into upper and lower soil levels may reveal the relative importance of shallow versus deeper root system in oats. Higher root biomass and root length density in the subsoil layer are thought to be possible features for wheat adaptation to water stress, as they boost the subsoil water extraction capability for grain filling and increased grain yield (Palta et al., 2011).

Plants are known to adjust the use of the photosynthates from metabolic activity to osmotic adjustment and storage compounds under drought (Hasibeder et al., 2015). During drought stress, the RWC also decreases. Maintaining RWC under drought stress can be considered a drought tolerance character (Rahman et al., 2016; Soltys-Kalina et al., 2016). The osmotic adjustment or the accumulation of solutes in response to drought is well recognized to play a role in plant adaptation to drought (Blum, 2017). In our study, the highest decrease in RWC was found in Saddle which also showed the highest decrease in seed yield in response to drought. On the other hand, SD140327 and Hayden showed a smaller decrease in RWC. The oat genotypes that can maintain RWC under drought may be able to produce various organic solutes. Gong et al. (2010) reported that drought tolerant oat genotypes maintained significantly higher RWC and osmotic potential in roots and leaves. While it is difficult to find a single trait responsible for yield advantage in different crops under drought conditions, Blum (2017) reported osmotic adjustments can sustain yield under drought in many crops. The variability in RWC under drought conditions in oat genotypes suggests that oat genotypes have different abilities to produce soluble sugars for osmotic adjustment under drought.

One of the first responses of plants to drought is to close the stomata to reduce transpiration. Stomata are small apertures that open and close to absorb photosynthetic carbon dioxide and to limit water loss through transpiration. Both increase and decrease in stomatal numbers in response to drought have been reported. (Changhai et al., 2010; Ghimire et al., 2021; Li et al., 2017). We observed a significant decrease in stomata number under drought in seven cultivars with Hayden showing the highest decrease. Although stomata number can impact transpiration, the degree of stomatal opening is also

an important factor that determines the resistance of CO_2 and water vapor between the leaf and the atmosphere. The increase in stomatal number under drought can be accompanied by a decrease in stomatal aperture (Ghimire et al., 2021) and smaller stomata are more dynamic in opening and closing and thus regulating transpiration more efficiently (Raven, 2014). Further studies on the size of stomata and how responsive the stomata are to the drought-induced abscisic acid might help better understand the role of stomata in drought tolerance in oats. Since the plant can produce abscisic acid under drought to initiate stomatal closure, the sensitivity of stomata to abscisic acid can determine the effectiveness of stomata in controlling gaseous exchanges.

The cultivar Checota was included in this study as a resistant check based on previous reports of its drought tolerance in field evaluation under rainfed and irrigated conditions (Akcura & Ceri, 2011). In our greenhouse study, Checota was intermediate for reduction in grain yield under drought conditions compared to other cultivars. However, it showed a sharp decrease in chlorophyll content and a relatively small decrease in RWC. Checota also showed a strong increase in root to shoot ratio. Smaller reduction in RWC is also observed in Hayden that showed smaller reduction in grain yield. This indicates that different oat cultivars may employ different mechanism to maintain yield under drought conditions. This is in agreement with the literature that suggests drought tolerance is affected by many small effects gene and thus pointing to a specific trait that contributes in overall drought tolerance in oats may be difficult.

Overall, we evaluated drought response in ten oat cultivars, and they responded differently depending on the trait evaluated. Based on our results, Hayden showed the lowest decrease in grain yield under drought conditions, and it also showed a relatively

smaller decrease in RWC, chlorophyll content, but a higher decrease in stomata number. These traits may be important in oats for coping with drought stress. Drought tolerance is a complex mechanism controlled by many small effect genes. Although it is difficult to find a single trait contributing to yield advantage in different crops under drought conditions, Blum (2017) reported osmotic adjustments can sustain yield under drought in many crops. Hayden maintained both yield and RWC relatively better compared to other cultivars. The decrease in overall plant growth under drought can be attributed to a decrease in stomatal conductance that limits CO_2 entry into the leaf. Thus, having an optimal number of stomata that can open and close more dynamically in response to environmental cues such as light and drought can help oat cultivars optimize the leaf stomatal conductance and thus optimize water use and photosynthesis under drought. A strong decrease in stomata number was observed in Hayden, suggesting small number of stomata may be regulated efficiency in response to drought stress to optimize gaseous exchange and maintaining yield under drought. Further study about the size of stomata and the responsiveness of stomata to abscisic acid may reveal the role of stomata in drought tolerance in oats. Our results suggest that having a larger root length, root area and root volume may not provide a yield advantage under drought conditions. Since root distribution in the subsoil layer is thought to be an adaptation feature in wheat for drought stress (Palta et al., 2011), further studies about the distribution of root mass into different soil layers may reveal the importance of deeper or shallower root system in oats and reveal if differential distribution of root mass in soil layers can balance the distribution of photosynthate into root and shoot to optimize the yield under drought.

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CHAPTER 5

Conclusion and future directions

In this study we evaluated the genetics of oat root traits and their response to drought stress and endophytic bacterial inoculation. We found that there is a genetic component in the variability in the root system architectural traits in oat and the response of oat genotypes to endophyte inoculation and drought stress varied depending upon the oat genotypes. The study on the response of oat cultivars to endophytic bacterial inoculation showed that the endophytic bacteria have the potential in improving oat growth. The response of oat cultivars is highly variable depending upon the plant genotypes, bacterial strains and the traits evaluated, and nitrogen fertilization level, thus it is challenging to find a specific bacterial strain that can promote overall plant growth. Multi-strain inoculation may provide better overall plant growth. Further studies into the multi-strain inoculation and field trials are needed to determine the potential application of bacterial endophytes in oat production. The genome-wide associated study on the root system architectural traits showed that a germination paper-based root phenotyping approach can be used to measure root traits that can be successfully used in genome wide association studies. The single nucleotide polymorphic markers significantly associated with various root traits were located in or near genes that are known to have a role in root development. Further studies into these genes can elucidate the biological function of these genes in root development and may facilitate the development of oat cultivars with an effective root system capable of acquiring soil resources more efficiently. The drought study evaluating the morphological and physiological response of oat cultivars to drought

stress revealed some key traits that may help oat cope with drought stress. Maintaining relative water content under drought conditions and a reduction in stomata number may help plants cope with drought. While our study showed that having larger root length, root area and root dry weight may not provide a yield advantage in drought conditions, conducting a study on the effect of root mass distribution in various soil layers may improve our understanding of the importance of deep or shallow root system for oat plants to cope with drought conditions. Since in field plants are in constant interaction with multiple factors such a drought, soil microbes, a further study into the response to root system with multiple factors might help better understand the overall root response to diverse environmental conditions.

Supplementary Table 2.1**.** Analysis of variance table for root length, root area, and root volume in root vigor assay.

Traits	Effects	P-value
Root length (cm)	Bacteria Genotype Bacteria * Genotype	4.34e-08 *** 2e-16 *** 0.000929 ***
Root area (cm^2)	Bacteria Genotype Bacteria * Genotype	7.31e-15 *** < 2e-16 *** 9.72e-06 ***
Root volume (cm^3)	Bacteria Genotype Bacteria * Genotype	3.54e-11 *** < 2e-16 *** 0.000189 ***

Significant Codes: 0 '***', 0.001 '**,' 0.01 '*': significant at p< 0.050.001, p<0.01, and p<0.05, respectively.

Supplementary Table 3.1. Oat cultivars and breeding lines are used in the genome-wide association of root system architectural traits of oat seedlings.

SN	Genotype	Breeding program
1	AAC_ALMONTE	Agriculture and Agri-Food Canada
2	AAC_OAKLIN	Agriculture and Agri-Food Canada
3	ANDREW	University of Minnesota
4	CLINTFORD	Purdue University
5	CLINTLAND64	Purdue University
6	COLT	South Dakota State University
7	DEON	University of Minnesota
8	GOLIATH	South Dakota State University
9	GOPHER	University of Minnesota
10	HAYDEN	South Dakota State University
11	HORSEPOWER	South Dakota State University
12	IL05_9931	University of Illinois
13	IL08_9201	University of Illinois
14	IL09_5239	University of Illinois
15	IL11_2353	University of Illinois
16	KAME	University of Minnesota
17	MN06120	University of Minnesota
18	MN06203	University of Minnesota
19	MN08138	University of Minnesota

20	MN08160	University of Minnesota
21	MN08211	University of Minnesota
22	MN08243	University of Minnesota
23	MN08252	University of Minnesota
24	MN08260	University of Minnesota
25	MN09103	University of Minnesota
26	MN09105	University of Minnesota
27	MN09115	University of Minnesota
28	MN09223	University of Minnesota
29	MN09230	University of Minnesota
30	MN09255	University of Minnesota
31	MN10121	University of Minnesota
32	MN10130	University of Minnesota
33	MN10209	University of Minnesota
34	MN10253	University of Minnesota
35	MN11110	University of Minnesota
36	MN11139	University of Minnesota
37	MN11211	University of Minnesota
38	MN11221	University of Minnesota
39	NATTY	South Dakota State University
40	ND070182	North Dakota State University
41	ND080816	North Dakota State University
42	ND090709	North Dakota State University

43	ND090868	North Dakota State University
44	ND100362	North Dakota State University
45	ND101473	North Dakota State University
46	ND102000	North Dakota State University
47	ND111357	North Dakota State University
48	NEWBURG	North Dakota State University
49	OA1331_6	Agriculture and Agri-Food Canada
50	P021A1_66_2	Purdue University
51	SD041405	South Dakota State University
52	SD081644	South Dakota State University
53	SD110304	South Dakota State University
54	SD110640	South Dakota State University
55	SD120069	South Dakota State University
56	SD120096	South Dakota State University
57	SD120261	South Dakota State University
58	SD120266	South Dakota State University
59	SD120296	South Dakota State University
60	SD120316	South Dakota State University
61	SD120419	South Dakota State University
62	SD120456	South Dakota State University
63	SD120553	South Dakota State University
64	SD140002	South Dakota State University
65	SD140003	South Dakota State University

66	SD140009	South Dakota State University
67	SD140027	South Dakota State University
68	SD140037	South Dakota State University
69	SD140054	South Dakota State University
70	SD140056	South Dakota State University
71	SD140098	South Dakota State University
72	SD140147	South Dakota State University
73	SD140156	South Dakota State University
74	SD140161	South Dakota State University
75	SD140166	South Dakota State University
76	SD140199	South Dakota State University
77	SD140201	South Dakota State University
78	SD140244	South Dakota State University
79	SD140253	South Dakota State University
80	SD140313	South Dakota State University
81	SD140327	South Dakota State University
82	SD140330	South Dakota State University
83	SD140337	South Dakota State University
84	SD140338	South Dakota State University
85	SD140344	South Dakota State University
86	SD140354	South Dakota State University
87	SD140355	South Dakota State University
88	SD140358	South Dakota State University

89	SD140361	South Dakota State University
90	SD140383	South Dakota State University
91	SD140384	South Dakota State University
92	SD140399	South Dakota State University
93	SD140404	South Dakota State University
94	SD140408	South Dakota State University
95	SD140410	South Dakota State University
96	SD140412	South Dakota State University
97	SD140427	South Dakota State University
98	SD140433	South Dakota State University
99	SD140435	South Dakota State University
100	SD140440	South Dakota State University
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275	SD150315	South Dakota State University
276	SHELBY427	South Dakota State University
277	STREAKER	South Dakota State University
278	SUMO	South Dakota State University
279	WIX10055_8	University of Wisconsin
280	WIX10088_6	University of Wisconsin
281	WIX9082_1	University of Wisconsin
282	WIX9414_1	University of Wisconsin
283	WIX9487_1	University of Wisconsin
284	WIX9528_1	University of Wisconsin
285	WIX9897_5	University of Wisconsin